Host Microbiota and Infection Outcomes in Thermally Extreme Environments

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Declaration

I declare that this thesis was composed by myself and that the work contained herein is my own except where explicitly stated in the text. This work has not been submitted for any degree or professional qualification except as specified.

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Publications and Contributions

The following published (and *in Prep*) papers have arisen from this thesis, and are presented in Chapters 2, 3, 4 and 5:

Chapter 2

Li J, Bates KA, Hoang KL, Hector TE, Knowles SCL, King KC. 2023. Experimental temperatures shape host microbiome diversity and composition. *Global Change Biology.* 29(1):41-56. doi:10.1111/gcb.16429

J.L. and K.C.K. conceived the study. J.L., S.C.L.K., and K.C.K. designed the study. J.L. conducted literature review, collected data, and performed the meta-analysis, with input from K.A.B., K.L.H., T.E.H., S.C.L.K., and K.C.K.. J.L. and K.C.K. wrote the manuscript. All authors contributed to reviewing and editing.

Chapter 3

Li J, Gao Y, Stevens EJ, King KC. 2023. Dual stressors of infection and warming can destabilize host microbiomes. *Philosophical Transactions of Royal Society B.* (*accepted*)

J.L and K.C.K. conceived and designed the study. J.L. conducted the experiment and analysed the data with input from E.J.S. and K.C.K.. J.L. and Y.G. performed DNA extractions. J.L. and K.C.K. wrote the manuscript. All authors contributed to reviewing and editing.

Chapter 4

Li J, Guttmann N, Drew GC, Hector TE, Wolinska J *, King KC *. Warming-induced excess deaths of infected animals depend on pathogen traits (*in prep*)

*Equal contribution

J.L., J.W., K.C.K. and G.C.D. designed the study. J.L. and N.G. conducted literature review and collected data. J.L. performed the meta-analysis, with input from J.W., K.C.K., G.C.D. and T.E.H.. J.L. and K.C.K. wrote the manuscript. All authors contributed to reviewing and editing.

Chapter 5

Li J, Chen J, King KC. Prolonged warming strengthens selection for host resistance to infection (*in prep*)

J.L. and K.C.K. conceived and designed the study. J.L. conducted the experiment and analysed the data, with input from K.C.K., J.L. and J.C. conceived the mathematical model. J.L. and K.C.K. wrote the manuscript. All authors contributed to reviewing and editing.

Thesis Abstract

Hosts, their collection of commensal and pathogenic microbes, and the environment are all interlinked in a disease pyramid. Their interactions contribute to host fitness, particularly relevant in the context of a changing world. Climate change is leading to higher average temperatures as well as shifting the global distribution of infectious diseases, posing great risk to wildlife, ecosystem biodiversity, and human health. Host microbiomes are increasingly regarded as conferring extended phenotypes, potentially buffering host organisms against abiotic and biotic stressors. In this thesis, I combine meta-analytical and experimental approaches to explore the impact of warming temperatures and infection on ecological dynamics (i.e., microbiomes, infection disease outcomes) and evolutionary dynamics (i.e., host gene-based resistance). Firstly, I used a meta-analytic approach to investigate whether and how experimental temperatures altered host microbiome structures, across a wide range of host species. I found that experimental warming and cooling drove microbiome diversity loss, with the magnitude affected more by host habitat and experimental protocols, rather than host biological traits. I then extended this work to empirically include a biotic factor – pathogen infection – to investigate changes in *Caenorhabditis elegans* microbiome composition. I revealed that warming and infection could destabilize microbiome communities within hosts, but their effects were not additive. Focusing more on the impact of warming to host-pathogen interactions, I subsequently used a meta-analytic approach to tackle the relationship between warming and disease outcomes across ectothermic animals. I found that experimental warming drove higher mortality of infected hosts, with larger temperature increases associated with more host deaths. The magnitude of these effects varied by pathogen taxonomy and their evolutionary history within the host. Lastly, I zoomed out to empirically capture the possible host evolutionary paths for resistance to pathogens due to warming. I competed two *C. elegans* genotypes (susceptible wild-type vs. resistant mutant) across 10 host generations, varying in pathogen presence and the timing of warming during their development. I detected a loss

of genetic-based resistance under periodic warming despite infection. I revealed that such host evolutionary trajectories could be driven by the combination of fitness constraints on genetic-based resistance, temperature-mediated host protection, infection severity, as well as the dilution of pathogen cells by resistant hosts. Work in this thesis is a timely contribution to our understanding of the diversity of consequences of warming to host-microbe interactions across the mutualist-parasite continuum. Biologists seeking to refine predictions of biodiversity amidst climate change should strongly consider the relationships of animals with their resident and attacking microbes.

Chapter 1:

Introduction

Introduction

1.1 Host-microbe interactions

Virtually all plants and animals are ubiquitously associated with microorganisms (Overstreet & Lotz 2016). These microorganisms can be bacteria, archaea, virus, and fungi, with bacteria being the most abundant taxonomic group and mostly studied (González & Elena 2021). Microbial associations, or 'symbiosis', can be harmful, beneficial, or neutral to the host organism, with rapid transitions possible across the mutualist-parasite continuum (Drew et al. 2021).

Mutualisms can occur when microbial symbionts engage in mutually beneficial relationships with their host. Microbial mutualists can play essential roles in host biology (Su et al. 2013). Historically, research on mutualistic relationships has focused on the interactions between hosts and their individual symbionts (Moran et al. 2008; Werren et al. 2008). However, this view has been broadened in recent years as symbiotic microbes can exist within a community harboured by the host, called the microbiome (Blaser 2014). Microbiomes can feed on host nutrients, and play important roles in host nutrient absorption, energy harvest, immunity, and defence against pathogen infection (Clemente et al. 2012; Nicholson et al. 2012; Marchesi et al. 2016). Moreover, host microbiomes can impact host physiology and adaptation abilities (Alberdi et al. 2016; Kohl & Carey 2016; Fontaine & Kohl 2020). A general change in microbiome compositions in response to perturbations can sometimes be described as a state of "imbalance" or "dysbiosis", indicating negative effects on host health (Hooks & O'Malley 2017). However, there is no robust association between unhealthy host conditions and microbial variations, nor has "dysbiotic" microbiota been clearly defined (Hooks & O'Malley 2017; Olesen & Alm 2016).

Microbial associations can also be harmful for the host. Pathogens (or parasites) can exploit host resources to cause harm. Some pathogens cause mild symptoms in their hosts, such as *Equine herpesvirus type 1*, which normally give horses mild to moderate respiratory

disease and a fever (Goehring et al. 2005). By contrast, other pathogens, such as the fungus *Batrachochytrium dendrobatids*, can cause chytridiomycosis in amphibians. This disease is thought to be a major player in global declines and extinctions of amphibian populations (Voyles et al. 2009, Wilber et al. 2017). Furthermore, the same pathogen can have highly variable effects on different hosts. The fungus, *Batrachochytrium dendrobatids*, kills some frog species within weeks, but only has mild negative effects on others (Skerratt et al. 2007).

The fitness outcome of host-microbe associations is not necessarily fixed. Host-associated microbes can shift along a mutualistic-parasitic continuum in ecological or evolutionary time, as the relative benefits and costs change (Drew et al. 2021). Abiotic factors, such as temperature (Baker et al. 2018), carbon and nitrogen availability (Hom & Murray 2014), and biotic factors, such as species interactions within the community (Oliver et al. 2003; Bates et al. 2021), can drive these transitions. For example, a parasitic microbe can shift from parasitic to mildly helpful and protective during coinfections with a more virulent pathogen (Kieran et al. 2021). Microbes can also rapidly adapt to their biotic and abiotic environments, fueling movements along the continuum (Sachs et al. 2011; King et al. 2016; Gibson et al. 2015).

1.2 The effect of temperature on host and microbes

Climate change is increasing average global temperatures and leading to more extreme thermal events. Temperature, as a crucial abiotic environmental factor affecting all levels of biology, directly or indirectly impacts the physiology and life-history for all organisms (Lagerspetz 1987). At an individual level, an organism's response to changes in body temperatures can depend on its thermal tolerance curves, which consisted of several key parameters, including the critical thermal maximum, minimum and optimum - the upper, lower and optimal temperatures at which it cannot function (Chown et al. 2010; Hoffmann et al. 2003; Huey et al. 2012). Thus, organism performance increases with temperature to

an optimum and declines after reaching the thermal maximum (Chown et al. 2010; Kingsolver & Buckley 2017). Typically for multicellular ectothermic species, these curves are often used to assess their vulnerability to climate warming, as warming directly changes their body temperatures, influencing their physiological sensitivity and fitness (Huey et al. 2012). The difference between ectotherm's thermal maximum and the temperature it regularly experiences is defined as heat safety margins (Sunday et al. 2014). Heat safety margins often increase with latitude (Deutsch et al. 2008). Ectothermic hosts from tropical areas are thought to have relatively narrow heat safety margins, implying their higher vulnerability to climate warming than their temperate counterparts (Deutsch et al. 2008; Hoffmann et al. 2012; Huey et al. 2009; Pinsky et al. 2019). Endothermic hosts can maintain constant body temperature mainly by physiological adjustment under diverse environmental conditions, under warming they may alter physiological functions such as increasing evaporative cooling (Boyles et al. 2011). As sessile organisms, plant hosts can modify their metabolism in response to temperature rise, given that most plants cannot regulate heat production (Zhu et al. 2023; Guihur et al. 2022).

Microbes are highly sensitive to temperatures. Temperature can influence bacterial motility, exotoxin production, nutrient acquisition, and host attachment (Lam et al. 2014). Temperature also directly mediates pathogen virulence and transmission (Blanford et al. 2003; Laine 2007; Vale and Little 2009; Kimes et al. 2012). For example, higher water temperatures can enhance the virulence of the coral pathogen *Vibrio coralliilyticus* by upregulating the expression of virulence factors (Kimes et al. 2012). For complex microbial communities, the effect of temperature can be mediated by altered physiology of individual microbes, and/or microbe-microbe interactions within the community (Burman & Bengtsson-Palme 2021). For host microbiomes, the effect of temperature can be indirect and through altered host-microbe interactions (Hague et al. 2022). It has been shown that, across a wide range of host species, experimental temperature change can disturb host microbiome compositions and decrease microbiome diversity (Li et al. 2023).

1.3 The disease pyramid

The disease outcome induced by pathogen infection can depend on the dynamic interactions between the host, the pathogen, and the environment (Stevens et al. 1960; Snieszko 1974; Allen & Little 2011; Agha 2018; Wolinska & King 2009). Outcome of such dynamic tripartite interactions is illustrated by a “disease triangle” (Stevens et al. 1960). The disease triangle was initially formalized in plant systems (Stevens et al. 1960) and is also useful for understanding the disease dynamics in animal hosts amidst climate change (Bernardo-Cravo et al. 2020). Periods of extreme heat can suppress components of animal host immunity, facilitating the occurrence of infections (Moriyama & Ichinohe 2019; Regnier et al. 1981; Lecchi et al. 2016; Lacetera et al. 2005). Pathogen infection can reduce host thermal maxima, by causing host stress and disrupting homeostasis (Hector et al. 2023). Host and pathogens can differ in their abilities to acclimate or adapt to thermal changes. Pathogens often have shorter generation times and faster metabolisms than hosts. They might thus adapt more rapidly to temperature shifts than their hosts. This discrepancy might increase pathogen growth within hosts and worsen infection outcomes (Gillooly et al. 2001).

Accumulating evidence has shown that microbiomes could mediate host tolerance to extreme temperatures, across a diversity of animal species (Chevalier et al. 2015; Hector et al. 2022; Moghadam et al. 2018). Specifically, microbiomes are associated with improved host thermal tolerance, potentially aiding host adaptation to warmer environments (Moghadam et al. 2018; Posadas et al. 2022; Fontaine et al. 2022 Ziegler et al. 2017). The microbiome can also influence disease outcomes. Microbiomes can interact directly with infecting pathogens (i.e. resource competition or interference competition), or modulate host immunity and defense responses (Kamada et al. 2012; Louca et al. 2016; González & Elena 2021). Conversely, the host microbiome might enhance pathogen infection and worsen disease severity, through modifying within-host environment and/or cross-feeding the invading pathogens (Stevens et al. 2021). As the microbiome is considered part of the extended phenotype of host defenses, it is now incorporated into the three-edged disease

triangle, making it a four-edged disease pyramid (Figure 1) (Bernardo-Cravo et al. 2020). Current climate-induced changes in species interactions are highlighting a timely need for the application of the disease pyramid. This disease pyramid enables the investigation of interactions among the host, host microbiomes, pathogen and the external environment (i.e. temperature). In sections below, I will introduce and illustrate the multi-way interactions shown by the disease pyramid (Figure 1).

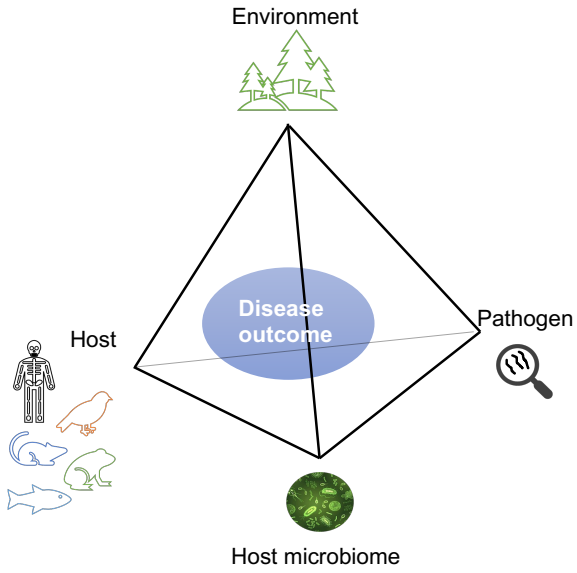

Figure 1. The disease pyramid, adapted from Bernardo-Cravo et al. 2020. The disease pyramid shows that the interactions among host, host microbiomes, pathogen and the environment together contribute to disease outcomes.

1.4 The role of temperature in structuring host microbiomes

Host microbiomes can be shaped by a diversity of abiotic environmental factors, such as nutrient availability, chemicals, and temperatures (Wang et al. 2018; Messyasz et al. 2021; Li et al. 2023; Zaneveld et al. 2016). For example, nutrient enrichment can increase both coral microbiome richness and community variations (Messyasz et al. 2021). The effects of temperature on microbiomes and the resulting fitness impacts on host have been tested more widely, given its close relevance to species persistence under climate change (Fontaine et al. 2022; Li et al. 2023).

Temperature stress can induce disturbance to microbiome structures or loss of microbiome diversity (Hylander & Repasky, 2019; Sepulveda & Moeller, 2020), which can be associated with diminished host health (Doering et al. 2021; Jaramillo & Castañeda, 2021). One study showed that experimentally depleting diversity in the tadpole gut microbiome—through environmental water sterilization—reduced tadpole acute thermal tolerance to both heat and cold (Fontaine et al. 2022). Another has revealed that microbiome transplantation from heat-tolerant *Drosophila melanogaster* to recipient flies could confer heat tolerance (Moghadam et al. 2018). In corals, higher abundances of particular bacterial taxa have been correlated with host fitness during short-term heat waves (Hartman et al. 2019; Ziegler et al. 2017). Microbiomes thus might be important for host thermal tolerance, and disruption of those microbiomes induced by thermal change can affect host key functions (Doering et al. 2021; Jaramillo & Castañeda, 2021).

As hosts and their microbiomes are constantly interacting (Foster et al. 2017), microbiome changes under different temperatures can also be mediated by host-related factors (Kers et al. 2018; Woodhams et al. 2020; Etemadi et al. 2018). Several hypotheses can be developed. The thermal safety margins of ectotherms are shaped by their adapted thermal environments (Hector et al. 2020; Kelly et al. 2012; Yampolsky et al. 2014), which may affect the microbiome changes. Thus, ectotherms from tropical areas may be more stressed and have more disrupted microbiomes under climate warming, given their relatively narrow heat safety margins (Deutsch et al. 2008; Hoffmann et al. 2012; Huey et al. 2009; Pinsky et al. 2019). Microbiome colonization site might affect the relative impact of temperature on microbiome structures. Compared with host internal microbiomes, external microbiomes (i.e. skin microbiomes) directly interact with external temperatures and might be more influenced by environments (Woodhams et al. 2020). The extent to which microbiomes change in response to temperatures are impacted by variable host and environmental factors may be important for predicting host performance under global change, which I tackle in Chapter 2.

1.5 The impact of temperature on host-pathogen interactions

Infectious diseases are affected by climate warming and increasingly regarded as great risks to human and wildlife (Altizer et al. 2013, Cavicchioli et al. 2019). Infection outcomes in any host-pathogen systems might be associated with fitness changes in both partners, under rising temperatures. Such fitness changes likely depend on their thermal tolerance (Chown et al. 2010; Kingsolver & Buckley 2017). A mismatch in host and pathogen thermal optima can change infection outcomes, such that cold-adapted pathogens might be less harmful in a hot-adapted host at higher temperatures (Cohen et al. 2017; Cohen et al. 2020). This situation could facilitate host jumping and worsening disease as pathogens expand their ranges under climate change (Roberts et al. 2018). Shifting global temperatures are changing the geographic distribution of both host and pathogens (Jones et al. 2008; Liang & Gong 2017), generating novel transmission opportunities (Roberts et al. 2018). Pathogens may expand or contract their host ranges, under changing global temperatures (Roberts et al. 2018). Transmission for a pathogen to become established in a new host species can lead to intensified disease outbreaks. For example, some of the deadly human pathogens including Ebola virus and SARS coronavirus, have been linked to the host switch from wild animal reservoirs to humans (Leroy et al. 2014; Pimentel et al. 2020; Villordo et al. 2015; Musso et al. 2014; Caminade et al. 2014; Tsetsarkin et al. 2011).

The infection outcomes and host persistence under changing temperatures can also depend on host resistance to pathogens. Resistance is broadly defined as host ability to suppress or eliminate the invading pathogens, including defenses such as physical barriers, behavioural avoidance, or rapid immune responses (Raberg et al. 2009). Within host populations in nature, individuals can vary in their resistance to infection (Thrall & Burdon 2000; Altermatt & Ebert 2008; Thrall et al. 2012; Laine 2004). Resistance is under continual selection by pathogens due to reduced relative fitness of infected individuals (Anderson & May 1982). Thus, resistance is not a fixed trait for host populations, instead it is constantly evolving, eventually impacting species outlook under infectious diseases and the changing

environments (Bérénos et al. 2009; Duffy & Sivars-Becker 2007). Virulent pathogens can strongly select for resistance, but fixation of resistance in host populations rarely happens in nature (Allen et al. 2004; Laine 2004). The persistence of susceptible hosts in the presence of pathogens can be explained by multiple mechanisms, such as loss of resistance due to selection against costly resistance (Koskella 2018). Genetic-based resistance commonly carries fitness cost (i.e. reduced fecundity), compared with susceptible individuals in the absence of infection (Sheldon & Verhulst 1996, Kraaijeveld & Godfray 1997).

Host resistance can be shaped by both biotic and abiotic factors. Biotic factors such as previous microbial exposure or infection history are shown to impact host resistance to secondary infection, through regulation of host immunity (Salgame et al. 2013; Desai et al. 2021; Mabbott 2018). Abiotic factors such as nutrition, can affect host resistance, such as that acute food deprivation prior infection can reduce resistance of American Robin (*Turdus migratorius*) to West Nile virus (Owen et al. 2021). Temperature, which shapes host physiological and immune responses, can impact host resistance to infection (Angilletta 2009; Elliot et al. 2002; Mitchell et al. 2005; Vale et al. 2008; Garbutt et al. 2014; Manzi et al. 2019). Warming can change the body temperatures of ectothermic hosts, influencing their physiological sensitivity and fitness (Huey et al. 2012; Deutsch et al. 2008), rendering them more susceptible to infections (Ben-Horin et al. 2013). For endothermic hosts that can maintain constant body temperatures under different conditions, under warming they may need to invest more energy in maintaining their internal temperature, thus investing less in anti-pathogen defenses (Rohr et al. 2013). Conversely, infection has also been shown to interfere with host responses to thermal stress (Sherman 2008; Bates et al. 2011; Linderman et al. 2012; Greenspan et al. 2017; Gehman et al. 2018), reducing host fitness under these dual stressors.

Temperatures experienced during host development can cue plastic responses in resistance. In *C. elegans*, early-stage heat stress confers resistance to infection later in life, likely due to heat stress activating the heat shock protein transcription and production, part of the host multi-pathogen defense pathways (Singh & Aballay, 2006; Prithika et al. 2016). The protective effect of early-stage heat exposure has been found in other host organisms, from broiler chickens to plants (Liew et al. 2003; Janda et al. 2019; Lee et al. 2012). For *C. elegans*, inheritable multigenerational plasticity has also been established (Moore et al. 2019; Palominos et al. 2017; Burton et al. 2020; Wibisono & Sun, 2023; Legüe et al. 2022; Baugh & Day, 2020). It was shown that previous pathogen exposure could lead to acquired pathogen avoidance behaviour and resistance, which transmitted across nematode generations (Baugh & Day, 2020; Ghildiyal & Zamore, 2009; Fire et al. 1998). Such nongenetic inheritance was via histone modifications and small non-coding RNA regulation in progeny gene expression (Ghildiyal & Zamore, 2009; Fire et al. 1998). It is however not clear whether warming could induce inheritable epigenetic phenotypic change to infection (Baugh & Day, 2020). It is largely unclear how infection outcomes and the evolutionary dynamics of host resistance will be shaped by warming which I tackle in Chapter 4 and Chapter 5.

1.6 The impact of pathogen infection on host microbiomes

When evading the host, pathogens are also interacting with the colonised microbiome (Pallen 2011). Host microbiomes generally confer protection against pathogen infection. Such microbiome-mediated protection has been widely found across multiple host species, through mechanisms such as resource competition (Kamada et al. 2012; Louca & Doebeli 2016; King et al. 2016) and modulation of host immune responses (Rooks & Garrett 2016; Brown et al. 2013). However, these benefits might decrease when the microbiome directly or indirectly facilitate pathogen infection and worsen disease severity (Broderick et al. 2006; Wei et al. 2017). For example, the pathogen might benefit from exploiting the metabolites produced by the microbiomes (Roman & Wagner 2018).

Reduced host fitness induced by infection can correlate with perturbation in the microbiomes, causing transition of beneficial or commensal microbes to become harmful to the host (Broderick et al. 2006; Wei et al. 2017). In humans, infection-induced perturbation of gut microbiomes has been associated with chronic diseases ranging from gastrointestinal inflammatory to respiratory illnesses (Sun & Jia, 2018; Durack & Lynch, 2019). Changes in microbiome structure as a result are increasingly recognized as meaningful indicators to altered host health under infection (Jani & Briggs 2014; Muletz-Wolz et al. 2019; Hill et al. 2012; Duvallet et al. 2017).

Pathogen infection can alter microbiome diversity, with the direction of change varying across species and context (Gaulke et al. 2019; Vlčková et al. 2018; Leung et al. 2018; Chang et al. 2008; Muletz-Wolz et al. 2019). For example, *Clostridioides difficile* infection in the human gut can decrease microbiome diversity (Chang et al. 2008), so is infection of *Batrachochytrium dendrobatidis* on red-backed salamanders (Plethodon) skin microbiomes (Muletz-Wolz et al. 2019), while *Mycobacterium tuberculosis* or helminth infection has the opposite effect on human gut microbiomes (Maji et al. 2018; Luo et al. 2017; Lee et al. 2014). Increased microbiome alpha diversity under infection is hypothesized to be caused by an altered gut immune environment with prominent inflammation (Gaulke et al. 2019; Lee et al. 2014; Broadhurst et al. 2012). Apart from altering host microbiome diversity, pathogen infection can also disrupt microbiome compositions and destabilize the community, through direct interactions with individual microbiomes or induced inflammation within host (Borton et al. 2017; Ma 2020). Destabilization can be characterized by increased interindividual variability in microbiome compositions, termed dispersion. Such a pattern normally indicates a loss of or lowered host ability to regulate community composition under stressful conditions (Zaneveld et al. 2017; Ma 2020; Lesser et al. 2016). It has been established in human and animal gut microbiomes that dispersed microbiome compositions

are more likely to occur in infected individuals than healthy individuals (Ma 2020; Lesser et al. 2016; Dey et al. 2013; Giongo et al. 2011).

1.7 Temperature X infection effects on host microbiomes

Stressors, biotic or abiotic, can be temporally and spatially variable in nature, but also occur simultaneously. Under current climate change, the pressures of temperature extremes, pollution and overexploitation can have cumulative effects to devastate biodiversity (Butchart et al. 2010; Barnosky et al. 2011). The cumulative effects of multiple stressors can be magnified by synergistic interactions, accelerating biodiversity loss (Côté et al. 2016; Brook et al. 2006).

Similarly, hosts and their microbiomes are often faced with multiple stressors. For example, marine invertebrates-associated microbiomes are under global stressors such as climate warming and local stressors such as pollution or overfishing (Lesser et al. 2016; McDevitt-Irwin et al. 2017; Zaneveld et al. 2016; Wang et al. 2018). Only a few studies empirically tested the individual and combined effects of multiple stressors on host microbiomes. Previous studies showed that the effects of multiple stressors (i.e. nutrient pollution (Zaneveld et al. 2016), simulated predation (Maher et al. 2019), overfishing (Zaneveld et al. 2016) and warming (Maher et al. 2019)) on coral tissue loss/mortality or microbiomes acted in a non-additive or 'opposing', rather than a synergistic or additive fashion (Zaneveld et al. 2016; Maher et al. 2019). Non-additive effects of infection and temperatures have also been observed in studies on skin microbiomes of red-backed salamanders (*Plethodon*) (Muletz-Wolz et al. 2019) and hemolymph microbiomes of Pacific oysters (Lokmer & Wegner 2015).

Animals are constantly faced with the dual threat of climate change-induced warming and infectious diseases, with warming also interacting with infection to shape host fitness consequences (Lafferty & Mordecai 2016). As host microbiomes play important roles in both host thermal tolerance (Jaramillo & Castañeda 2021; Doering et al. 2021) and pathogen resistance (Stevens et al. 2021; King et al. 2016), they might be critical in

mediating host responses to multiple stressors (Fontaine et al. 2022). Changes in the dynamics and composition of host microbiomes under warming and infection might thus be meaningful indicators for predicting host persistence under climate change and outbreaks (Jani & Briggs 2014; Ribas et al. 2023; Astudillo-García et al. 2019). I investigate the extent to which warming and infection interact to shape host microbiomes in Chapter 3.

1.8 Experimental system

In this thesis, I conducted experimental work using the model host organism *Caenorhabditis elegans* and its natural pathogen *Leucobacter musarum* sp. nov. subsp. musarum subsp. nov strain CBX152T (*L. musarum*). The host microbiome I used was CeMbio community. Each component in this experimental system is elaborated in sections below.

1.8.1 *Caenorhabditis elegans* as a model animal host

Caenorhabditis elegans is a free-living nematode species that is normally found in soils or rotting compost (Kurz & Ewbank 2000). The nematodes are simultaneous hermaphrodites capable of self-fertilization, and only <0.2% of the progeny are males under lab conditions (Hodgkin & Doniach 1997). This trait enables easy maintenance of homogenous lines of *C. elegans* hermaphrodites in lab. The nematode has rapid life cycle, composed of six distinct stages (Figure 2, egg, L1-L4 larve stages, and adult), which occurs in around 3.5 days at 20°C (Brenner 1974; Hope 1999).

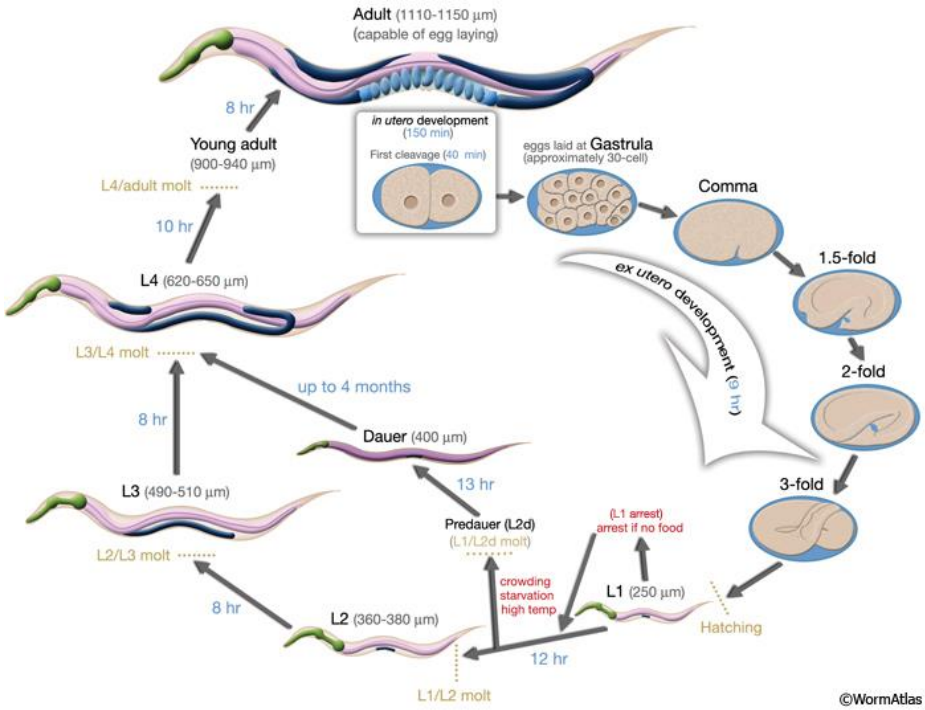

Figure 2. *Caenorhabditis elegans* life cycle. *C. elegans* hermaphrodite develop from eggs to adults producing their own fertilized eggs in approx. 3 days at 22°C (wormbook.org). The first larval stage (L1) can arrest their development if insufficient food is available and enter into a stress-resistant larval stage known as the dauer stage (Golden and Riddle 1984). With sufficient food (normally Escherichia coli strain OP50), L1 nematode then goes through 3 more larval stages (L2, L3 and L4) each lasting around 8-12 hours before becoming an adult. Adults can live for several weeks. In the figure, 0 hours represents fertilization, and the blue numbers correspond to the duration of each developmental stage. The body length of nematode is noted in brackets. The image is taken from wormatlas.org.

Wild nematodes eat bacteria in their natural environments (Schulenburg & Félix 2017). Under lab conditions, *C. elegans* are typically fed *E. coli* strain OP50 bacteria on Nematode Growth Media (NGM) petri dish, which has a well-established recipe (wormbook.org). The gut of *C. elegans* can be colonized by a wide variety of microbial species, which affect host life history traits, making it a good animal host for studying host-microbiome interactions (Clark & Hodgkin 2014; Kumar et al. 2020). *C. elegans* can be infected by several species of pathogens, which are mostly culturable and easily maintained in lab (Schulenburg & Félix 2017). Naturally associated bacterial pathogens can either infect host by cuticle attachment and rectal invasion such as *Leucobacter spp* (Hodgkin et al. 2013), or colonization of the gut lumen such as *Pseudomonas aeruginosa* (Tan & Ausubel 2000) and *Bacillus thuringiensis* (Schulte et al. 2011). Host intestinal damage can occur from gut accumulation of pathogenic bacteria, which excreted toxin producing spores (Schulenburg & Félix 2017;

Kurz et al. 2003) and expressed virulence factors (Tan & Ausubel 2000), eventually kill the nematode host. Fungal pathogens such as *Drechmeria* and *Harposporium* can infect *C. elegans* by ingested spores or hyphae adherence to host cuticle (Engelmann et al. 2011). Moreover, viruses such as Orsay virus (Félix et al. 2011), and microsporidia, *Nematocida parisii*, can infect *C. elegans* by injecting DNA or RNA through the intestinal cells resulting in loss of host gut structure (Balla et al. 2015). Numerous *C. elegans* mutants with mutations in surface-affecting genes were shown to be resistant to various bacterial pathogens (O'Rourke D et al. 2023). For example, the N2-mutant srf-2 (CB6850 = ccIs4251 srf-2(yj262)), which has altered surface glycosylation, is resistant to *M. nematophilum* and *L. musarum*.

Caenorhabditis elegans has been extensively used to study host-pathogen interactions and the evolutionary dynamics with opportunistic pathogens, such as *Staphylococcus aureus* (Ford et al. 2017) and *Enterococcus faecalis* (Ford et al. 2016) in lab experiments. The mechanisms of *C. elegans* immune or cellular responses under pathogen attack, have provided useful insights into immune functions of more complex animals, which are otherwise difficult to measure (Schulenburg et al. 2004; Gray & Cutter 2014). Such experiments are normally carried out on the canonical lab strain N2, but there are over 150 different wild strain isotypes and genetic mutants, archived by the Caenorhabditis Genetics Center (CGC). The wild nematode strains are collected globally and thus adapted to different environmental conditions.

Temperature, in particular, can impact *C. elegans* fitness (Aprison & Ruvinsky 2014) and evolution (Hoang et al. 2021), as well as their susceptibility to pathogens (Zhou et al. 2019). Nematodes are normally reared at 15-20°C. N2 worms are heat-stressed at 25°C and above with reduced fecundity (Gouvêa et al. 2015). When temperature increases to 27°C, nematode fertility drops dramatically which makes long-term maintenance becomes impossible (Petrella 2014; Begasse et al. 2015). Temperatures within the 15-25°C

cultivation range also have major effects on worm developmental time or longevity (Xiao et al. 2013; Klass 1977). For example, N2 worms completes the life cycle in around 5 days at 15°C, whereas at 25°C worm has faster development and compete life cycle in around 2.5 days. Similarly, compared to worms maintained at 20°C, the mean life span of *C. elegans* extends by 60% at 16°C or shortens by 50% at 24°C (Klass 1977). Moreover, temperature's effect on worm longevity can depend on worm lifestage under exposure (Zhang et al. 2015). For example, nematode exposure to low temperatures 15°C during adult stage prolongs lifespan whereas low temperatures at the larval stage reduces lifespan, compared to standard cultivation temperature 20°C (Zhang et al. 2015). Besides lab N2 strain, some other wild *C. elegans* have been isolated from tropical areas where the maximum annual mean temperature can reach up to 25°C, which may enable different climatic adaptability of these isolates (Andersen et al. 2012). Together, this host animal will enable us to investigate the impact of warming, infection, and host background on host microbiome assembly, using controlled lab experiments.

1.8.2 Worm microbiome

In nature, *Caenorhabditis elegans* nematodes feed on bacteria which proliferate on decaying organic matter. The interactions of wild *C. elegans* with associated microbiomes were characterized for the first time in 2016 (Berg et al. 2016; Dirksen et al. 2016; Samuel et al. 2016). These studies showed that bacterial taxa such as *Enterobacteriaceae, Pseudomonaceae, Xanthomonodaceae* (in *Gammaproteobacteria*) and *Sphingobacteriaceae, Weeksellaceae, Flavobacteriaceae* (in *Bacteroidetes*) dominated associated microbiome communities of *C. elegans*. Rare taxa like *Acetobacteriaceae* were also consistently found. *C. elegans* microbiota have been shown to enhance host growth and resistance to both abiotic and biotic stressors including temperatures, osmotic stress and pathogen infection (Dirksen et al. 2016; Samuel et al. 2016). Moreover, the microbiome composition can impact *C. elegans* population sizes in natural habitats (Samuel et al. 2016). For example, communities rich in *Alphaproteobacteria* (e.g. several *Acetobacteriaceae*)

can promote *C. elegans* population growth, while *Bacteroidetes* or pathogenic bacteria were associated with non-proliferating nematode populations (Samuel et al. 2016). Thus, the associated microbiome community is likely a key determent of *C. elegans* life history, impacting a variety of nematode physiological and adaptation functions.

A community of 12 bacterial isolates found naturally associated with *C. elegans* has been assembled (CeMbio kit) (Dirksen et al. 2020). The CeMbio community is a simplified core microbiome derived from a previous meta-analysis on wild *C. elegans* (Zhang et al. 2017), based on data from the three studies (Berg et al. 2016; Dirksen et al. 2016; Samuel et al. 2016). Each CeMbio species is readily culturable, with its full genome sequenced (Dirksen et al. 2020). These species can colonize the worm gut individually or comprise a robust community during host development and potentially affect nematode life-history (Dirksen et al. 2020). No pathogenicity was observed for any of the CeMbio strains. Exposure to the CeMbio community has been shown to accelerate *C. elegans* development, compared with the food OP50. Whether or how the CeMbio community could impact host resistance to pathogen infection has not been tested explicitly.

1.8.3 Pathogen – Leucobacter musarum

The gram-positive bacterium, *L. musarum* sp. nov. subsp. musarum subsp. nov strain CBX152^{T} (Verde2), is a natural pathogen isolated from wild *Caenorhabditis* collected on rotting banana trunks, on the island of San Antao, Cape Verde (Republic of Cape Verde) (Hodgkin et al. 2013; Clark & Hodgkin 2015). The genus *Leucobacter* consists of 25 gram-positive, rod-shaped bacterial species. Species in this genus have been isolated from diverse environments including the bovine rumen, phyllosphere and nematodes (Bates & King 2021). *Leucobacter* species are globally distributed spanning free-living and host-associated niches. When associated with hosts, *Leucobacter spp.* can be parasitic or commensal (Bates et al. 2021). For example, *L. musarum* and another species, *L. celer* CBX151^{T} (Verde1), both cause mortality to nematodes, whereas Verde1 infection could

also confer protection for host against Verde2 (Hodgkin et al. 2013; Bates et al. 2021). A recent experimental evolution study also showed that the interactions between these two *Leucobacter* species and *C. elegans* can shape host resistance evolutionary trajectory to infection (Bates et al. 2021).

Parasitic *Leucobacter spp.* can exhibit different levels of virulence in *C. elegans* hosts (Hodgkin et al. 2013; Clark & Hodgkin 2015; Bates & King 2021). *Leucobacter musarum* is particularly virulent. It causes lethal rectal infections in *C. elegans*, by inducing vacuole formation in host body cavity and subsequent lysis of internal structures (Hodgkin et al. 2013). The virulence of this pathogen species can vary across abiotic factors, such as moisture of substrates and temperatures. Whilst in liquid environments, the pathogen is not harmful, at higher temperatures (25°C) on solid media, *L. musarum* causes the highest level of mortality exhibited (Hodgkin et al. 2013). It kills most young adult worms exposed within 36h at this temperature (Hodgkin et al. 2013). Alteration in *C. elegans* surface glycosylation (in srf-2 knock-out) can confer resistance to this bacterial pathogen (O'Rourke et al. 2023).

1.9 Thesis outline

In this thesis, I will develop on above themes. Specifically:

In Chapter 2, I investigate the general effects of experimental warming and cooling on host microbiomes. I analysed 16S sequencing data from published studies across a diversity of host species and regions. As a result, I find decrease in host microbiome phylogenetic diversity and alteration of microbiome compositions under both experimental warming and cooling. I show that the host habitat and experimental factors affected microbiome changes more than host biological traits. The extent to which microbiome respond to changing temperatures impacted by different factors can help predict persistence of variable host species under climate warming.

I then extend work in Chapter 2, by including a biotic factor – pathogen infection, to address the effects of warming and infection dual stressors on host microbiomes. In Chapter 3, I took an experimental approach to investigate the alteration of host microbiomes under warming and infection. I included several wild *C. elegans* isolates spanning a range of latitudes, upon warming and infection treatments. I find that host microbiome diversity decreases, and dispersion increases over time, with the former more prominent in uninfected hosts and the latter more so during infection. I further reveal that infection and warming during infection, individually increased microbiome dispersion, indicating destabilized microbial communities under stressors. However, exposing infected hosts to warm temperatures does not have an additive destabilizing effect on their microbiomes, indicating the two stressors interacting in a non-additive way. Investigation on the interactive effects of abiotic and biotic factors on microbiomes sheds light on animal performance amidst climate change and epidemics.

In Chapter 4, I continue to focus on temperature, but solely on host-microbial pathogen relationships. Using a meta-analytical approach, I find broad evidence that experimentally increased temperatures drove higher pathogen-induced host mortality. Though the magnitude of these effects is not impacted by host life-stage or immune complexity, and consistent across variable experimental protocols, it is affected by pathogen type and host-pathogen evolutionary history. I show that the severity of established bacterial infection might be exacerbated the most by warming. Together, I suggest that global warming could cause excess deaths in infected animal populations, but with the effects differing by pathogen type.

Given our finding that higher temperatures can lead to more harmful infection outcomes, I test the selective impacts of warming on the evolution of host resistance to infection in Chapter 5. I experimentally passaged susceptible and resistance lines of *C. elegans* nematodes and tracked their frequencies across different warming and infection regimes. I

find that warming during host development, then continuing into maturity during infection selected for genetic-based host resistance. Periodic warming within host lifetime drove the loss of genetic-based resistance, despite pathogen presence. Moreover, warming during host development resulted in plastic temperature-mediated protection which weakened the selection for more costly genetic-based resistance, upon cooling to ambient levels. These findings improve our understanding of the evolutionary implications for patterns of infection and host persistence, during global temperature change.

Finally, in Chapter 6, I summarise the main findings from Chapters 2-5. I discuss the wider implications of investigating the impacts of biotic and abiotic factors on host microbiomes, as well as host persistence amidst climate change and epidemics. I conclude by suggesting future research directions.

Chapter 2:

Experimental temperatures shape host microbiome diversity and composition

This Chapter has been published in *Global Change Biology.*

Experimental temperatures shape host microbiome diversity and composition

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Abstract

Global climate change has led to an increase in extreme thermal events. Plants and animals harbour diverse microbial communities which may be vital for their physiological performance and help them survive stressful climatic conditions. The extent to which microbiome communities change in response to warming or cooling may be important for predicting host performance under global change. Using a meta-analysis of 1377 microbiomes from 43 terrestrial and aquatic species, we found a decrease in ASV-level microbiome phylogenetic diversity and alteration of microbiome composition under both experimental warming and cooling. Microbiome beta dispersion was not affected by temperature changes. We showed that host habitat and experimental factors affected microbiome diversity and composition more than host biological traits. In particular, aquatic organisms – especially in marine habitats – experienced a greater depletion in microbiome diversity under cold conditions, compared with terrestrial hosts. Exposure involving a sudden long and static temperature shift was associated with microbiome diversity loss, but this reduction was attenuated by prior-experimental lab acclimation or when a ramped regime (i.e., warming) was used. Microbial differential abundance and co-occurrence network analyses revealed several potential indicator bacterial classes for hosts in heated environments and on different biome levels. Overall, our findings improve our understanding on the impact of global temperature changes on animal and plant microbiome structures across a diversity of habitats. The next step is to link these changes to measures of host fitness, as well as microbial community functions, to determine whether microbiomes can buffer some species against a more thermally-variable and extreme world.

Keywords: Global warming, host microbiome, temperature, species persistence, thermal tolerance, microbiome disturbance, meta-analysis

Introduction

Managing the impacts of climate change is one of the biggest challenges of the 21st century. Climate change has led to an increase in extreme thermal events, from heat waves to cold snaps, causing population decline and local extinction of animals and plants (Maxwell et al., 2019). Extreme heat and cold have consequences across all levels of biology, including physiology (Guschina and Harwood 2006; Beker et al., 2018), behaviour (Taylor et al., 2021; Sformo et al., 2009), reproduction (Hansen 2009; Tong et al., 2018), and evolution (Chakravarti and van Oppen 2018; Mesas et al., 2021; Sinclair et al., 2003). Moreover, an organism's response to temperature can depend on their thermal tolerance limits, which is quantified by the upper and lower temperatures at which they cannot function (Hoffmann et al., 2003, Chown et al., 2010; Huey et al., 2012).

A role for the microbiome in the ability of animals to tolerate extreme temperatures is emerging across a diversity of species (Hector et al., 2022; Moghadam et al., 2018; Chevalier et al., 2015). Host microbiomes are important for host physiological functions and overall health, and many studies show that disturbance or loss of microbiome diversity can be associated with diminished host health (Mohajer et al., 2018). A recent study found that experimentally depleting diversity in the tadpole gut microbiome – through environmental water sterilization – reduced tadpole acute thermal tolerance to both heat and cold (Fontaine et al., 2022). Another found that microbiome transplantation from heat-tolerant *Drosophila melanogaster* to recipient flies could confer heat tolerance (Moghadam et al., 2018). Moreover, in corals, the abundances of particular bacterial taxa have been correlated with host fitness during short-term heat waves (Hartman et al., 2019; Ziegler et al., 2017). Microbiomes seem to be important for host thermal tolerance, and disruption of those microbiomes induced by thermal change can affect host key functions (Jaramillo and Castañeda 2021; Doering et al., 2021).

Environmental temperatures can directly cause changes in a host's microbiome structure (eg., Hylander & Repasky 2019; Sepulveda & Moeller 2020), but whether general changes in microbiome composition then feed-back into mediating host thermal responses remains unclear. Given host and resident microbes are likely constantly interacting (Foster et al., 2017), perhaps differences in microbiome structure at different temperatures are driven by both host biological and environmental factors (Woodhams et al., 2020; Kers et al., 2018). The strength of these respective factors may vary. For example, it has been shown that hosts with adaptive immune systems have higher microbiome diversity than hosts with only innate immunity (Woodhams et al., 2020), suggesting different levels of host control over the microbiome in the two groups. Microbiome site may additionally affect the relative impact of environmental temperature as a structuring force. Compared with host internal microbiomes, external microbiomes directly interact with environmental temperatures and are therefore less influenced by host factors (Woodhams et al., 2020). Host biology and environment, not only have separate impacts, but may also interact to shape the microbiome. The ambient temperature and thermal variation can affect the adaptive thermal tolerance of organisms (Kelly et al., 2012; Yampolsky et al., 2014; Hector et al., 2020). Hosts from tropical areas may be more stressed under climate change (Hoffmann et al., 2012; Pinsky et al., 2019; Deutsch et al., 2008; Huey et al., 2009; Sunday et al., 2014; Janzen 1967; Stevens 1989, Scholander et al., 1950), given their relatively narrow thermal safety margins (the difference between a species' maximum tolerance to heat and its current regularly experienced temperature as defined in Sunday et al., 2014). Consequently, they will have more disrupted microbiomes at thermal extremes. Similarly, marine ectotherms have smaller thermal safety margins than terrestrial ectotherms (Pinsky et al., 2019), potentially leaving them more susceptible to disturbed microbiomes under thermal changes.

Using a formal meta-analysis, we assessed how experimental warming and cooling altered the diversity, composition, and stability of host microbiomes and the generality of their

effects. We searched the published literature for experimental studies and established a standardized metabarcoding analysis pipeline to re-analyze the sequencing data to assess the degree to which host microbiome diversity and composition change across thermal regimes. With the associated metadata, we tested whether host biological traits, environmental variables, and experimental approaches were associated with host microbiome responses to temperature change. We hypothesized that experimental warming and cooling would be associated with a decrease in host microbiome diversity and stability, thereby indicating a disturbed community in a stressful environment (Zaneveld et al., 2017). We additionally tested the moderating effects of host thermal buffer capacity (i.e., endothermic vs. ectothermic, aquatic vs. terrestrial), thermal characteristics of host habitat, as well as experimental opportunity for host and microbiome acclimation (i.e., ramping vs. static) on effect sizes. Finally, we assessed the presence of microbiome species enriched in thermal treatment groups, across hosts and in different biomes, their potential importance in the corresponding microbial co-occurrence network, and whether any predicted functional pathways were enriched in microbiomes across thermal treatments. Understanding the generality of these effects across the tree of life is important for projections of species persistence in an increasingly thermally-variable world (Hector et al., 2022; Sepulveda & Moeller 2020). If warming and cooling towards thermal maxima generally cause microbiome diversity loss or disturbance, microbiome characteristics could help predict the impacts of climate change on host health and survival.

Materials and Methods

Literature search and Data collection

A literature search was performed on Web of Science, Google Scholar, and the National Center for Biotechnology Information (NCBI) PubMed databases using the following search terms: “host microbiome/microbiota”, “temperature”, “16S” and “TOPIC: (microbio*) AND TOPIC: (16S sequenc*) NOT TITLE: (soil) NOT TITLE: (food) AND TOPIC: (temperature) ”. We then performed forward and backward reference searches on papers of interest. The

same search terms were used to search in NCBI BioProject databases, the European Nucleotide Archive (ENA) database (Leinonen et al., 2011), and Qiita (Gonzalez et al., 2018) to retrieve any relevant datasets and associated studies.

We then filtered and kept studies from the search that fitted the following criteria: (i) published in the last 10 years (between 2010 and 2021), a period of time when high-throughput sequencing methods have been fairly standardized and commonly used to measure microbial structure and composition; (ii) measured microbial composition by 16S or shotgun sequencing at different temperature levels with other variables controlled; (iii) were experimental studies conducted in a lab, constituting controlled tests of the effect of temperature; (iv) used Illumina next generation sequencing (NGS); and (v) had sequencing data and associated metadata available publicly or shared by the author by June 2021.

Studies that fulfilled criteria for the meta-analysis were evaluated for confounding variables. For studies that manipulated multiple variables besides temperature (pH, humidity, etc.), only samples/data arising from the temperature manipulation were retained, and all other samples were discarded. For longitudinal studies, only start/baseline and endpoint samples were analyzed. For studies that had multiple temperature levels, we only included data from the extreme (highest or lowest) treatment group and control group. This allowed us to capture the greatest microbiome alteration under temperature change. All studies included herein investigated the impact of temperature and host microbiomes using controlled experiments. Compared to observational field studies, experiments can control for confounding factors and allow for more confident inference from the results.

Sequence processing per study

To make each published dataset fully comparable, we downloaded and processed raw sequencing reads using a standardized pipeline on each study separately. Data were downloaded from the Sequence Read Archive (SRA) in NCBI (Sayers et al., 2020), ENA (Leinonen et al., 2011), and/or Qiita repositories (Gonzalez et al., 2018). 16S sequencing

data were processed following the standard operating procedure suggested by Quantitative Insights into Microbial Ecology (QIIME2 2020.11 distribution) (Bolyen et al., 2019) and sequences were resolved to amplicon sequence variants (ASVs) using the Deblur workflow (Amir et al., 2017). Taxonomy was assigned to ASVs using a classifier trained on the full-length 16S rRNA gene SILVA v138 database and phylogenetic trees were built using SEPP (Quast et al., 2013). Shotgun sequencing data was aligned with a 16S rRNA database using SortMeRNA (Kopylova et al., 2012), before extracted 16S rRNA reads were processed in the same way as 16S sequencing data. Sequencing region for 16S rRNA sequences that were extracted from shotgun reads was labeled as “full length” when comparing them with 16S sequencing data. A detailed description of the bioinformatic pipeline can be found in Supplementary methods.

Sample metadata and predictor variables

For each study, we collated information on a comprehensive set of abiotic and biotic moderator variables, take either directly from the original paper or from publicly available databases (Supplementary Table 1). Several variables were specific to either terrestrial or marine hosts respectively. We classified these moderator variables into three categories: host-related biological variables (including host higher rank taxonomy, host body site, thermal type (ectotherm or endotherm), host lifespan, host immune type (innate only or adaptive and innate)), environmental variables (including host habitat, host region, mean and range of annual temperature within host region), and experimental variables (experimental exposure time, lab acclimation, newly collected or lab-reared, experimental temperature change, ramping or static) on microbial alteration under warming and cooling exposures. Full details are in the Supplementary methods.

Diversity analysis

Unless specified, all analyses were conducted in R 4.1.0 (RStudio 1.4.1717). We performed alpha and beta diversity analysis within each individual study. To generate alpha diversity

metrics, we rarefied data. We selected a minimum sampling depth at which estimated diversity had plateaued in rarefaction curve, which in most cases was the lowest sample depth within each study. Four alpha diversity indices (Shannon's index, Richness, Evenness and Faith's phylogenetic distance, Faith's PD) were computed in package *phyloseq* (McMurdie and Holmes 2013). Since Faith's PD is not statistically independent of species richness, to obtain a measure of phylogenetic diversity that is independent of species richness, we calculated PD values corrected for unequal richness across samples (Ses.pd). For beta diversity analysis, community distance matrices of weighed and unweighed UniFrac, CLR Euclidean distance (Euclidean distance between zero replacement CLR-transformed compositions), Bray-Curtis dissimilarity, and PhILR Euclidean distance were calculated using package *vegan* (Dixon 2003). Permutational analysis of variance (PERMANOVA) was conducted with 9999 replications on each distance metric to evaluate differences in microbiome structure and composition between treatments. For each beta diversity metric, beta dispersion was calculated using the *betadisper* function in the *vegan* package.

Effect size calculation and meta-analysis

Effect sizes were calculated from warming-control comparisons (in warming exposures) and cooling-control comparisons (in cooling exposures) separately. And the meta-analysis was conducted on warming and cooling data separately. For each alpha diversity metric, effect size Hedge's g was calculated as the standardized mean difference (SMD) in diversity between temperature treatments. We used Hedge's g because it has better small sample properties than Cohen's d. This metric is also more suitable for our data type as the sample sizes of some studies were low (Hedges 1981). For beta diversity, we used the omega-squared (ω^2) from PERMANOVA (adonis function in R) as the effect size, which estimated the variation in microbiome composition that can be explained by temperature treatment. We tested the difference in microbiome dispersion between treatments using Tukey's 'Honest Significant Difference' method (TukeyHSD). Then Hedge's g was used as

the effect size and quantified the difference between average distance to median in different treatment groups. To increase the robustness of our results, we performed both frequentist multi-level meta-analysis models and Bayesian hierarchical meta-analysis models using rma.mv (metafor package, Viechtbauer 2010) and brm functions (brms package, Bürkner 2017), respectively. Study was used as a random factor. We did not include host species-level phylogenetic tree in the random factor as the phylogeny was not resolved for 11 out of 43 species. We then classified host species to higher taxonomic rank and tested its effect in moderator analysis. Full details of effect size calculation and modelling are in Supplementary methods.

Moderator analysis

We kept the most relevant temperature-related climatic variables (annual mean temperature and annual temperature variation) among all the retrieved climatic metadata (Supplementary Table 1). We assessed the multicollinearity of categorical variables by calculating the generalized variance inflation factor (GVIF, *VIF* function in 'regclass' R package v1.6, GVIF^(1/(2*Df)) > 2 indicated collinearity) (Supplementary Table 3). We tested the influence of moderator variables using univariate mixed effect models. Due to the limited number of effect sizes, model selection did not support including all the relevant moderators together. Linear mixed-effects models with a study-level random effect were built using rma.mv for Hedge's g and lmer function for ω^2 (lme4 package, Bates et al., 2015), and statistical analysis was carried out in R 4.1.0 using the appropriate functions (with prior distribution checked and p <=0.05 as significant). All statistic models and summary results are in Supplementary Table 3.

Tests for differentially abundant taxa and functions

Within each study, we performed non-parametric Wilcoxon rank-sum test on taxa between treatment and control groups using ALDEx2 package (Fernandes et al., 2014) and ANCOM (Mandal et al., 2015) in R (details in SI Methods). Though different tools can identify

different numbers and sets of significant ASVs, these two methods were previously shown to be the most reliable (Nearing et al., 2022). We used PICRUSt2 (Douglas et al., 2020), which is a widely used functional prediction tool on 16S sequencing data (Bharti & Grimm 2021; Zhang et al., 2019; Ashton et al., 2017), to predict the metagenomic function of the microbiomes within each study and identified differential abundant functions using two-sided Wilcoxon rank-sum test (wilcox.test function). We obtained consensus results based on multiple differential analysis methods (Nearing et al., 2022). We only kept significant differential bacterial taxa and pathways that were found more than twice in the same treatment group, across different studies/species. To evaluate the importance of differential ASVs in the microbial community network, we established microbial network using Sparse Correlations for Compositional data (SparCC, Friedman and Alm 2012) in different treatment groups. Details are in Supplementary methods.

Results

Summary of included studies and samples

From the 75 highly relevant studies yielded from literature search, we incorporated data from 41 studies which contained data available meeting our inclusion criteria. These data covered 43 host species, ranging from mammals to corals and insects (Fig. 1, Supplementary Table 1). Apart from animals, algae and plant hosts have also been included in our analysis. Both aquatic and terrestrial habitats consisted of a variety of host species (Fig. 1d). We analyzed a total number of 1377 microbiome samples out of 4038 samples (details in Supplementary Results and sample metadata in Supplementary Table 1).

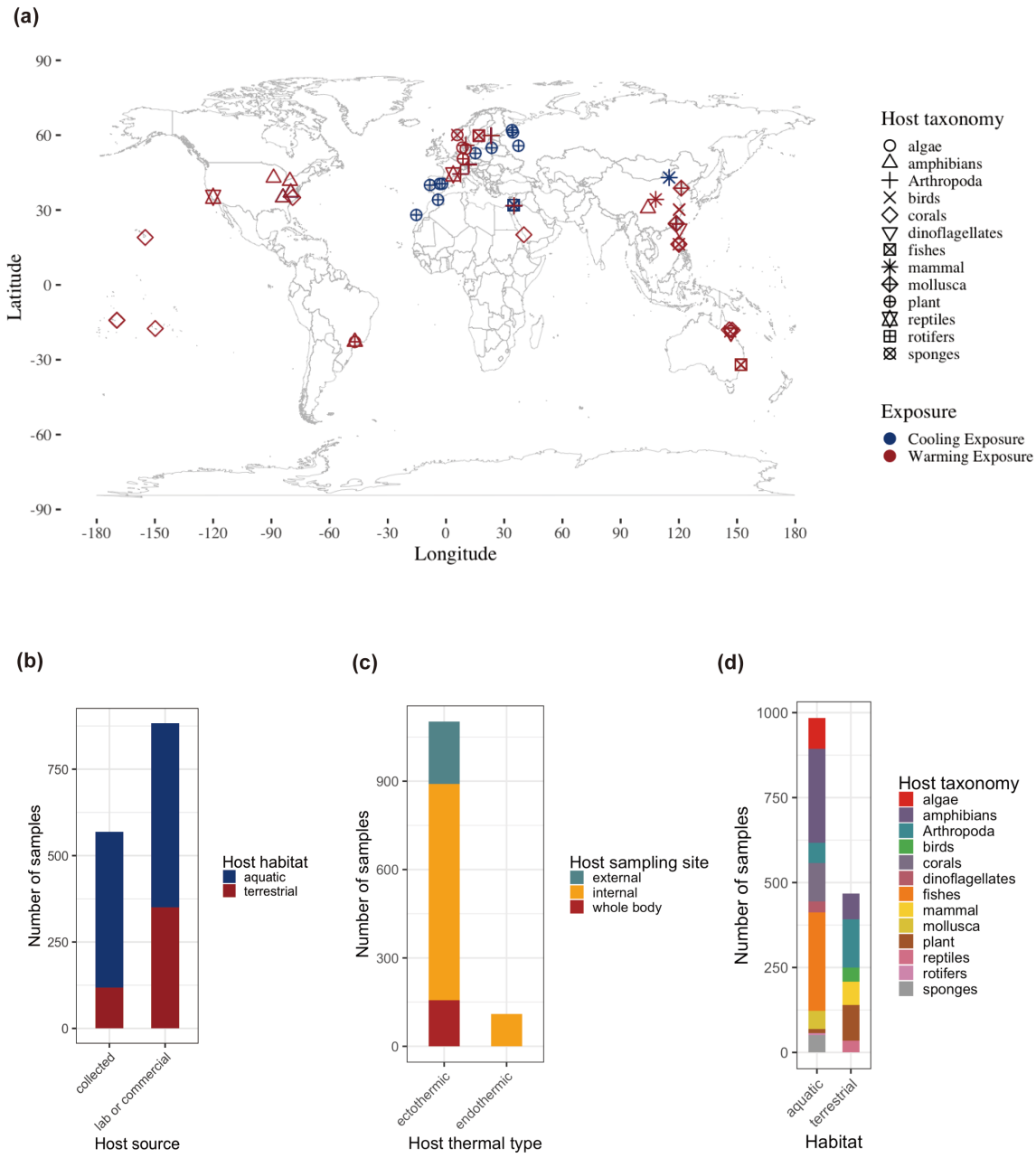


Fig. 1: Study and sample characteristics. (a) Geographical locations of field-collected hosts (map lines delineate study areas and do not necessarily depict accepted national boundaries). Shape represents different host taxonomy, color represents warming or cooling exposures. (b) Number of samples for different host types (aquatic vs. terrestrial; newly collected from the wild or reared in lab). (c) Number of samples for different host body sites (internal or external) from ectothermic and endothermic hosts. (d) Number of samples from different host taxa in aquatic or terrestrial habitats.

Warming and cooling alter microbiome phylogenetic diversity

In total, from 57 warming-control comparisons and 39 cooling-control comparisons, we calculated 462 effect sizes (ESs) (Hedge's g, five alpha diversity metrics, used to quantify

microbiome diversity change) for microbiome alpha diversity, 320 effect sizes for beta dispersion (Hedge's g, four beta diversity metrics, used for assess change in microbiome stability) and 336 effect sizes for beta diversity (ω^2, four beta diversity metrics, used for evaluate microbiome compositional change). To test for small-study bias, we fitted a multilevel meta-regression with sample size as the moderator. We did not observe a significant effect of sample size in both alpha and beta diversity effect sizes (meta-analytic model, $p>0.2$ in all models, Supplementary Table 3). However, effect sizes from beta dispersion were influenced by sample size ($p<0.0033$ for two metrics in warming and cooling, Supplementary Table 3). We thus added sample size as a random factor in the subsequent moderator analysis.

We coded the data such that negative Hedge's g indicated thermal manipulation (warming or cooling) decreased host microbiome diversity. We observed a heterogeneous distribution of effect sizes for alpha diversity across studies, and summary effect sizes were negative but close to zero (Fig. 2). Across all studies, summary effect sizes for the impact of warming and cooling on microbiome alpha diversity were consistently negative (Supplementary Table 2), with phylogenetic diversity measures decreasing significantly - Faith's PD under warming and Ses.pd under cooling (summary Faith's PD ES in warming: -0.398, 95% C.I. (-0.768, -0.028); summary Ses.pd ES in cooling: -0.531, 95% C.I. (-1.003, -0.059)). These results suggested a decrease of host microbiome diversity under warming and cooling, but for some alpha diversity metrics the degree of loss did not pass the significance threshold. Visual inspection of forest plots did not indicate strong variation in effect size across host taxa (Fig S1).

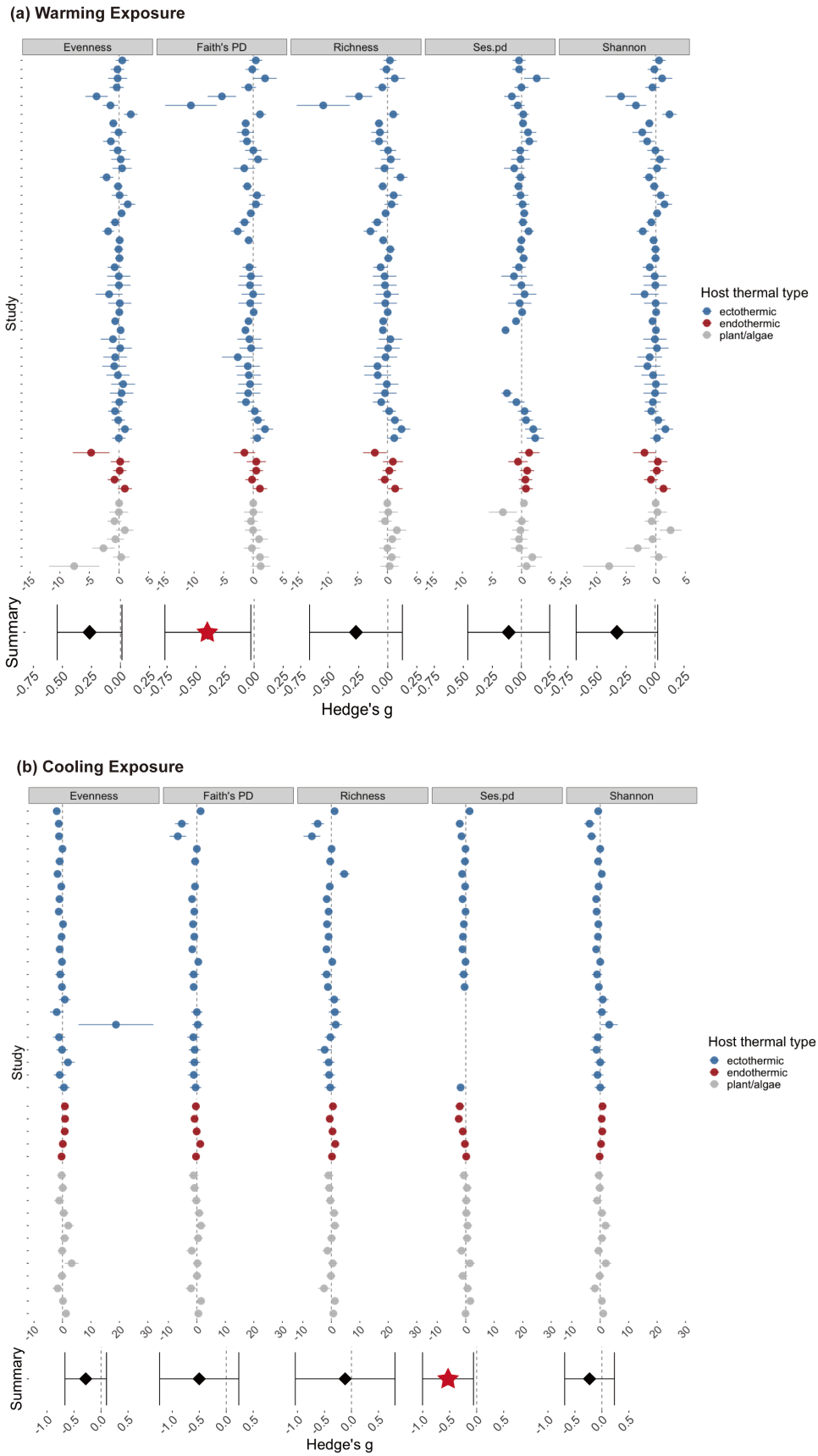


Fig. 2: Forest plots of Hedge's g calculated for five alpha diversity metrics. (a) Warming vs. Control, vertical panels are different alpha diversity metrics; (b) Cooling vs. Control, vertical panels are different alpha diversity metrics. Circles and error bars indicate individual Hedge's g and 95% CIs,

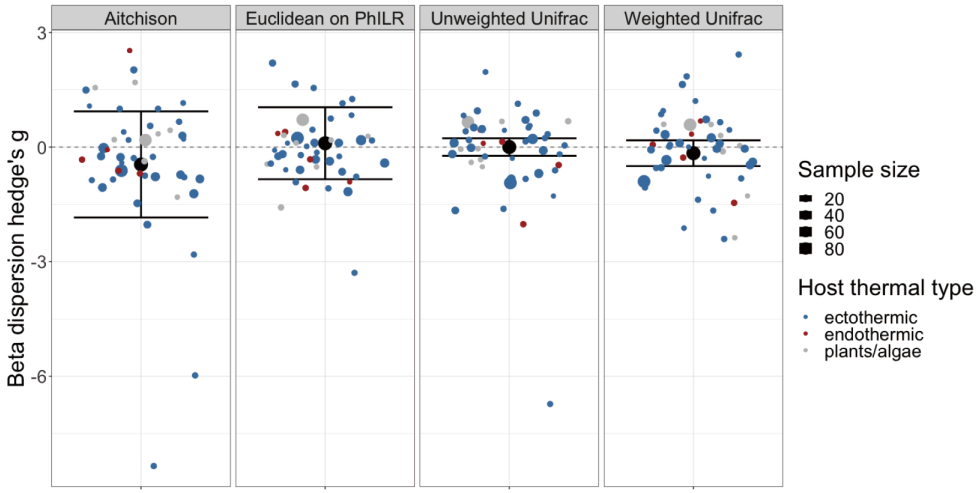
and different colors indicate different host thermal types. Summary effect sizes are shown at the bottom of forest plots (filled diamonds, significant summary ES is labeled as filled red star). All summary ESs are negative, indicating decreased microbiome alpha diversity under thermal manipulation.

Warming and cooling affect microbial community composition rather than dispersion

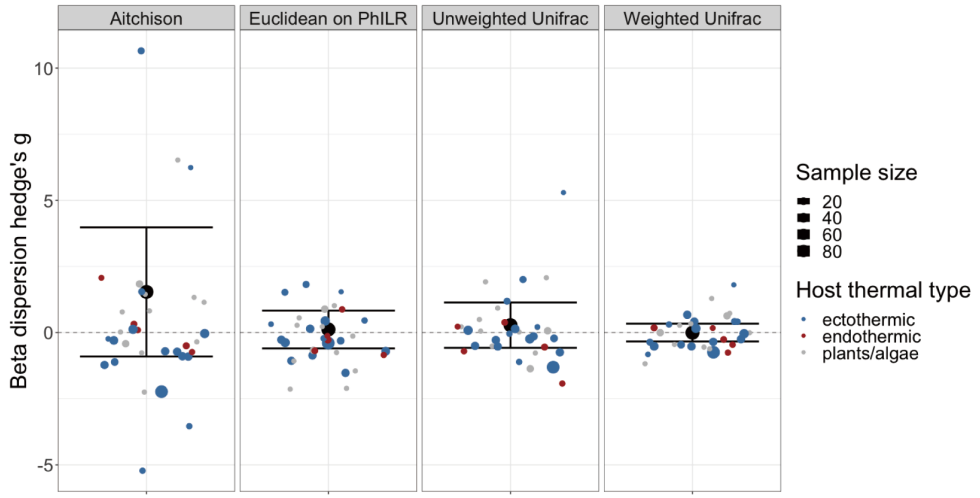
Beta diversity ω^2 were calculated as the variation of microbiome composition that can be explained by thermal treatment, thus ω^2 were positive values with a range of (0, 1). We found the summary median and mean ω^2 were both around 0.1, for all four beta diversity metrics (Fig. 3c), showing medium to large effects of microbiome compositional change under thermal manipulation.

For beta dispersion analysis, we coded the data such that positive Hedge's g indicated that warming/cooling increased microbiome dispersion. The summary Hedge's g for beta dispersion was not significantly different from 0, under both warming and cooling (Fig. 3a, 3b), meaning that microbiome dispersion was not distinct under thermal manipulations.

(a) Warming Exposure



(b) Cooling Exposure



(c)

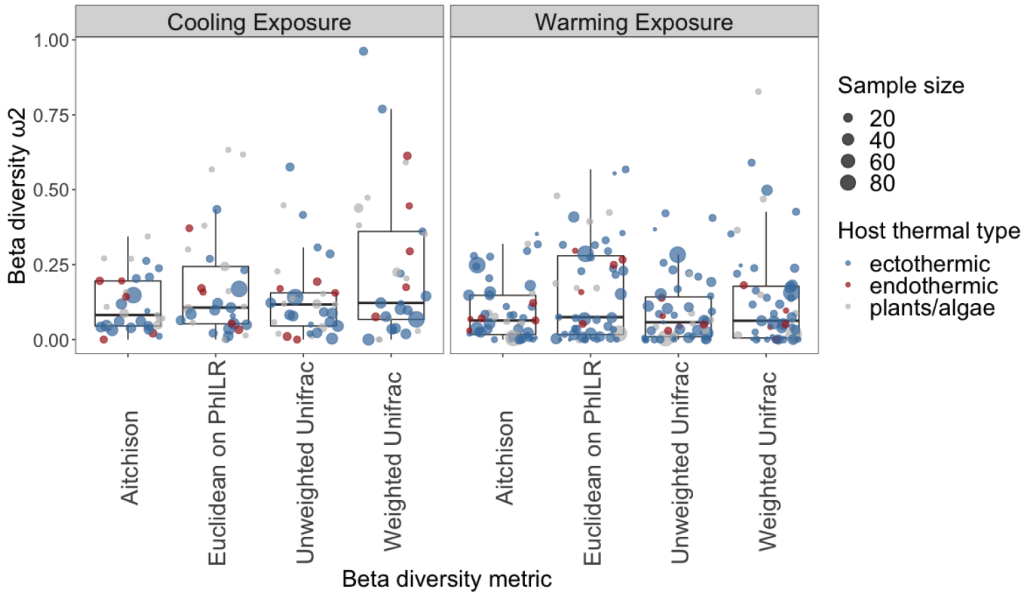


Fig. 3: Effect sizes for beta dispersion and beta diversity in warming and cooling exposures, data were calculated from four beta diversity metrics. (a) Beta dispersion effect sizes (Hedge's g) under warming exposure. (b) Beta dispersion effect sizes (Hedge's g) under cooling exposure. Bold black point with 95% confidence interval represents summary effect size, and individual Hedge's g are displayed as jittered points. Jittered points are colored by host thermal type. Hedge's g not different from 0 meant that beta dispersion was not distinct under thermal treatments (Hedge's g > 0: greater microbiome dispersion under cooling; Hedge's g < 0: greater microbiome dispersion under control treatment). Point size indicates sample size of the study from which the individual effect size was calculated. (c) Beta diversity effect sizes (ω2) under warming and cooling exposures. Higher effect sizes indicated greater microbiome compositional change under thermal treatments. Point size indicated sample size of the study from which the individual effect size was calculated.

Experimental design affects the impact of temperature on microbiomes

Experimental studies included in our analysis varied in protocols such as prior experimental host acclimation in the lab and experimental exposure time. We found that prior-experimental lab acclimation time influenced how microbiome richness responded to warming, with Hedge's g significantly larger under long acclimation time (more than a year) than that under short-time lab acclimation (days), meaning smaller decrease in microbiome diversity under warming for hosts acclimated in lab for longer time (Fig S5A, $p=0.004$). However, longer lab acclimation was associated with increased microbiome dispersion under warming (Fig S5B, $p<0.0001$). We further found that longer-acclimated hosts in the lab had significantly lower baseline microbiome diversity (for four alpha diversity metrics), even without treatment, compared with newly collected hosts (Fig S6, $p<0.009$ for four metrics).

Apart from acclimation time, we found that ramping or static thermal regime, which often had a substantial influence on thermal responses (John et al., 2011), also impacted host microbiome changes under warming. Host microbiome diversity showed a small reduction under ramped warming compared to static warming treatments (Fig. 4a, $p=0.001$ for Richness, $p=0.002$ for Faith's PD, $p=0.047$ for Ses.pd). Similar effects of ramping regimes were observed in one metric under cooling (Fig S2, $p=0.001$ for Richness). Though the rate of temperature change was important for host microbiome richness, the temperature range (the difference between warming/cooling and control temperature) was less important for host microbiome diversity (Supplementary Table 3, no significant effect of temperature

range on alpha diversity Hedge's g was observed: $p>0.2$ for all metrics). When static thermal regime was used, we found that longer exposure time was associated with greater microbiome diversity loss (Fig. 4b, warming: $p=0.03$ for Shannon, $p=0.034$ for Evenness; cooling: $p=0.036$ for Richness, $p=0.019$ for Faith's PD, $p=0.032$ for Shannon), but this trend was not observed for ramping regimes (Fig S3, $p>0.054$ for all metrics).

Compared to microbiome diversity, the scale of microbiome compositional change was more robust to static and ramping regimes (Fig S4, beta diversity effect sizes, NS for 3 metrics in warming, and NS for all 4 metrics in cooling). We nevertheless found that larger beta diversity effect sizes were associated with longer exposure time, specifically greater microbiome compositional change with longer warming exposures (Fig. 4c, $p=0.003$ for Aitchison, $p=0.039$ for PhILR, $p=8.43e-5$ for Weighted Unifrac).

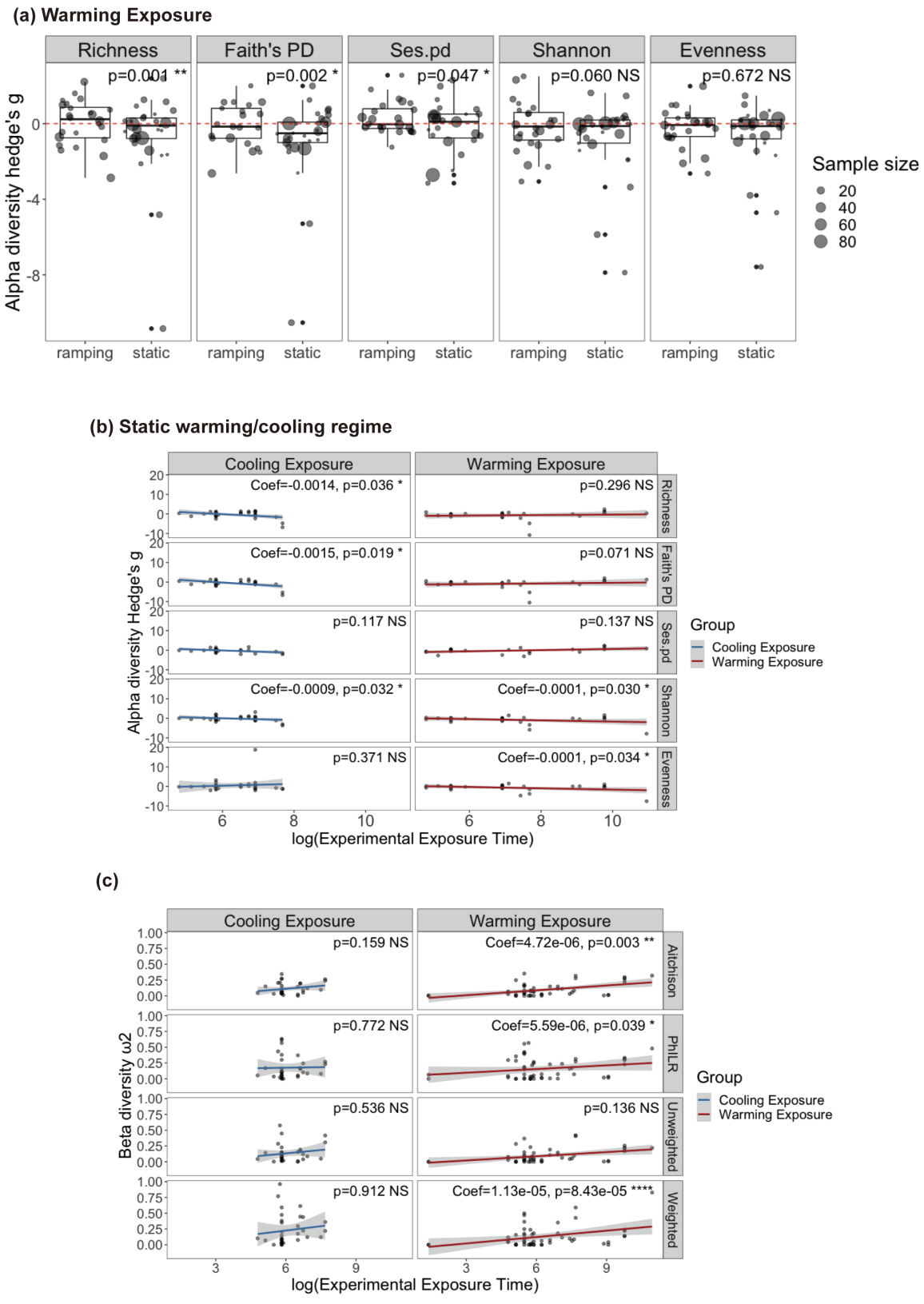


Fig. 4: Experimental variables influenced microbiome diversity and composition response to warming and cooling. The significance level (p value) is shown on the top right corner of each faceted plot: NS ($p > 0.05$); * ($p < 0.05$); ** ($p < 0.01$). Coefficient value is shown on (b) and (c) when there is a significant association. (a) Impact of ramping and static regimes on host microbiome alpha diversity with warming. Vertical panels are different alpha diversity metrics. Points are individual effect size (Hedge's g), with sizes indicating sample size used for Hedge's g

calculation. Points under the red dotted line means Hedge's g < 0: decreased alpha diversity under warming treatments. (b) Association between experimental exposure time and change of host microbiome alpha diversity under static warming and cooling regimes. Vertical panels are different thermal exposure, and horizontal panels are different alpha diversity metrics. Exposure time on x-axis was log-transformed for clearer visualization. Points are individual effect size (Hedge's g), and blue (under cooling exposure) or red (under warming exposure) smooth lines show a positive or negative association between exposure time and microbiome alpha diversity change. Positive association: longer exposure was associated with less microbiome diversity loss; Negative association: longer exposure associated with greater microbiome diversity loss. (c) Association between experimental exposure time and host microbiome compositional change (beta diversity effect sizes). Horizontal panels are different beta diversity metrics. Points are individual effect sizes (ω2), and blue (under cooling exposure) or red (under warming exposure) smooth lines show a positive or negative association between exposure time and microbiome compositional change. Positive association: longer exposure was associated with greater microbiome compositional change; Negative association: longer exposure associated with less microbiome compositional change.

Host habitat impacts microbiome responses to temperature

We did not observe a significant effect of host-related traits (host taxonomy, body site of microbiomes, host lifespan, host immune complexity) on microbiome responses to thermal changes (Supplementary Table 3, $p>0.05$ for all metrics, alpha and beta diversity effect sizes of plant and rotifer showed dubious significance under cooling with few effect sizes in each group). Overall, there was little evidence that changes of microbiome alpha and beta diversity varied strongly according to the mean temperature and temperature range of the environment from which a host was collected (Supplementary Table 3). We only found significant effect of temperature range on one alpha diversity metric under cooling (Fig S8, $p=0.0268$), indicating less diversity loss with wider habitat temperature range. We did not observe a consistent effect of mean temperature or temperature range on microbiome beta dispersion, across different metrics (Supplementary Table 3).

We found that aquatic hosts experienced more microbiome diversity loss under cooling, compared with terrestrial hosts (Supplementary Table 3, $p=0.0027$ for Richness, $p=0.0063$ for Faith's PD, $p=0.0035$ for Shannon). Among the three habitat types on which we focused – freshwater, marine, and land – we found that marine hosts had the greatest microbiome loss (though there were only two effect sizes in marine group), followed by hosts living in freshwater habitats. Terrestrial hosts had the least altered microbiome diversity (Fig. 5, $p<0.0019$ for all sea vs. freshwater and terrestrial vs. freshwater comparisons). Similar

results held for ectotherms across these habitats (Fig S7, p<0.024 for all sea vs. freshwater and terrestrial vs. freshwater comparisons).

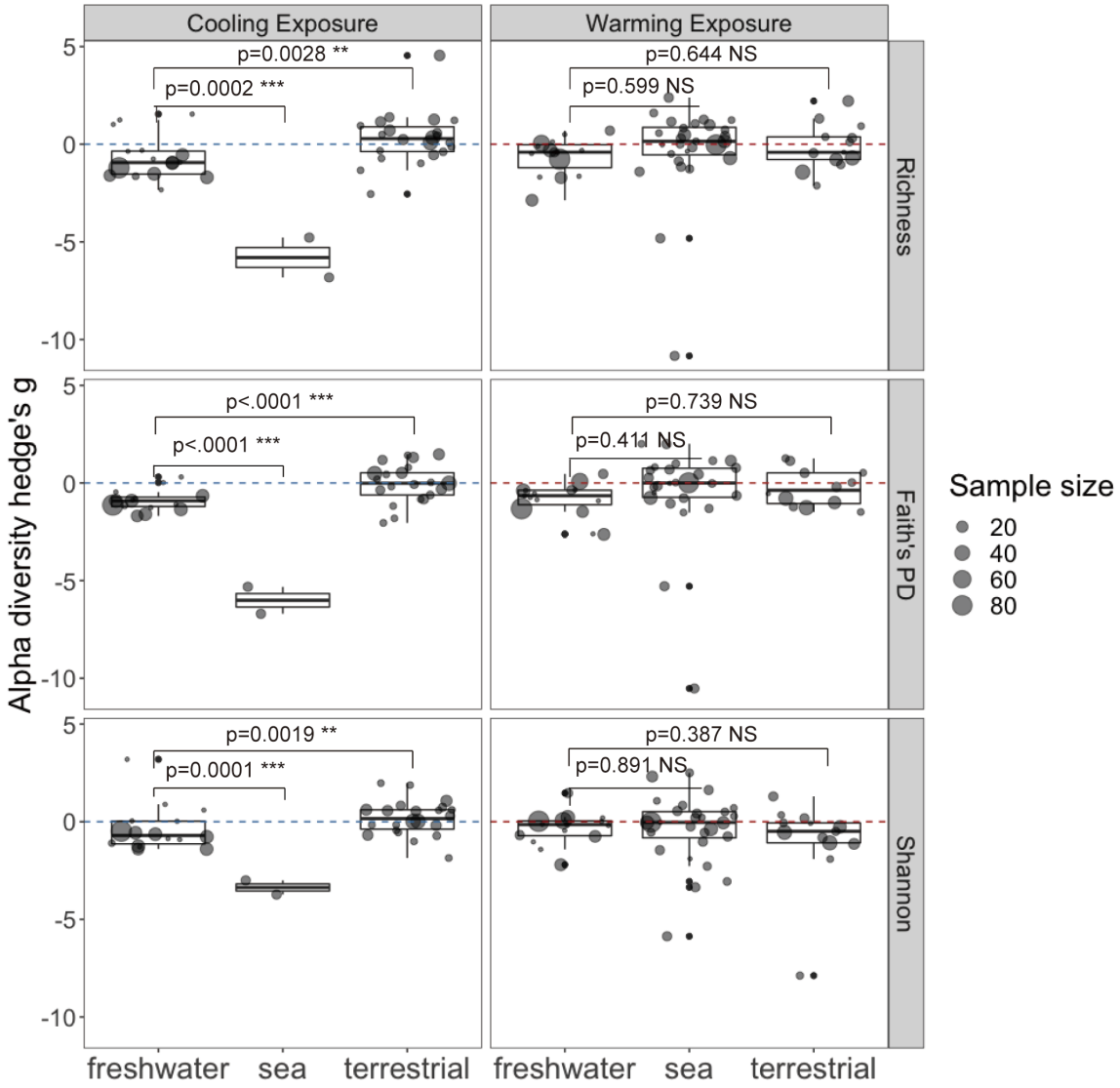


Fig. 5: The impact of host habitat on the response of host microbiome alpha diversity to thermal change. Points are individual Hedge's g, with sizes showing sample size used for Hedge's g calculation. Points below the blue (under cooling exposure) or red (under warming exposure) dotted line mean Hedge's g < 0: decreased alpha diversity under warming or cooling treatments. Vertical panels are different thermal exposures, and horizontal panels are different alpha diversity metrics. The significance levels for freshwater vs. sea and freshwater vs. terrestrial are shown above respective brackets (NS: p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001).

Differential analysis and functional analysis

An average of 27 differential ASVs were identified per warming-control comparison and an average of 14 per cooling-control comparison, with numbers of differential ASVs ranging from 1-99 in warming-control comparisons and 2-52 in cooling-control comparisons. Phyla

Proteobacteria, Bacteroidota and Firmicutes had the largest proportion of differential ASVs (Fig S9). Most of the differential ASVs belong to one of the four classes Gammaproteobacteria, Alphaproteobacteria, Bacterodia, and Bacilli (Fig S10A, B). We found that more differential bacterial taxa were enriched in marine host compared with terrestrial hosts under warming, but under cooling more differential taxa were enriched in terrestrial hosts (Fig S10E, F). However, the numbers of host species included from both habitats were not high (less than five). We evaluated the importance of these differential ASVs by assessing their centrality in the microbial co-occurrence network. We found that these ASVs enriched under warming or cooling treatment did not show higher degree or betweenness centrality in the corresponding microbial networks (Fig S11). This result indicated little altered importance in the microbiome community under either warming or cooling. However, in certain hosts (*Aiptasia* corals (strain *CC7, H2*), *Notophthalmus viridescens* newts), we found differential ASVs from classes Myxococcia and Polyangia were not only enriched under warming, but also showed a higher degree and betweenness centrality in microbial networks under warming (Fig S10C, Fig S11A). For the newts, differential ASVs from Polyangia showed significant enrichment in the cooling group (Fig S10D, Fig S11B). Differential functions identified were mostly associated with metabolism such as glycolysis and amino acid biosynthesis. We found that more functional pathways were enriched in warming in terrestrial, compared with aquatic (freshwater, sea) host microbiomes, while more functions were enriched in cooling in aquatic than terrestrial host microbiomes (Fig S12C, D). Details can be found in the Supplementary Results.

Discussion

Climate change can affect the fitness and geographic distribution of a species (Malhi et al., 2020). Increasingly, microbiomes are being revealed as critical to their animal and plant host's ability to withstand thermal stress (Hector et al., 2022; Moghadam et al., 2018). Studying the general response of resident microbiomes to temperature changes could thus

shed light on the resilience of different host species to a changing world (Chevalier et al., 2015; Moghadam et al., 2018; Rosado et al., 2019).

Microbiome diversity and compositions are distinct across species. Despite some variation across studies and systems (higher temperatures, decreases in diversity: Bestion et al., 2017, Zhu et al., 2019, Fontaine et al., 2018; higher temperatures, no change in diversity: Tajima et al., 2007; Kohl and Yahn 2016), our meta-analysis found broad support for a reduction in host microbiome phylogenetic diversity under experimental warming and cooling. Reduced microbiome diversity has been associated with detrimental effects on host health, with accumulated evidence from human gut microbiomes (Pickard et al., 2017; Kriss et al., 2018; Valdes et al., 2018). The similar dysbiotic effect of thermal stress on host microbiome diversity could be an indicator of diminished host health under global change (Mohajer et al., 2018). We also detected a general microbiome compositional change induced by experimental warming and cooling. As our meta-analysis involved short-read 16S sequencing data (the most widely used microbiome characterization method), we were unable to resolve warming/cooling-enriched bacterial species to strain level, and heterogenous functional roles were identified for bacteria within the same class (e.g., Alphaproteobacteria, Bacteroidia). However, we observed that more differential bacterial taxa, but not more functional pathways, were enriched in marine hosts compared with terrestrial hosts under warming, but the contrary under cooling. Though challenging, future studies using metagenomic sequencing will help resolve common indicator taxa to species/strain level. This in-depth approach is key for predicting general host performance under temperature stress, as well as the functional capacity of the community. It will further aid the discovery of probiotic microbial species for use in improving species thermal tolerance as climate change progresses (Rosado et al., 2019; Morgans et al., 2020; Doering et al., 2021).

Our meta-analysis showed that across systems, there was no significant increase on microbiome beta dispersion under either warming or cooling. This finding contrasts with the Anna Karenina Principle. This principle, which was adapted from the opening line of Leo Tolstoy's book Anna Karenina "all happy families are alike; each unhappy family is unhappy in its own way", was used to predict consequences for animal microbiomes with dysbiosis (Zaneveld et al., 2017). Zaneveld et al., (2017) proposed that perturbations induced stochastic changes in microbiome compositions and therefore transited microbiome community from stable to unstable states. Essentially, dysbiotic individuals have more dispersed (or less stable) microbial community composition than the microbiomes of healthy individuals, with supporting evidence from disease-associated human gut microbiomes (Giongo et al., 2010; Gilbert et al., 2012; Dey et al., 2013). However, studies testing this hypothesis on non-human animals revealed diverging results (Ahmed et al., 2019; Lavrinienko et al., 2020). While Ahmed et al., (2019) showed evidence supporting the Anna Karenina effect of long-term temperature stress on coral microbiomes, Lavrinienko et al., (2020) found no effect after radiation exposure on microbiomes of bank voles. However, in moderator analysis, we found that compared with newly collected hosts, lab-acclimated hosts – with an already lower baseline microbiome richness – exhibited dispersed microbiomes under warming exposure. Lower microbiome diversity has been associated with a dysbiotic status (Pickard et al., 2017; Kriss et al., 2018; Valdes et al., 2018) and was shown to be the most important factor associated with microbiome instability (Frost et al., 2021). Thus, a stress-induced Anna Karenina effect might be context-dependent. Here, hosts with low initial microbiome diversity are more likely to exhibit structures in support of the Anna Karenina effect. Species with less diverse microbiomes could be disproportionately vulnerable under global warming if the increased instability of their microbiomes contributes to diminished host health (Zaneveld et al., 2017; Giongo et al., 2010; Gilbert et al., 2012; Dey et al., 2013).

We found that, across the tree of life, microbiome changes under thermal treatments were likely to be determined by host habitat and not by host biological traits. That said, the sample size for some host types was relatively low or some samples were collected from a single study (e.g., zooplankton), which might limit our ability to detect any significant effect of host traits. Endotherms experienced a similar level of decrease in microbiome diversity as ectotherms with temperature changes. It is predicted that marine organisms have narrower thermal safety margins, which increase their susceptibility to global warming compared with terrestrial organisms (Pinsky et al., 2019). Our results focusing on marine host microbiome support this prediction. In general, we find that aquatic hosts (and marine hosts to a greater extent) experience more reduction in microbiome diversity under cooling. As reductions in microbiome diversity can indicate dysbiosis with accompanying detriments to host health (Pickard et al., 2017; Kriss et al., 2018; Valdes et al., 2018), our result suggests aquatic species might be less tolerant to extreme cooling. If so, global change driving colder extremes may hinder host ability to expand their range towards new habitats in polar regions (Hastings et al., 2020). Hosts collected from less thermally variable habitats (regions with smaller annual temperature variation) also showed greater loss of microbiomes under cooling. The natural environments (aquatic or terrestrial ecosystems; temperature variation in the environment) to which hosts are adapted play a big role in the degree of microbiome perturbation under thermal change.

A significant challenge in assessing general patterns in host microbiomes is the variety of experimental designs used, even among studies on the same species. For example, among coral microbiome studies, the length of thermal acclimation ranged from 10 days to two years across 3-7°C differences (Hartman et al., 2019; Ziegler et al., 2017; Gajigan et al., 2017; Ahmed et al., 2019). We found a longer lab acclimation period before experimental warming led to a lower baseline microbiome richness and better maintenance of microbiome richness. It remains unclear whether longer lab acclimation facilitates adaptive evolution within host microbiomes, with the outcome of helping hosts cope better with

thermal stress. Similarly, we found that ramping temperature treatments resulted in less microbiome diversity loss than static warming. This result suggests a gradual and longer time for host acclimation (John et al., 2011) can aid microbiome stability. Future global change studies aiming to mimic natural temperature variation should consider using ramping rather than static temperature treatments unless the goal is to simulate heat shock (Terblanche et al., 2007). We are unsure whether longer ramped thermal treatments will result in similar declines in microbiome diversity, or rapid microbiome adaptation will ultimately overcome the environmental shifts (Voolstra & Ziegler 2020). Longitudinal studies, within and between seasonal time scales, are needed to elucidate relevant microbiome dynamics with host health status in response to climate change (Levy et al., 2020).

Assessing microbiome changes in populations might prove a promising factor in predicting their persistence, and for informing microbiome-based interventions for increasing species resilience during global climate change (Rosado et al., 2019; Morgans et al., 2020; Doering et al., 2021). However, our work has revealed that the range of temperatures and host species utilized in tackling the connection between host microbiomes and temperature should certainly be expanded. Whilst evidence has accumulated on the thermal effect on microbiomes in ectothermic and aquatic animals (Moghadam et al., 2018; Posadas et al., 2022; Fontaine et al., 2022; Hartman et al., 2019; Ziegler et al., 2017), there is a gap in our understanding of the temperature-microbiome relationship in endotherms, terrestrial species, and plants (Etemadi et al., 2018; Chevalier et al., 2015; Zhu et al., 2019). Moreover, among the empirical studies we surveyed, few explored the causal relationship and mechanisms underpinning host microbiomes and thermal tolerance. Microbiome transplantation, the process of transferring the microbiome of a healthy or thermal-tolerant donor to a diseased/stressed individual, has been invaluable for the treatment of some human infectious diseases during the last decade (Ooijevaar et al., 2019; Zhang et al., 2018). In non-human hosts, such an approach has been established in corals (Rosado et

al., 2019; Morgans et al., 2020; Doering et al., 2021) and Drosophila *melanogaster* fruit flies (Moghadam et al., 2018) – both showing an increase in host heat tolerance. Lastly, microbiome transplantation has also been used in mice to establish a protective role against cold stress (Chevalier et al., 2015). Future experimental work using microbiome transplantation might help with conservation of hosts during global climate change (Guo et al., 2020). Lastly, while controlled experiments as analysed here provide the best evidence for causal effects of temperature change on microbiomes, their lab-based nature means we cannot be certain how well these findings will translate to the real world, where environments are more complex. Future experimental warming studies in semi-natural settings will useful in this context.

Acknowledgements

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Data availability

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.21078217.v1.

Conflicts of interest

The authors declare that they have no conflict of interest.

Author contributions

JDL and KCK conceived the study. JDL, SCLK, and KCK designed the study. JDL conducted literature review, collected data, and performed the meta-analysis, with input

from KAB, KLH, TEH, SCLK, and KCK. JDL and KCK wrote the manuscript. All authors contributed to reviewing and editing.

Supplementary Material

Chapter 2

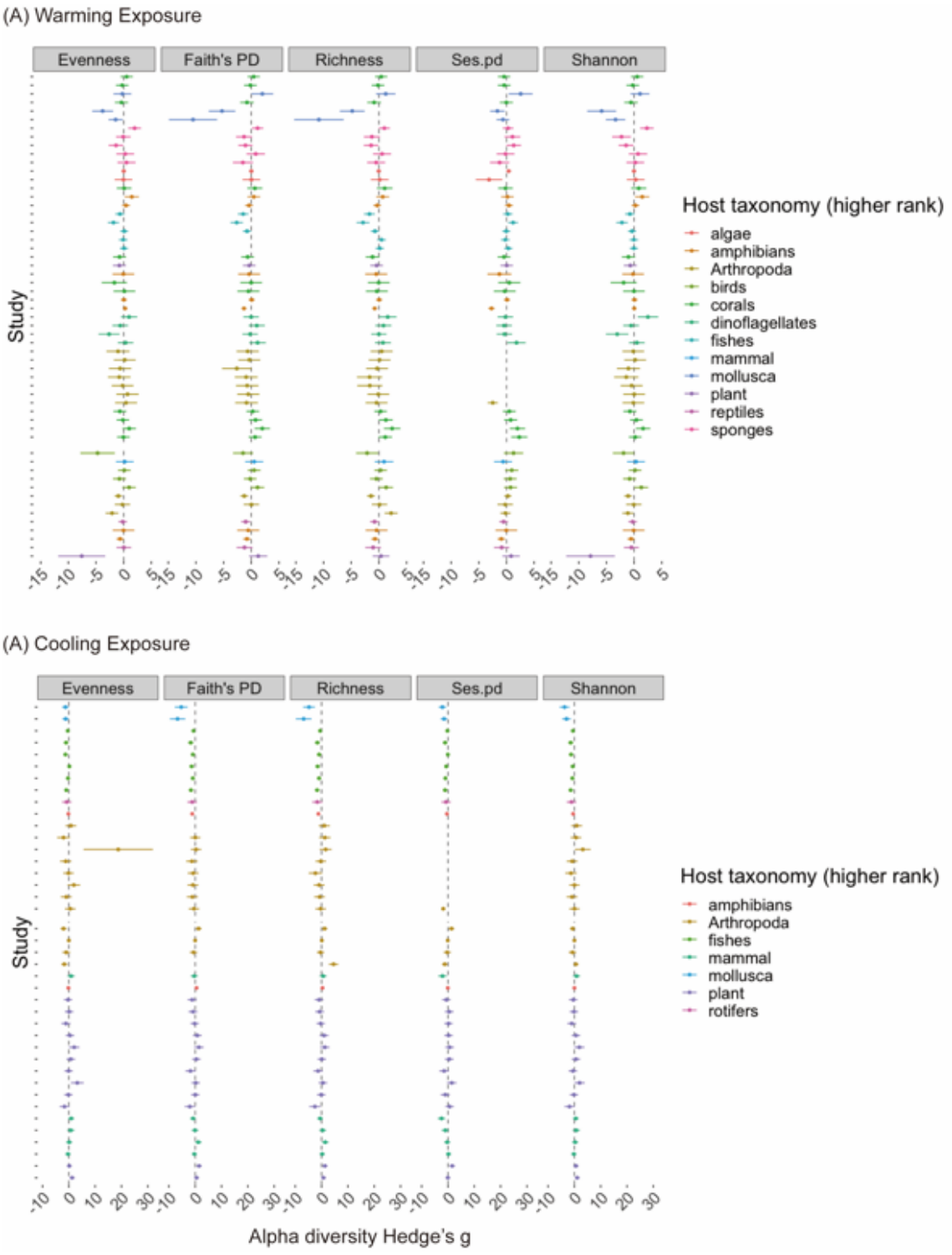


Fig S1. Forest plots of individual Hedge's g calculated for five alpha diversity metrics. (A) Warming vs. Control; (B) Cooling vs. Control. Hedge's g and 95% CIs are defined as circles and error bars. Vertical panels are different alpha diversity metrics. Different colors indicate different host taxa.

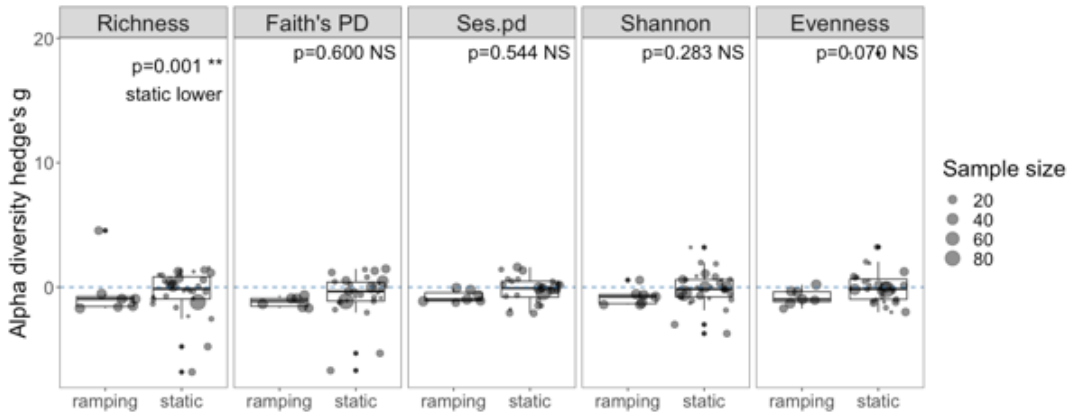


Fig S2. Impact of ramping and static regimes on host microbiome alpha diversity change in cooling. Vertical panels are alpha diversity metrics. Points are individual effect size (Hedge's g), with sample sizes used for Hedge's g calculation indicated. Points under the blue dotted line mean Hedge's g < 0: decreased alpha diversity under warming treatments. The significance level (p value) is shown on the top right corner of each faceted plot: NS (p > 0.05); * (p < 0.05); ** (p < 0.01).

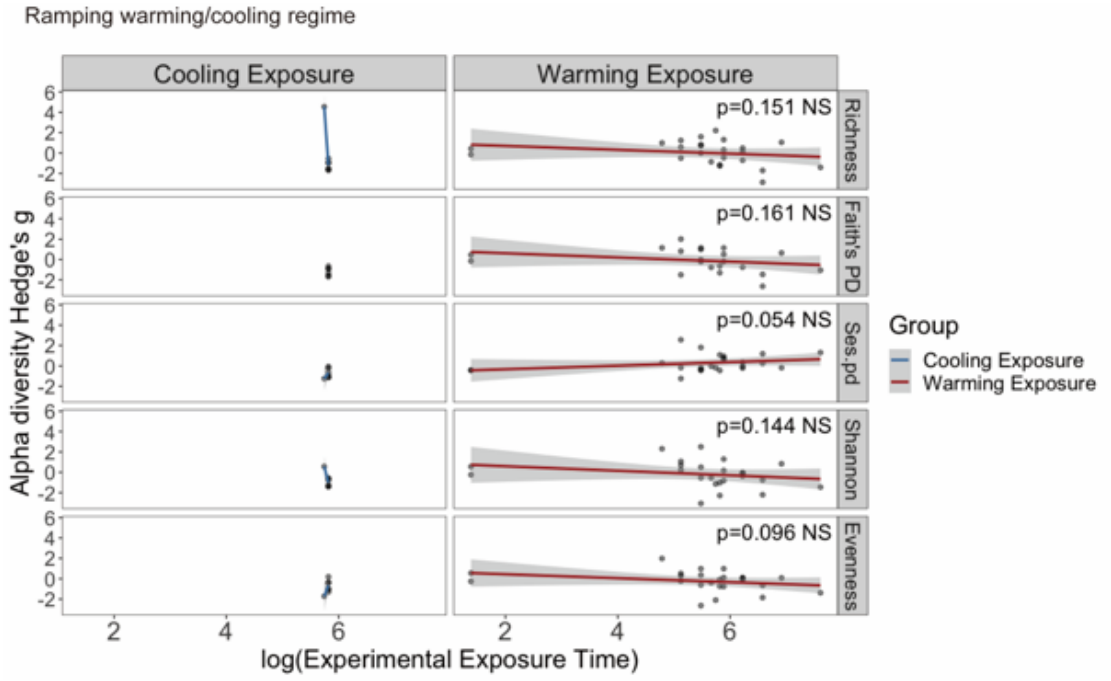


Fig S3. Association between experimental exposure time and change of host microbiome alpha diversity under ramping warming and cooling regimes. Vertical panels refer to different thermal exposures, and horizontal panels show different alpha diversity metrics. Exposure time on x axis is log-transformed for clearer visualization. Points represent individual effect sizes (Hedge's g), and a blue (cooling exposure) or red (warming exposure) smooth line is added to show a positive or negative association between exposure time and microbiome alpha diversity change. Positive association: longer exposure is associated with less microbiome diversity loss; Negative association: longer exposure is associated with greater microbiome diversity loss.

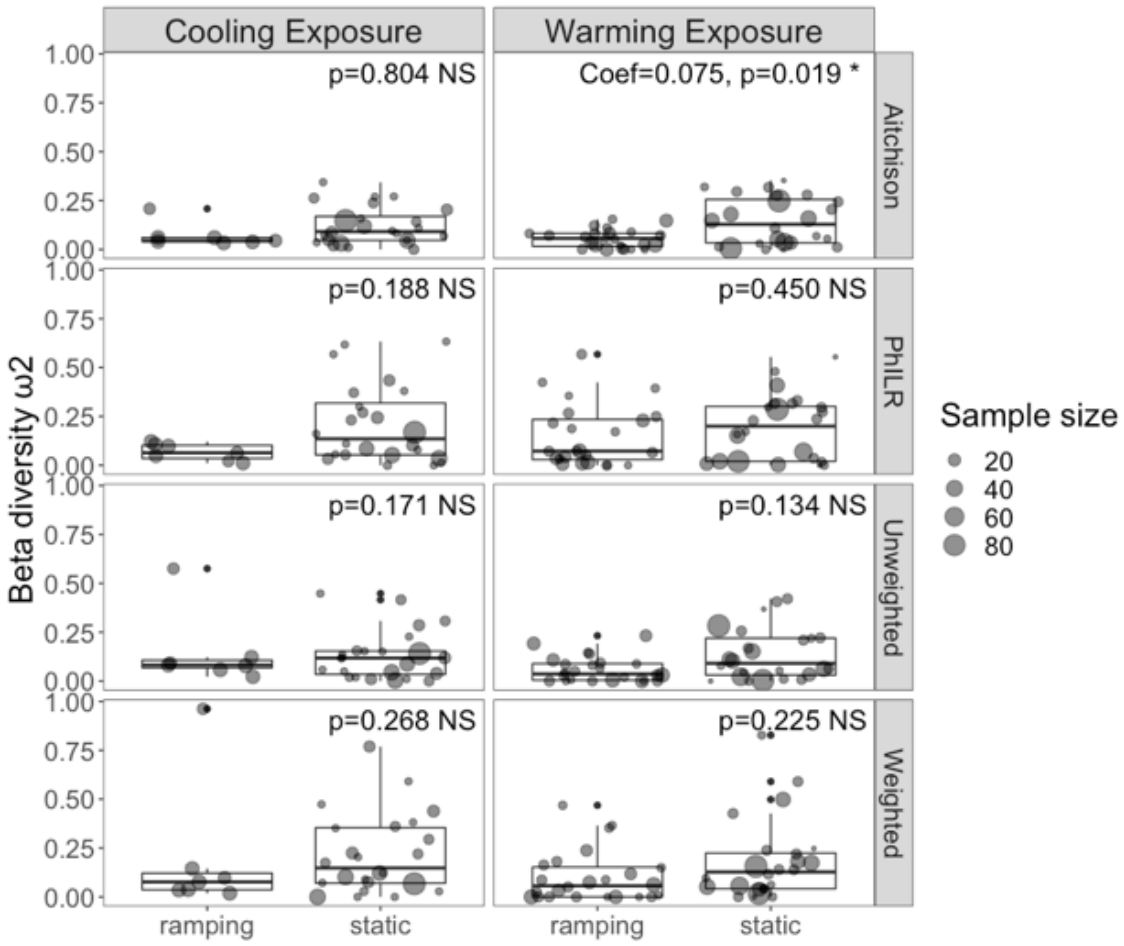


Fig S4. Impact of ramping and static regimes on host microbiome beta diversity in warming and cooling. Vertical panels are thermal exposure type and horizontal panels are beta diversity metrics. Points represent individual effect sizes (ω2), with sample sizes used for ω2 calculation indicated. The significance level of the difference between ramping and static treatments is shown on the top right corner of each faceted plot: NS ($p > 0.05$); * ($p < 0.05$); ** ($p < 0.01$). Only one metric under warming reveals that static regime is associated with larger microbiome compositional change ($p=0.019$).

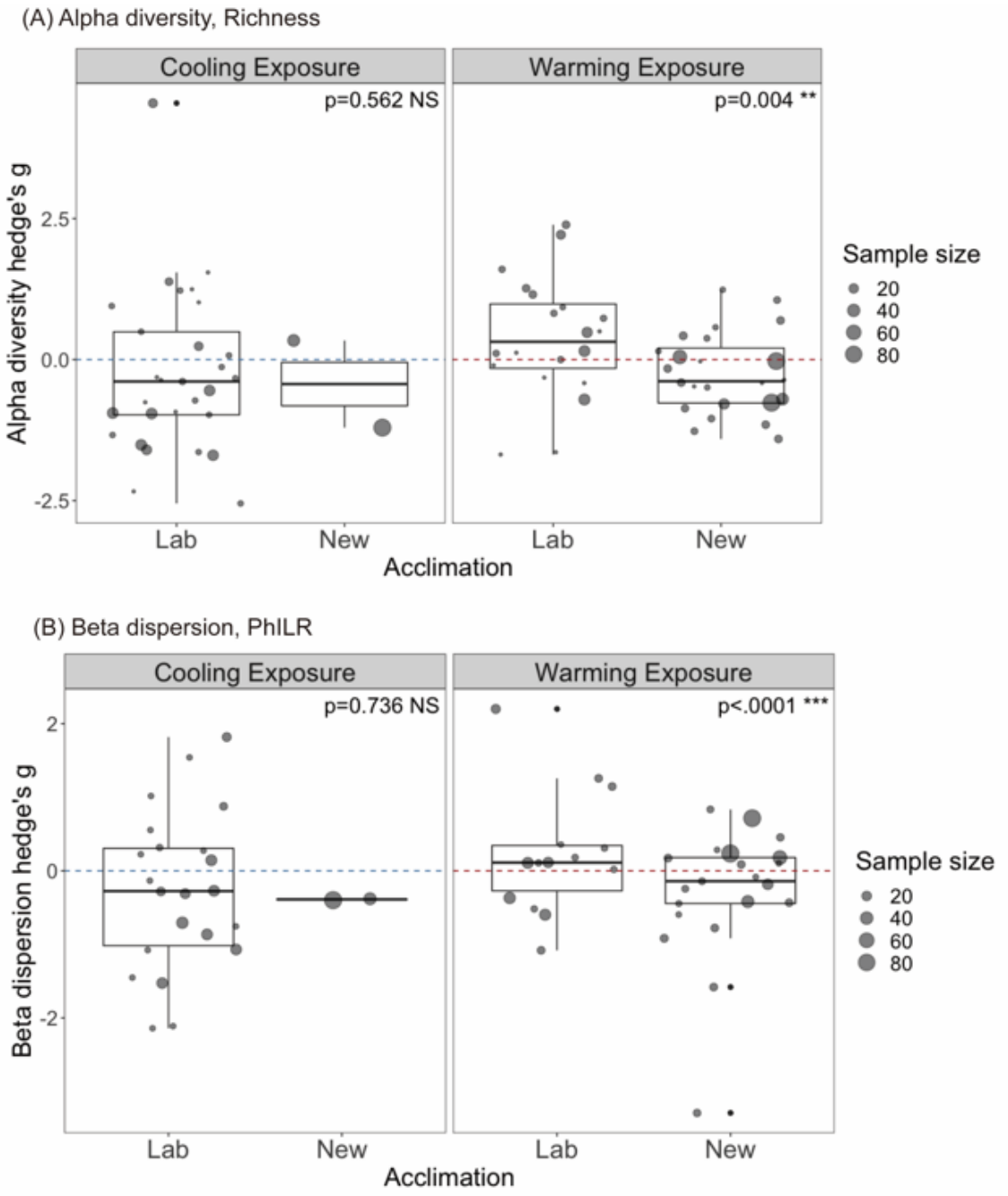


Fig S5. Impact of experimental lab acclimation on host microbiome diversity and dispersion. The significance level is shown on the top right corner of each faceted plot: NS ($p > 0.05$); * ($p < 0.05$); ** ($p < 0.01$). (A) Impact of host acclimation time (New: newly field-collected; Lab: maintained and acclimated in lab for generations) on host microbiome richness. Points under the blue (cooling exposure) or red (warming exposure) dotted line mean Hedge's $g < 0$: decreased alpha diversity under treatments. (B) Impact of host acclimation time (New: newly field-collected; Lab: maintained and acclimated in lab for generations) on host microbiome dispersion (PhILR metric). For all figures, vertical panels show different thermal treatments. Points represent individual effect sizes (Hedge's g), with sample sizes used for Hedge's g calculation indicated. Points above the blue (cooling exposure) or red (warming exposure) dotted line mean Hedge's $g > 0$: microbiomes more dispersed under treatments.

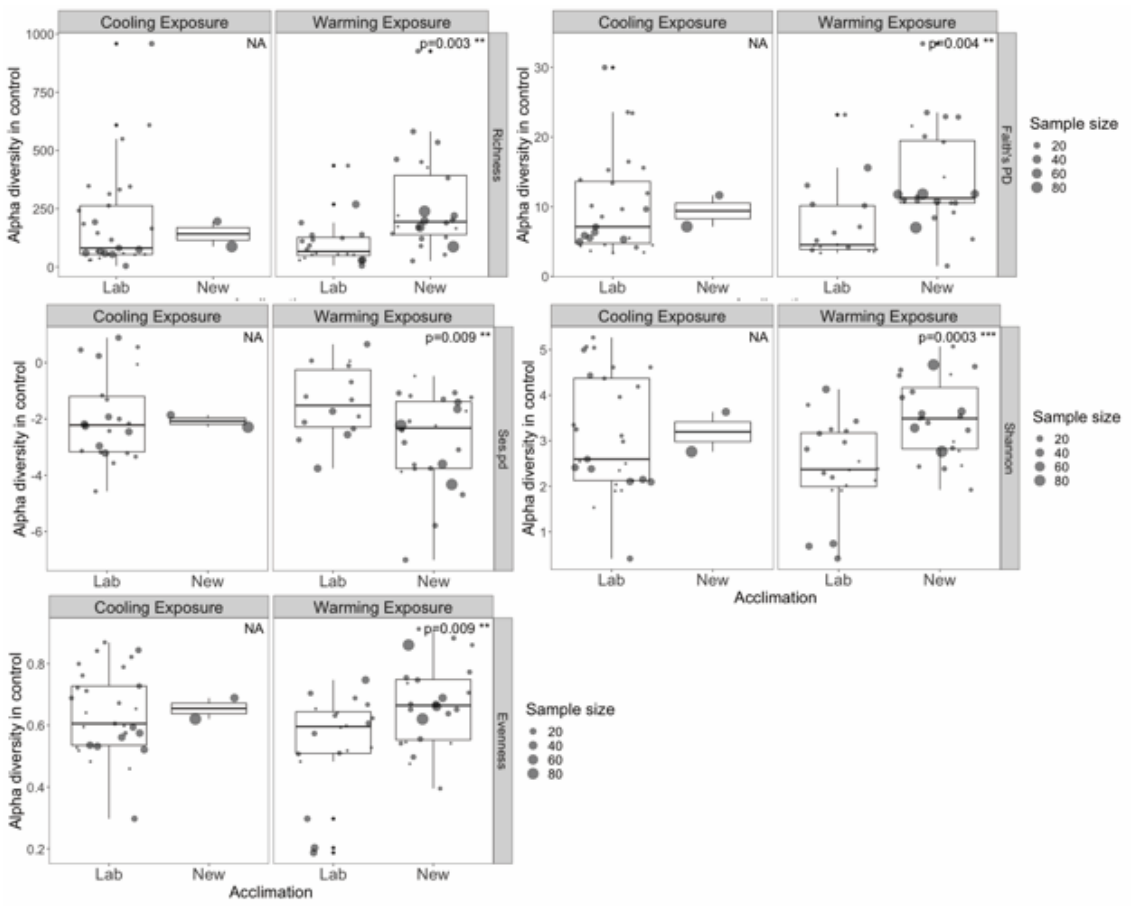


Fig S6. Impact of prior-experimental lab acclimation on host baseline microbiome diversity in control groups (without warming or cooling treatments). Each plot presents results for an alpha diversity metric. Vertical panels in individual plots show different thermal exposure. Points represent individual alpha diversity measures, with sample sizes indicated. New: newly field-collected; Lab: maintained and acclimated in lab for generations. The significance level of difference between New vs. Lab is shown on the top right corner of each faceted plot: NS ($p > 0.05$); * ($p < 0.05$); ** ($p < 0.01$). The results are consistent across all alpha diversity metrics except for Ses.pd.

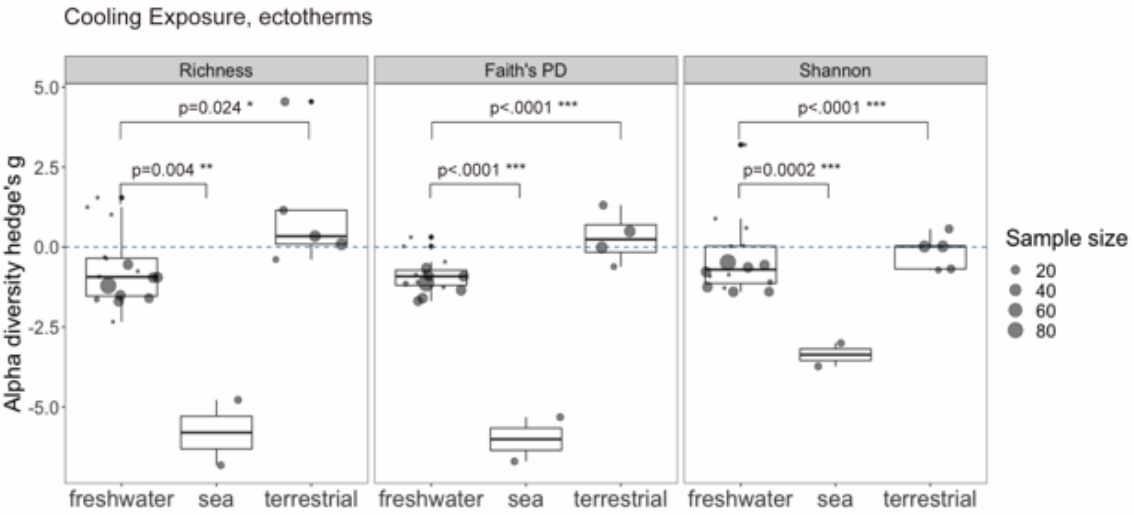


Fig S7. Impact of host habitat on the response of ectothermic host microbiome alpha diversity to thermal change. Points represent individual Hedge's g, with sample size used for Hedge's g calculation indicated. Points below the blue dotted line mean Hedge's g < 0: decreased alpha diversity under warming or cooling treatments. Vertical panels show different thermal exposure, and horizontal panels show different alpha diversity metrics. The significance levels for both sea vs. freshwater and terrestrial vs. freshwater comparisons are shown on the top middle of pairwise boxes (NS: p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001).

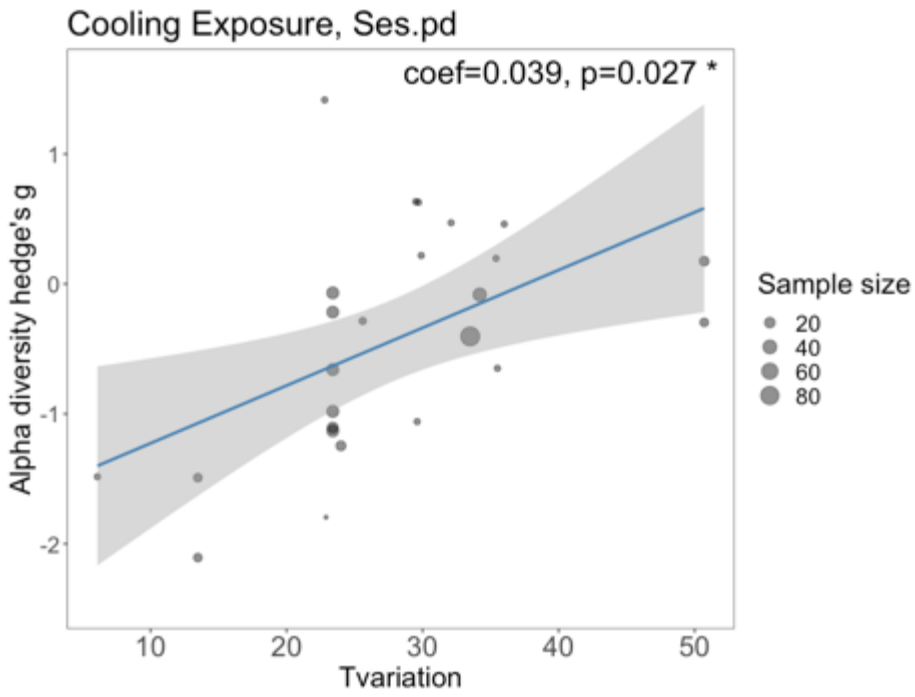


Fig S8. Association between the annual temperature variation (Tvariation) of host native habitat and host microbiome alpha diversity (Ses.pd) under cooling. Points represent individual Hedge's g, with sample size used for Hedge's g calculation indicated. A blue smooth line is added to show that larger Hedge's g is positively associated with greater Tvariation. The coeffient and significance level of the association are shown on the top right corner of the plot (coef=0.039, p=0.027).

Supplementary Methods

Sequence processing per study

Downloaded files were converted to match a format that was compatible with QIIME2 (zipped FASTQ files with specified names). FastQC (Andrews 2010) and MultiQC (Philip et al. 2016) were used for initial visualization of read quality, adapters or/and primers (if not already removed) were removed using Cutadapt (Martin 2011). Additional requests for raw sequencing data were made to the authors of studies where the forward and reverse reads were already joined. Where paired reads were available, reads were joined using vsearch (Rognes et al., 2016). If > 50% of reads could be overlapped, the merger was carried

forward for analysis; otherwise, where low reverse quality led to too few reads remaining, only the higher-quality forward reads were used. All low-quality reads were then filtered using default quality thresholds before starting Deblur workflow to denoise and classify sequences into ASVs. Within Deblur, trimming length was determined by manually viewing the quality plot for each study.

Within each study, taxonomy of the resolved ASVs was assigned using a classifier trained on the full-length 16S rRNA gene SILVA v138 database. Then, ASVs taxonomically assigned as “mitochondria” and “chloroplast”, and those not identifiable at the Kingdom level were removed, or present at a cumulative abundance of 10 or fewer within individual study were removed. Within individual study, the phylogenetic tree of the remaining ASVs were built using SEPP with a reference phylogeny (sepp-refs-gg-13-8).

Sample metadata and predictor variables

Whenever possible, data on moderator variables of interest was obtained from information from NCBI. When not possible, this information was obtained from the BioSample accessions and publications, and in a few cases, by directly asking the authors. To test some of our hypotheses, we also added metadata categories not in the original studies: (i) host habitat was classified as terrestrial or aquatic, with aquatic habitats further classified as freshwater or marine; (ii) whether host species was collected from the wild, purchased from a commercial institute, or from standardized lab stock. Collected host species were further classified into “collected”, meaning collected shortly before conducting the experiments, “lab_with_known_source”, meaning collected and maintained in the lab for a long time prior to experiments with source known. (iii) Host body site: whether the host microbiome was sampled from an internal body site (eg. intestinal tract, tissue, etc.), an external site (skin) or the whole body; (iv) Immune complexity: whether the host species has both innate and adaptive immunity or innate immunity only; (v) Host thermal type: whether the host species was ectothermic or endothermic; (vi) Thermal regime: whether the temperature change was ramping or static; (vii) elevation data, region and climate

information from the host native habitat were extracted using FreeMapTools (https://www.freemaptools.com/about.htm) with the latitude and longitude of where host was originally collected. Furthermore, for terrestrial hosts, bioclimatic variables representing temperature and precipitation (2.5 km resolution) of host native habitat were extracted for each unique host original geographic location from the WorldClim database (extract function in raster package R 3.6). For marine hosts, we extracted marine and geophysical variables from the MARSPEC database (Sbrocco and Barber 2013). For those with data missing in the MARSPEC database, associated max, min and mean temperature was extracted from Bio-ORACLE database (Tyberghein et al. 2011). We classified host into “corals”, “reptiles”, “mammal”, “amphibians”, “fishes”, “Arthropoda”, “sponges”, “birds”, “rotifers”, “mollusca”, “dinoflagellates”, “algae” and “plant” based on taxonomy of the hosts.

Effect-size calculation and meta-analysis

We performed microbiome diversity analysis within each study using a standardized pipeline and calculated effect-sizes using methods below.

For alpha diversity analyses, to calculate the effect size Hedge’s g, we calculated the mean of each alpha diversity metric and its standard deviation for each treatment. We then calculated the standard mean difference between groups using the escalc function (metafor package). For studies that had three treatment groups (warming, cooling and control), two effect sizes were calculated from each study: one for the difference between warming vs. control and the other from cooling vs. control. Multi-level mixed effect meta-analysis model and Bayesian hierarchical meta-analysis model were built using rma.mv (metafor package) and brm functions (brms package). For the latter method, we visually evaluated chain convergence and also made sure that Rhat was 1. Since the results from the frequentist mixed effects model and Bayesian hierarchical model were similar (possibly due to the fact we did not have enough evidence to support a strong prior and thus used a less informative prior), we presented summary effect sizes from the former model in the main text. We

focused on the less computationally intensive frequentist method in subsequent moderator analysis.

For beta dispersion, we also used Hedge's g as the effect size. Hedge's g was calculated to quantify the difference between average distance to median in different thermal treatments. Multi-level mixed effect meta-analysis model was built using rma.mv (metafor package).

For beta diversity analyses, effect sizes were calculated to quantify microbiome compositional change under thermal treatment. From each warming vs. control or cooling vs. control comparison, we used omega-squared for PERMANOVA as the effect sizes (adonis_OmegaSq function from https://github.com/Russel88/MicEco/). Effect sizes were calculated from four different beta diversity metrics - weighted UniFrac, unweighted UniFrac and Bray-Curtis. Before effect size calculation, we first evaluated whether sample sets could be merged for multiple host genotypes or multiple sampling body sites (eg. variable gut parts) within individual study when sample size for each genotype/sampling body site group was small (two samples in each group, which means it was hard to compare them statistically). We assessed the microbiome compositions between different genotypes/sampling body sites by performing PERMANOVA (adonis function, *vegan* R package) on all the four beta diversity dissimilarity metrics and merged sample sets if p-value was larger than 0.05 for all the metrics.

Tests for differentially abundant taxa and functions

We performed differential species analysis at both ASV and class level using ALDEx2 (clr based method) and ANCOM (alr based method), both methods used non-parametric test but had different normalization strategies. Within each study, the differential analysis was either performed on ASV level or on collapsed Class level, depending on the resolution of taxonomic classification. We performed non-parametric Wilcoxon rank-sum test on each of the taxa between the two treatment groups using aldex.ttest in ALDEx2 package in R. Taxa

with Benjamini & Hochberg (BH) adjusted p-value < 0.05 was considered to be differently enriched between groups. Then the aldex.effect function was used to calculate the expected value of the difference between distributions of each treatment group (median log2 difference), the expected value of the pooled group variance (median log2 dispersion) and the standardized effect size on the taxa abundance difference between two treatment groups. Taxa with effect-size > 0.3 was considered to have a large difference between the two groups. The cutoff of W value in ANCOM was set 0.6. Within each study, differentially abundant KEGG (Kanehisa and Goto 2020) orthologs (KOs), enzymes (Enzyme Commission Number, EC) and MetaCyc (Caspi et al. 2020) pathways between the treatment and control group were identified using Wilcoxon rank-sum test (wilcox.test function, BH adjusted p value <=0.05). Identified differential taxa and functional pathways were compared between studies as well as grouped by moderator variables.

Network analysis

By establishing the microbial network and calculating the centrality metrics for each node (or bacterial species), we quantified the potential importance of each bacterial member in the microbial community in both treatment and control groups. We established microbial co-occurrence network (on both ASV and class level) of each experimental group (warming group, cooling group and control group) within each study using SparCC program wrapped in SpiecEasi R package, which is robust to any distribution of community abundances. Threshold for SparCC correlation matrix was set as 0.3 and spurious links were removed from the network. Nodes in each network correspond to ASVs/Class and edges correspond to direct signed interactions between ASVs given treatment group. We then assessed the node degree, betweenness centrality and closeness centrality of the differential taxa identified. We extracted the property information of the differential ASVs and summarized it to class level.

Supplementary Results

Summary of included studies and samples

The search yielded 75 potential studies for inclusion. Of these 75 studies, 14 did not have sequencing data of appropriate fastq format available, four lacked sample metadata, two had evaluated mixed effects of temperature and other variables, and 12 did not use Illumina NGS for sequencing. Forty-three studies remained for inclusion in our meta-analysis (details in Supplementary Table S1). Thirty-eight had data available in the public database, whilst for five we requested data directly from the authors. After standardized processing, Aydogan et al. 2020 and Ziętak et al. 2016 were removed before further analysis due to only 1 or no sample retained in the treatment or control group. As studies varied in technical considerations such as sequencing region, sequencing depth, sample size and metabarcoding pipelines, we downloaded and processed the raw reads on per study basis using a standardized Deblur workflow, the parameters used for primer removal, quality filtering and trimming within each study can be found in Supplementary Table S1.

Differential taxa in warming and cooling

We tested for a set of indicator species useful for predicting host microbiome responses to warming and cooling for each study. Using ALDEx2 (Wilcoxon rank sum test, significance level: p-value < 0.05), we identified 438 differential ASVs from fourteen comparisons in warming exposure and 226 differential ASVs from nine comparisons in cooling exposure. Using ANCOM (W cutoff = 0.6), we identified 577 differential ASVs from 30 comparisons in warming exposure and 279 differential ASVs from 19 comparisons in cooling exposure. The identified species were similar between the two methods (347 overlapped ASVs in warming, and 128 overlapped ASVs in cooling). We thus focused on differential species that repeatedly identified using both methods in the subsequent analysis. We summarized differential ASVs to class level for better visualization. Overall, we found that most differential ASVs were from the prevalent bacterial phyla (such as Proteobacteria, Bacteroidota and Firmicutes), and there was only a small proportion of differential ASVs in

these bacterial phyla (Fig S9). Most ASVs did not change significantly in abundance under either warming or cooling.

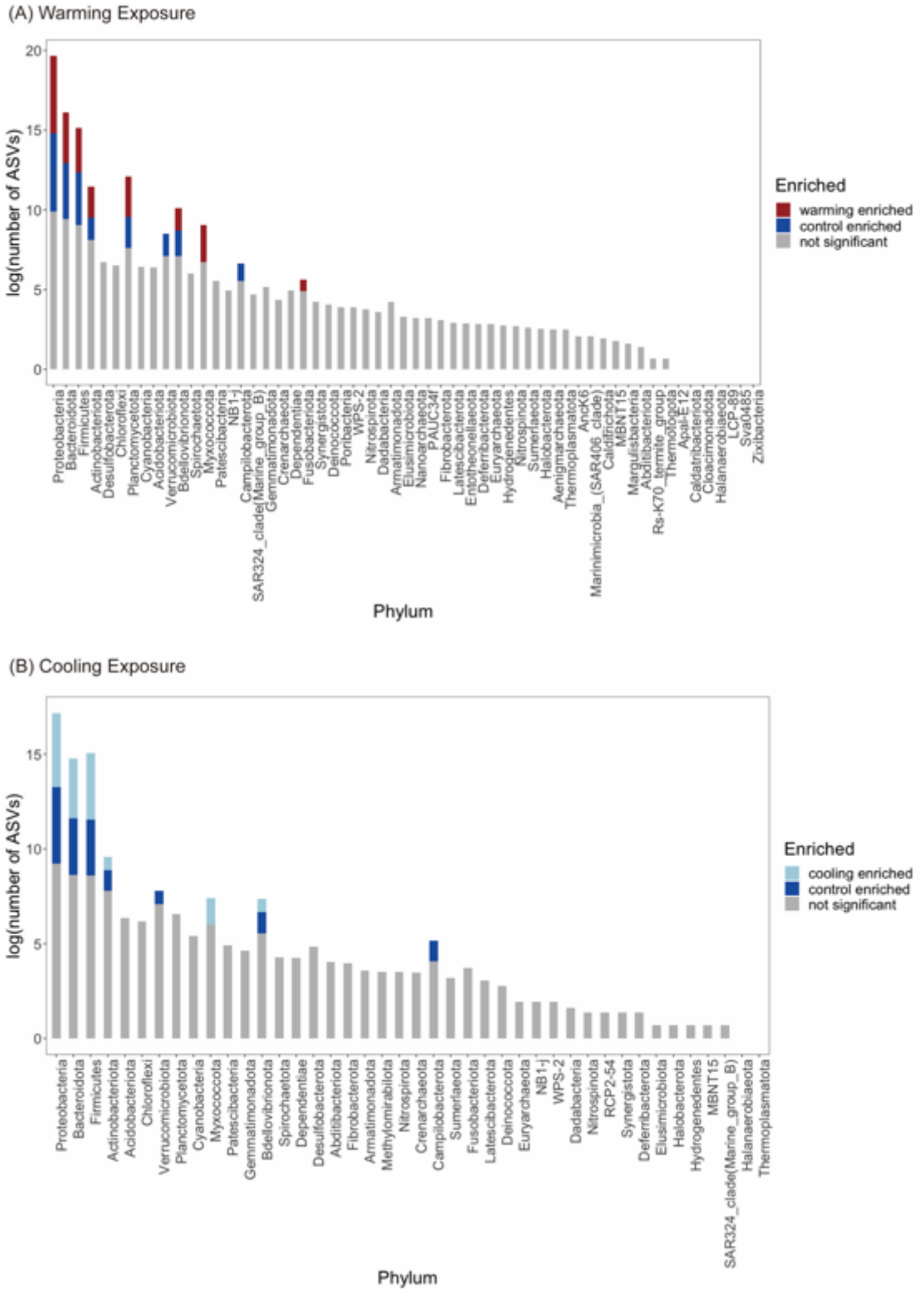


Fig S9. Proportion of differential ASVs in individual bacterial phylum under both warming and cooling. (A) Number of ASVs per bacterial phylum under warming. Each stacked bar is filled by a combination or different colors – red indicates enrichment in warming group, dark blue indicates enrichment in control group, and grey represents ASVs that were not differentially enriched in

either group. (B) Number of ASVs per bacterial phylum under cooling. Each stacked bar is filled by a combination or different colors – light blue indicates enrichment in cooling group, dark blue indicates enrichment in control group, and grey represents ASVs that were not differentially enriched in either group.

We found that the distribution of differential ASVs were highly heterogeneous in the four common classes Gammaproteobacteria, Alphaproteobacteria, Bacterodia, and Bacilli. Nearly half the ASVs in each class were enriched in warming or cooling groups and the other half enriched in control group (Fig S10A,B). When we mapped the pattern of differential taxa to individual host species, we found that enriched taxa were highly heterogeneous across hosts (Fig S10C,D). Though pacific and hybrid abalone showed quite similar differential taxa in their microbiomes, they were from the same study (Wang et al. 2020) and may be subject to study or author bias. When grouped by biome (terrestrial, sea, freshwater), we found that more differential bacterial taxa were enriched in marine rather than terrestrial host microbiomes under warming, while under cooling, more differentia bacteria were enriched in terrestrial hosts (Fig S10E, F). Freshwater host microbiomes did not show a more similar pattern of differential taxa with marine hosts, under either warming or cooling. These results revealed the challenges in identifying common indicator taxa used to predict host performance under temperature stress.

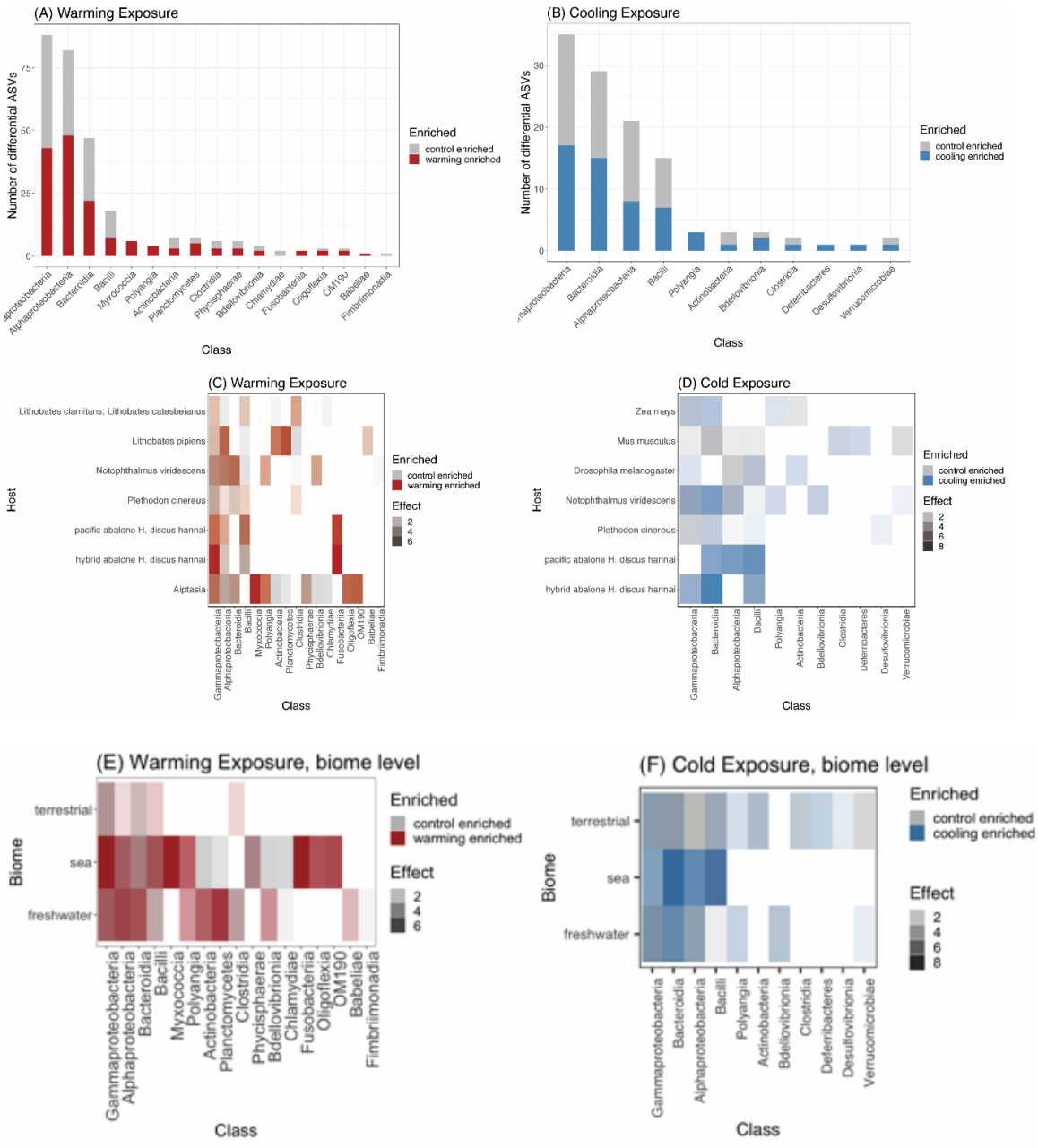
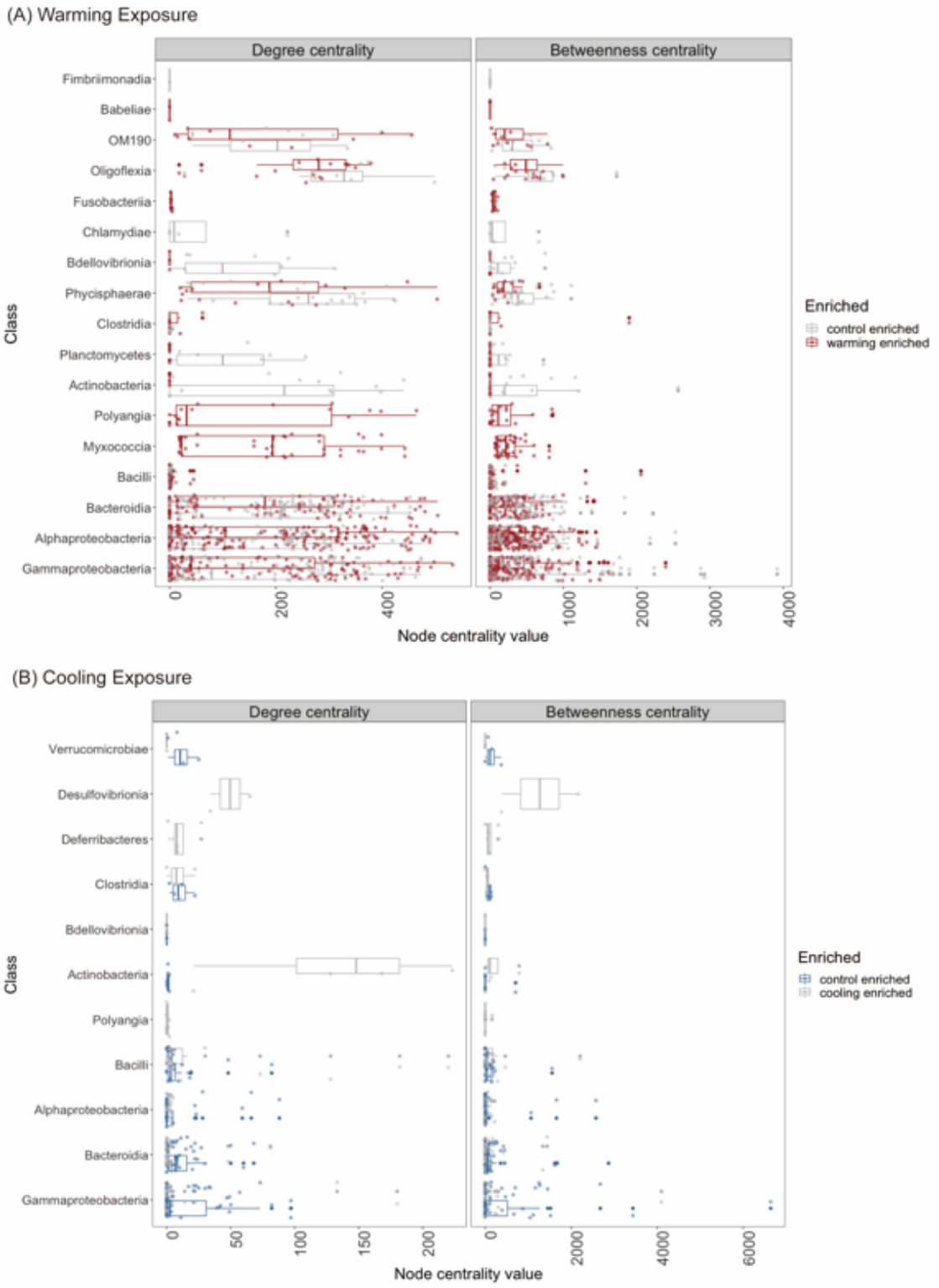


Fig S10. Differentially enriched bacterial classes under warming and cooling, across different hosts. (A) Bacterial class-level classification of ASVs that were differentially abundant in warming comparisons. Red indicates enrichment in the warming group, grey indicates enrichment in the control group. (B) Differential ASVs in each class-level taxonomic group in cooling exposure. Blue indicates enrichment in cooling group, grey indicates enrichment in control group. (C) Heatmap of differential taxa across hosts in warming exposure. Shade indicates the abundance difference of the differential taxa between warming and control groups, which is shown by the effect. (D) Heatmap of differential taxa across different hosts in cooling exposure. (E) Heatmap of differential taxa across different biome in warming exposure. (F) Heatmap of differential taxa across different biome in Cooling exposure.

Differential taxa in the microbial network

We further assessed the centrality measures of differential taxa in the microbial co-occurrence network. We found that differential ASVs from the most prevalent classes

Gammaproteobacteria, Alphaproteobacteria, Bacterodia and Bacilli did not change greatly in their degree or betweenness centrality in the microbial networks under either warming or cooling (Fig S11). This finding might suggest that generalists in the microbial community changed in a stochastic way under temperature perturbation, but did not become more important in the microbial communities. However, differential ASVs in two less prevalent classes Myxococcia and Polyangia – highly enriched in the warming compared to the control group (Fig S10C) – also showed a higher degree and betweenness centrality in the warming group. These taxa become more abundant under warming, but also could potentially play more important roles in the microbial communities as “hubs” or keystone taxa. When grouping the differential taxa by host region, we found that these two potential “hub” taxa were shown as hubs in tropical and subtropical regions, but not temperate regions under warming (Fig S11C), indicating their environmental adaptability.


(A) Warming Exposure
Degree centrality
Betweenness centrality
Fimbriimonadia
Babeliae
OM190
Oligoflexia
Fusobacteriia
Chlamydiae
Bdellovibrionia
Phycisphaerae
Clostridia
Planctomycetes
Actinobacteria
Polyangia
Myxococcia
Bacilli
Bacteroidia
Alphaproteobacteria
Gammaproteobacteria
Class
0
200
400
0
1000
2000
3000
4000
Node centrality value
Enriched
control enriched
warming enriched
(B) Cooling Exposure
Degree centrality
Betweenness centrality
Verrucomicrobiae
Desulfovibrionia
Deferribacteres
Clostridia
Bdellovibrionia
Actinobacteria
Polyangia
Bacilli
Alphaproteobacteria
Bacteroidia
Gammaproteobacteria
Class
0
50
100
150
200
0
2000
4000
6000
Node centrality value
Enriched
control enriched
cooling enriched

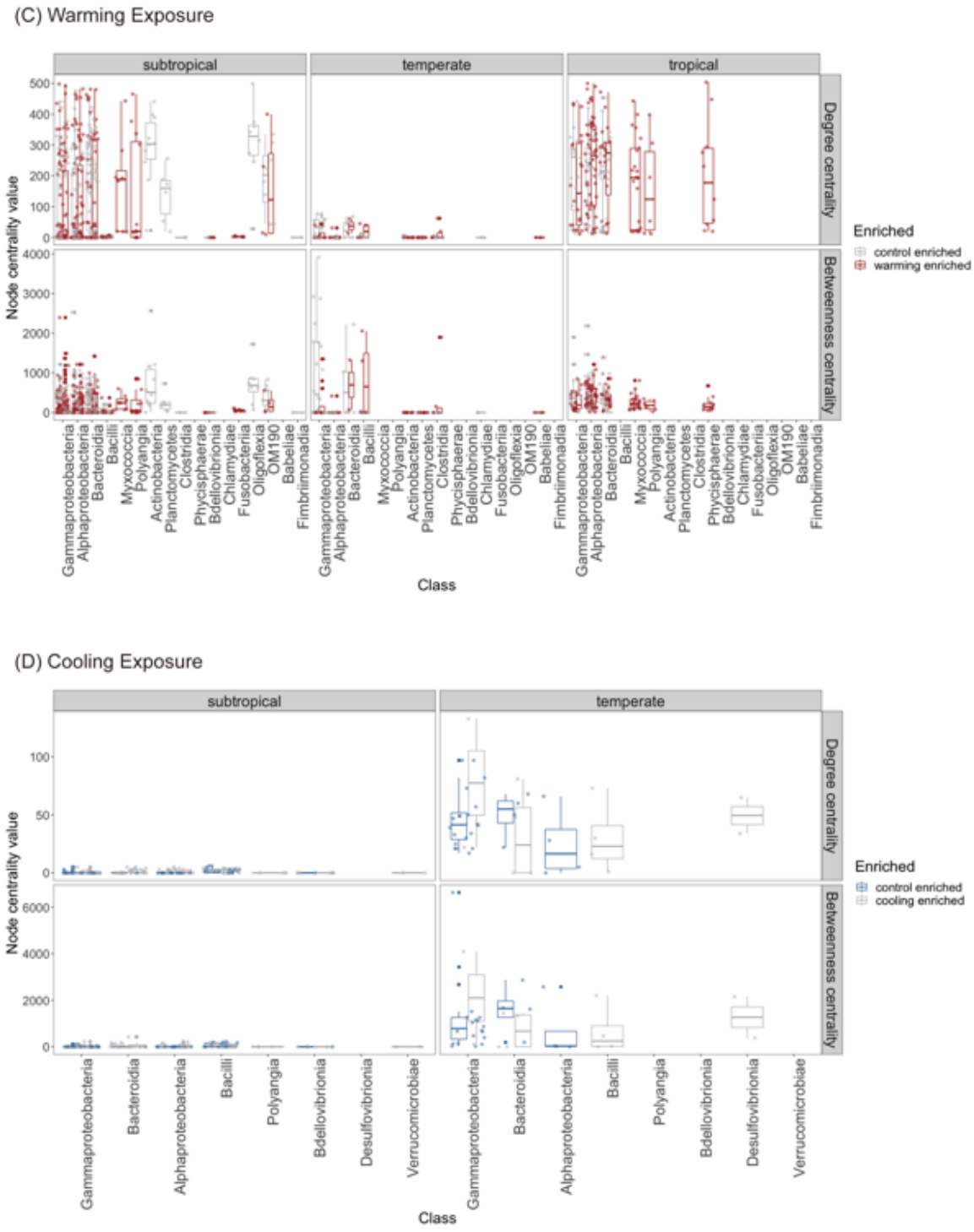
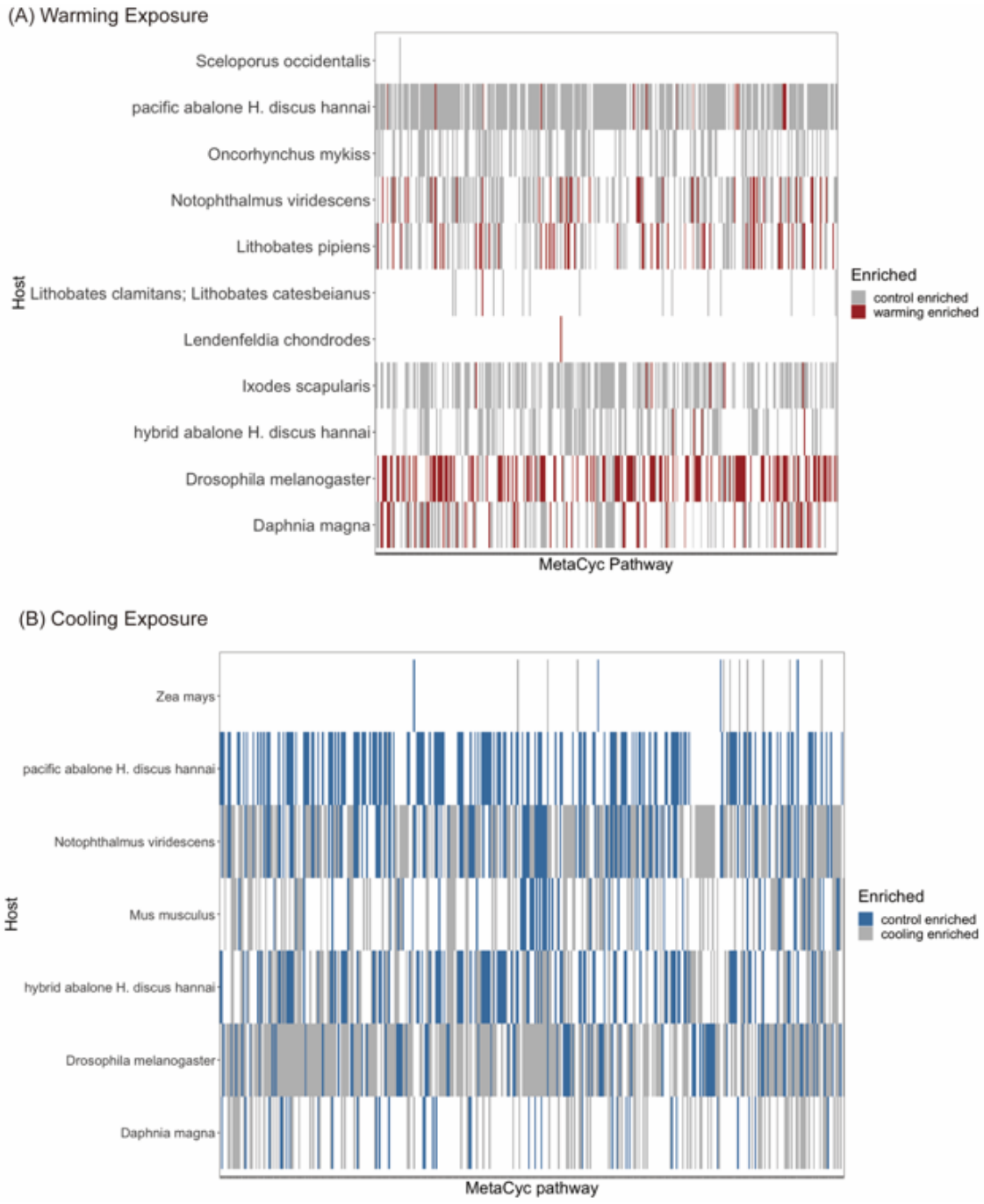


Fig S11. Degree centrality and betweenness centrality of differential taxa in microbial network under both warming and cooling exposures. (A) Centrality measures of differential ASVs on class level. Red and grey nodes are ASVs enriched in warming and control, respectively. (B) Degree centrality and betweenness centrality of each differential taxa in microbial network in cooling and control groups. Blue and grey nodes are ASVs enriched in cooling and control, respectively. (C) Centrality measures of differential ASVs (in warming exposure) on class level. Figure facetted by different host region (tropical, subtropical and temperate). (D) Centrality measures of differential ASVs (in cooling exposure) on class level. Figure facetted by different host region (subtropical and temperate).

Differential predicted functional pathways

We identified 619 differential MetaCyc pathways from eleven comparisons in warming exposure, with 243 pathways enriched in warming group and 376 enriched in control group. We have identified 682 differential MetaCyc pathways from nine comparisons in cooling studies (adjusted p-value < 0.05). It is worth noting that the functional analyses are predictive and the performance might degrade outside of human datasets, as shown in Sun et al. 2020.

After filtering differential pathways that have spurious results, we found that three pathways – starch degradation V (PWY-6737), glycogen degradation I (GLYCOCAT_PWY) and glycogen biosynthesis I (from ADP-D-Glucose) (GLYCOGENSYNTH_PWY) – were significantly enriched in both warming and cooling groups compared with controls. Results on differential pathways showed heterogeneity between hosts (Fig S12A, B), but we found that more functional pathways were enriched under warming in terrestrial host microbiomes, compared with that in marine or freshwater hosts. And in cooling, more functional pathways were enriched in aquatic (especially marine) host microbiomes rather than terrestrial hosts (Fig S12 C, D).


(A) Warming Exposure
Sceloporus occidentalis
pacific abalone H. discus hannai
Oncorhynchus mykiss
Notophthalmus viridescens
Lithobates pipiens
Lithobates clamitans; Lithobates catesbeianus
Lendenfeldia chondrodes
Ixodes scapularis
hybrid abalone H. discus hannai
Drosophila melanogaster
Daphnia magna
Host
MetaCyc Pathway
Enriched
control enriched
warming enriched
(B) Cooling Exposure
Zea mays
pacific abalone H. discus hannai
Notophthalmus viridescens
Mus musculus
hybrid abalone H. discus hannai
Drosophila melanogaster
Daphnia magna
Host
MetaCyc pathway
Enriched
control enriched
cooling enriched

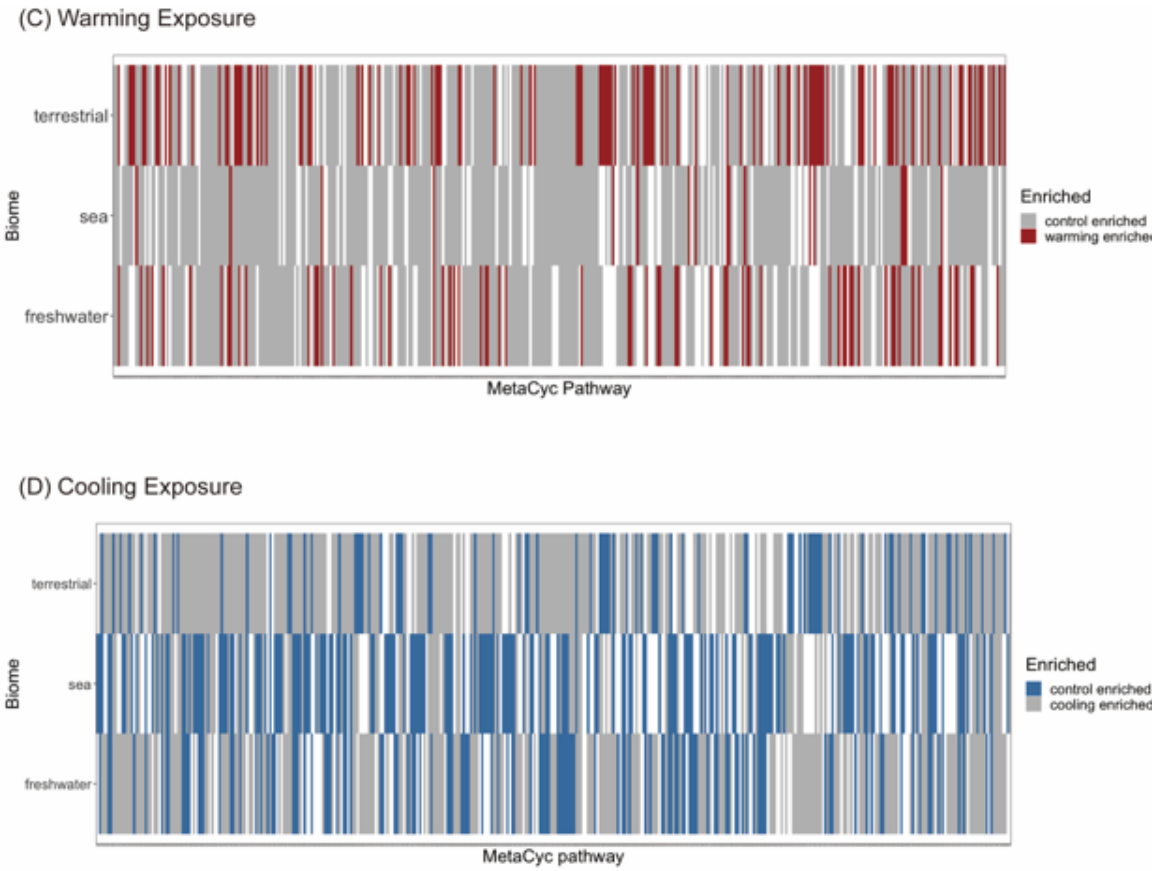


Fig S12. Differential functional pathways across hosts and distinct habitats. (A) Differential MetaCyc functional pathways under warming exposure. Filled tiles indicate pathway enrichment in either warming (red) or control groups (grey). (B) Differential MetaCyc functional pathways under cooling exposure. Filled tiles indicate pathway enrichment in either cooling (blue) or control (grey) groups. (C) Different functional pathways across host habitats (sea, freshwater, terrestrial) under warming. (D) Different functional pathways across host habitats under cooling.

Supplementary Table 1: Summary of studies included in the meta-analysis

Study name	Host	Taxa	Latitude	Longitude	Habitat	Bioproject	stress	acclimation	temp_diff	gradual	exposure_time	lab_or_collected
Hartman2019	Exaiptasia diaphana	coral	-18.15	147.483333	Marine	PRJNA576764	heat stress	10d	7	Yes	14d	collected
Ziegler2017	Acropora hyacinthus	coral	-14.183333	-169.6	Marine	PRJNA319637	heat stress	17month	6	Yes	6h	collected
Gajigan2017	Acropora digitifera	coral	16.2912778	120.012278	Marine	PRJNA341929	heat stress	7weeks	5	No	10d	collected
Ahmed2019_host1	Aiptasia	coral	19	-155	Marine	PRJNA497709	heat stress	NA	7	No	2years	collected
Ahmed2019_host2	Aiptasia	coral	35	-79	Marine	PRJNA497709	heat stress	NA	7	No	2years	collected
Ahmed2019_host3	Aiptasia	coral	20.082778	40.133056	Marine	PRJNA497709	heat stress	NA	7	No	2years	collected
Camp2020	Symbiodiniaceae	dinoflagellates	-19.135864	146.842356	Marine	PRJNA565838	heat stress	14d	6	Yes	10d	lab
Maher2019	Pocillopora meandrina	coral	-17.490567	-149.82642	Marine	PRJNA549489	heat stress	24h	3	Yes	21d	collected
Horlick2020	Seriola lalandi	fish	-32	152	Marine	PRJNA492935	heat stress	NA	4	Yes	21d	lab/collected
Fontaine2018	Plethodon cinereus	amphibian	37	-80	Terrestrial	PRJNA477306	heat and cold stress	4weeks	-5	No	5d	collected
Etemadi2018_host1	Arabidopsis	plant	62	34	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Etemadi2018_host2	Arabidopsis	plant	61.1	34.5	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Etemadi2018_host3	Arabidopsis	plant	55.75	37.35	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected

Etemadi2018_host4	Arabidopsis	plant	54.9	23.54	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Etemadi2018_host5	Arabidopsis	plant	52.73	15.15	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Etemadi2018_host6	Arabidopsis	plant	28	-15.3	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Etemadi2018_host7	Arabidopsis	plant	34.1	-4.12	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Etemadi2018_host8	Arabidopsis	plant	40	-8.25	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Etemadi2018_host9	Arabidopsis	plant	40.48	-3.22	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Etemadi2018_host10	Arabidopsis	plant	40.5	-2	Terrestrial	PRJNA399644	cold stress	42d	-14~16	No	56d	collected
Wessels2017	Lobophytum pauciflorum	coral	-18	146	Marine	PRJNA350227	heat stress	2weeks	4	Yes	12d	collected
Moghadam2018	Drosophila melanogaster	insect	55.9451278	10.2125861	Terrestrial	PRJEB14535 PRJEB18474	heat and cold	NA	-0.8	Yes	eggs to eclosed flies	collected
Moeller2020	Sceloporus occidentalis	reptile	35.3	-120	Terrestrial	PRJEB39005	heat stress	3d in lab and 7d in constant temperature at 25	10	No	>7 days	collected
Chevalier2015	Mus musculus	mammal	NA	NA	Terrestrial	PRJNA299679	cold stress	NA	NA	No	4 weeks	lab
Kohl2016	Lithobates pipiens	amphibian	42.9229972	-88.8377	Freshwater	PRJNA289102	warm and cool,	NA		No	from embryo to approxim	collected

							no stress				ately Gosner stage 25	
Bestion2017	Zootoca vivipara	reptile	44.2641556	3.71220278	Terrestrial	https://doi.pangaea.de/10.1594/PANGAEA.874404, asked	heat stress		+2~3		early june 2012 to may 2013	collected
Zhu2019	Gallus gallus	bird	33.3227444	120.529903	Terrestrial	PRJNA476844	heat stress				NA	lab/collected
Huyben2018	Oncorhynchus mykiss	fish	59.81606	16.91101		PRJNA408116	heat stress		7	Yes	6 weeks	lab/collected
Zare2018	Drosophila melanogaster	insect	NA	NA	Terrestrial	PRJNA435961	cold stress		-7	No	until get F2 generation flies	lab
Kokou2018	Oreochromis aureus	fish	NA	NA	Freshwater	PRJNA431528	cold stress	NA	-12	Yes	~14d	lab
Raimondi2020	Hermetia illucens	insect	44.389442	7.547778	Terrestrial	PRJNA573042	heat and cold stress	NA	-1.167	No	the whole rearing phase	lab
Vargas2020	Lendenfeldia chondrodes	other invertebrate	NA	NA	Marine	PRJEB35927	heat stress	4d	5.6	Yes	5 days	lab
Ramsby2018	Cliona orientalis	other invertebrate	-18.627778	146.493333	Marine	PRJNA407695	heat stress	17d	9	Yes	4 months	collected
Thapa2018	Ixodes scapularis	other invertebrate	NA	NA	Terrestrial	PRJNA471905	heat and cold stress			No	10 days	lab
Strand2017	Geodia barretti	other invert	60.0608	5.815	Marine	https://qiita.ucsd.edu/download_ra	heat stress	2 weeks + 5d	0.1	Yes	79d (65d recovery	collected

		ebrate				w_data/10533					period at 7)	
Tian2020	Anas platyrhynchos	bird	30.16	120.12	Terrestrial	PRJNA592979	heat stress	20d under conditions of natural light and at 25 degrees	10	Yes	15d	commercial
Bricker2020	Daphnia magna	planktonic crustacean	NA	NA	Freshwater	https://drive.google.com/drive/folders/1UvKnGedL0GjzPd1TTQPmPwDO6zqCgHiW	heat and cold stress	minimum of 2 weeks (acclimated to the experimental temperature)	-2	NA	~6 weeks (3 generations)	lab
Fontaine2020	Lithobates clamitans; Lithobates catesbeianus	amphibian	41.6427	-80.4277	Freshwater/Terrestrial	PRJNA601186	heat stress	2 weeks	5	NA	10d	collected
Li2019	Xenopus tropicalis	amphibian	30.67	104.07	Freshwater/Terrestrial	PRJNA497428	heat stress	NA	5	NA	from eggs to 3 days after post-metamorphosis (froglet)	collected
Aydogan2020	Arrhenatherum elatius; Galium album	plant	50.526667	8.695	Terrestrial	PRJNA578545	heat stress	NA	2	NA	6 years	collected
Aydogan2018	Galium album	plant	50.526667	8.695	Terrestrial	PRJNA400850	heat stress	NA	2	NA	6.5 years	collected
Wang2020	Haliotis discus hannai	other invertebrate	24.53	118.6	Marine	PRJNA530882	heat and cold stress	NA	NA	NA	3 months	lab

Mensch2020	Fucus vesiculosus	algae	54.4541531	10.1977461	Marine	PRJNA321820	heat stress	NA	5	NA	1 year	collected
Beirinckx2020	Zea mays	plant	NA	NA	Terrestrial	PRJNA524079	cold stress	NA	0.444	NA	5 weeks	lab
Yifeng2019	Mytilus galloprovincialis	other invertebrate	38.75	121.216667	Marine	PRJNA541804	heat stress	1 week	6	Yes	7 days	collected
Zhong2019	Capra aegagrus hircus	mammal	34.272117	108.084732	Terrestrial	PRJNA491591	heat stress	30d		NA	16*5d	lab/collected
Mensch2016	Fucus vesiculosus	algae	55.0283889	8.43316667	Marine	PRJNA310780	heat stress	NA	5	NA	>11 weeks	collected
Bo2019_experiment1	Lasiopodomys brandtii	mammal	NA	NA	Terrestrial	PRJNA555499	cold stress	NA	19	No	8 weeks	collected
Bo2019_experiment2	Lasiopodomys brandtii	mammal	NA	NA	Terrestrial	PRJNA555506	cold stress	NA	19	No		collected
Krisko2020	Mus musculus	mammal	NA	NA	Terrestrial	PRJNA589879	heat and cold stress	4 weeks		Yes (for cold exposure)		lab
Greenspan2020			-22.8316	-46.9651		PRJNA613682	heat stress					
Ziętak2016	Mus musculus	mammal			Terrestrial	PRJNA321010	heat and cold stress			No	4 weeks	lab
Carter2021	Notophthalmus viridescens	amphibian	35	-84		requested from author	no stress	1 week			45-90d minus 1 week	
Eckert2021	Lecane inermis	zooplankton	45.93	8.55	Freshwater	PRJNA655592	no stress	75d		No	1 month	lab
Posadas2021_host1	Neopetrosia compacta	sponge	16.296	120.014	Marine	PRJNA689294	NA	7 days	4	Yes	7 days	collected
Posadas2021_host2	Leucetta chagosensis	sponge	16.296	120.014	Marine	PRJNA689294	NA	7 days	4	Yes	7 days	collected

Supplementray Table 2 Modelling details for alpha-diversity and beta-dispersion effect-sizes

Multi-level mixed effects meta-analysis model
(random factor: study)

Metric	Variance	SMD	xmin	xmax	Group
Richness	0.0416	-0.274	-0.6738	0.125763	Warming
Shannon	0.0321	-0.328	-0.6792	0.023163	Warming
Faith's PD	0.0356	-0.398	-0.7678	-0.02819	Warming
Evenness	0.0202	-0.266	-0.5446	0.012568	Warming
Ses.pd	0.0324	-0.109	-0.4618	0.2438	Warming
Richness	0.222	-0.117	-1.0405	0.806491	Cooling
Shannon	0.0553	-0.229	-0.6899	0.231913	Cooling
Faith's PD	0.14	-0.5	-1.2334	0.233365	Cooling
Evenness	0.0385	-0.283	-0.6676	0.10158	Cooling
Ses.pd	0.058	-0.531	-1.003	-0.05897	Cooling

Bayesian Hierarchical meta-analysis model
(priors: c(prior(normal(0,1), class = Intercept),
prior(cauchy(0,1), class = sd)); iter = 4000;
random factor: study**)**

Metric	Estimate	Est.Error	l-95% CI	u-95% CI	Group
Richness	-0.26	0.21	-0.68	0.15	Warming
Shannon	-0.31	0.18	-0.68	0.04	Warming
Faith's PD	-0.37	0.19	-0.76	0.01	Warming
Evenness	-0.26	0.15	-0.55	0.02	Warming
Ses.pd	-0.1	0.18	-0.46	0.25	Warming
Richness	-0.09	0.43	-0.9	0.8	Cooling
Shannon	-0.2	0.24	-0.7	0.27	Cooling
Faith's PD	-0.43	0.35	-1.15	0.26	Cooling
Evenness	-0.27	0.2	-0.69	0.12	Cooling
Ses.pd	-0.5	0.24	-0.99	-0.02	Cooling

Beta dispersion

Metric	Estimate	Est.Error	l-95% CI	u-95% CI	Group
Euclidean on PhILR	0.102	0.4809	-0.8405	1.0445	Warming
Unweighted Unifrac	0.0021	0.1169	-0.227	0.2312	Warming
Weighted Unifrac	-0.1598	0.172	-0.4968	0.1773	Warming
Aitchison	-0.4542	0.7087	-1.8432	0.9349	Warming
Euclidean on PhILR	0.1175	0.3652	-0.5983	0.8333	Cooling
Unweighted Unifrac	0.2815	0.4374	-0.5759	1.1388	Cooling
Weighted Unifrac	-0.0035	0.1737	-0.334	0.337	Cooling
Aitchison	1.5379	1.2456	-0.9034	3.9792	Cooling

Supplementary Table 3 Modelling details for moderator analysis.

Test for multicolinearity				
Alpha diversity effect sizes				
Moderators tested	**Type of moderators**	**Metric**	**GVIF^(1/(2*Df))>2(indicating multicollinearity)**	**Group**
Host thermal type + body site + host lifespan + habitat + region + Ramping + Collected_or_lab + immune_complexity	categorical	Richness	body site, host lifespan	Warming
		Shannon	body site, host lifespan	
		Faith's PD	host lifespan	
		Evenness	body site, host lifespan	
		Ses.pd	host lifespan	
		Richness	body site, host lifespan, immune complexity	Cooling
		Shannon		
		Faith's PD		
		Evenness		
		Ses.pd		
Beta-diversity effect sizes				
Moderators tested	**Type of moderators**	**Metric**	**GVIF^(1/(2*Df)) > 2 (indicating multicollinearity)**	**Group**
Host thermal type + body site + host lifespan + habitat + region + Ramping + Collected_or_lab + immune_complexity	categorical	Aitchison	host lifespan	Warming
		Euclidean on PhILR		
		Weighted Unifrac		
		Unweighted Unifrac		
		Aitchison	host lifespan, immune complexity, body site	Cooling
		Euclidean on PhILR		
		Weighted Unifrac		
		Unweighted Unifrac		

Beta-dispersion effect sizes

Moderators tested	Type of moderators	Metric	GVIF^(1/(2*Df)) > 2 (indicating multicollinearity)	Group
Host thermal type + body site + host lifespan + habitat + region + Ramping + Collected_or_lab + immune_complexity	categorical	Aitchison	host lifespan	Warming
		Euclidean on PhILR		
		Weighted Unifrac		
		Unweighted Unifrac		
		Aitchison	host lifespan, immune complexity, body site	Cooling
		Euclidean on PhILR		
		Weighted Unifrac		
		Unweighted Unifrac		

Host microbiome alpha diversity

Moderator	Type of moderator	Levels	Metric	estimate	se	pvalue	Group	random factor
sample size	continuous	NA	Richness	-0.0061	0.0094	0.5155	Warming	study
			Shannon	0.0036	0.0082	0.664		
			Faith's PD	-0.0067	0.0084	0.4268		
			Evenness	0.0053	0.0062	0.3941		
			Ses.pd	-0.0095	0.008	0.2361		
			Richness	-0.0078	0.0223	0.7247	Cooling	
			Shannon	-0.0007	0.0112	0.9494		
			Faith's PD	-0.0006	0.0176	0.9748		
			Evenness	0.0024	0.0092	0.791		
			Ses.pd	0.0088	0.0112	0.4328		
Host taxonomy (higher rank)	categorical	algae, amphibians,	Richness			0.8141	Warming	study
			Shannon			0.8155		

		Arthropoda, birds, corals, dinoflagellates, fishes, mammal, mollusca, plant, reptiles, sponges	Faith's PD	NS for all levels, so put the p-value from "Test of Moderators"	0.6797		
			Evenness		0.3358		
			Ses.pd		0.7597		
			Richness	only 1 level showed difference	0.2178	Cooling	
			Shannon	only 1 level showed difference	<.0001		
			Faith's PD	only 1 level showed difference	0.0018		
			Evenness	NS for all levels, so put the p-value from "Test of Moderators"	0.1086		
			Ses.pd	NS for all levels, so put the p-value from "Test of Moderators"	0.5866		
Host thermal type	categorical	ectotherms (ref), endotherms, plant	Richness	NS for all levels	0.8848	Warming	study
			Shannon		0.5907		
			Faith's PD		0.676		
			Evenness		0.4771		
			Ses.pd		0.7102		
			Richness		0.8968	Cooling	study, host_taxa, host habitat
			Shannon		0.3598		
			Faith's PD		0.8656		
			Evenness	two levels sig	0.0076		
			Ses.pd	NS for all levels	0.1171		
Host body site	categorical	internal, external, whole_body	Richness	NS for all levels	0.4077	Warming	study
			Shannon		0.8194		
			Faith's PD		0.7676		
			Evenness		0.8645		
			Ses.pd		0.2278		

			Richness	1 level sig		0.0004	Cooling	study, host_taxa, host habitat
			Shannon	NS for all levels		0.4287		
			Faith's PD	1 level sig		0.0003		
			Evenness	NS for all levels		0.5155		
			Ses.pd			0.7812		
Amplicon region	categorical	full length (ref), V1-V2, V1-V3, V3, V3-V4, V4, V4-V5, V5-V6	Richness	1 level sig		0.3191	Warming	study
			Shannon	NS for all levels		0.8524		
			Faith's PD	NS for all levels		0.7784		
			Evenness	1 level sig		0.0791		
			Ses.pd	NS for all levels		0.9026		
		V1-V3(ref), V3, V3-V4, V4	Richness	1 level sig		0.0069	Cooling	study, host_taxa
			Shannon	NS for all levels		0.192		
			Faith's PD	NS for all levels		0.8564		
			Evenness	1 level sig		0.0102		
			Ses.pd	NS for all levels		0.8941		
Host immune complexity	categorical	innate, adaptive (ref)	Richness	0.2867	0.3853	0.4568	Warming	study
			Shannon	-0.3028	0.3496	0.3864		
			Faith's PD	0.3399	0.3557	0.3394		
			Evenness	-0.3132	0.3233	0.3327		
			Ses.pd	0.2461	0.3495	0.4813		
			Richness	-0.7282	1.5965	0.6483	Cooling	study, host_taxa
			Shannon	-0.5914	0.9782	0.5455		
			Faith's PD	-0.8737	1.5366	0.5696		
			Evenness	-0.4194	0.5094	0.4103		
			Ses.pd	0.3122	0.5047	0.5362		
Host habitat	categorical	terrestrial, aquatic (ref)	Richness	0.0834	0.4266	0.845	Warming	study
			Shannon	-0.3717	0.3788	0.3265		
			Faith's PD	-0.0607	0.4079	0.8818		

			Evenness	-0.5425	0.3165	0.0865		
			Ses.pd	0.0124	0.3856	0.7478		
			Richness	2.3149	0.7715	0.0027	Cooling	study, host_taxa
			Shannon	1.128	0.3863	0.0035		
			Faith's PD	1.4845	0.5439	0.0063		
			Evenness	0.2185	0.4049	0.5894		
			Ses.pd	0.8263	0.466	0.0762		
Host native region	categorical	tropical, subtropical, temperate	Richness	NS for all levels, so put the p-value from "Test of Moderators"		0.7243	Warming	study
			Shannon			0.5721		
			Faith's PD			0.4505		
			Evenness			0.7196		
			Ses.pd			0.9754		
		subtropical (ref), temperate	Richness	-0.4542	0.4978	0.3616	Cooling	study, host_taxa, host habitat
			Shannon	-0.1505	0.3915	0.7007		
			Faith's PD	0.221	0.4799	0.6451		
			Evenness	0.0674	0.3045	0.8249		
			Ses.pd	0.0277	0.3792	0.9418		
Annual mean temperature	continuous	NA	Richness	0.0163	0.0253	0.52	Warming	study
			Shannon	0.0187	0.0206	0.3643		
			Faith's PD	0.0194	0.0235	0.4084		
			Evenness	0.0056	0.0165	0.7358		
			Ses.pd	0.0065	0.0245	0.7909		
			Richness	0.0354	0.0395	0.3694	Cooling	study, host_taxa, host habitat
			Shannon	0.0141	0.035	0.6875		
			Faith's PD	-0.0043	0.04	0.9137		
			Evenness	-0.0198	0.0223	0.3742		
			Ses.pd	-0.0176	0.0305	0.5632		
	continuous	NA	Richness	-0.0078	0.0168	0.6434	Warming	study

Annual temperature range			Shannon	0.0002	0.0133	0.9905		
			Faith's PD	-0.0103	0.0152	0.4999		
			Evenness	0.0033	0.0102	0.7425		
			Ses.pd	-0.0084	0.0156	0.5897		
			Richness	0.0188	0.0301	0.5315	Cooling	study, host_taxa, host habitat
			Shannon	0.0196	0.0233	0.402		
			Faith's PD	0.051	0.0288	0.0766		
			Evenness	0.022	0.0133	0.0995		
			Ses.pd	0.039	0.0176	0.0268 *		
Experimental exposure time	continuous	NA	Richness	0	0	0.4668	Warming	study
			Shannon	-0.0001	0	0.0262		
			Faith's PD	0	0	0.1061		
			Evenness	-0.0001	0	0.0363		
			Ses.pd	0	0	0.2104		
			Richness	-0.002	0.0007	0.0077	Cooling	study, host_taxa, host habitat
			Shannon	-0.0007	0.0004	0.135		
			Faith's PD	-0.0017	0.0006	0.0043		
			Evenness	-0.0001	0.0004	0.7564		
			Ses.pd	-0.0008	0.0004	0.0524		
Experimental temperature span	continuous	NA	Richness	-0.0596	0.0653	0.361	Warming	study
			Shannon	-0.035	0.0587	0.551		
			Faith's PD	-0.064	0.0603	0.2887		
			Evenness	-0.0373	0.0447	0.4038		
			Ses.pd	0.0326	0.0583	0.5757		
			Richness	0.0387	0.0829	0.6406	Cooling	study, host_taxa, host habitat
			Shannon	-0.0037	0.0324	0.9099		
			Faith's PD	-0.0106	0.0592	0.8574		
			Evenness	0.04	0.0348	0.2499		

			Ses.pd	-0.0239	0.0431	0.5793		
Experimental temperature change rate	categorical	ramping (ref), static	Richness	-0.9853	0.309	0.0014	Warming	study, exposure time
			Shannon	-0.6202	0.3302	0.0604		
			Faith's PD	-0.9846	0.3214	0.0022		
			Evenness	-0.1308	0.3085	0.6715		
			Ses.pd	-0.7184	0.361	0.0466		
			Richness	-3.2633	1.0185	0.0014	Cooling	study, host_taxa, host habitat, experimental time
			Shannon	-0.5978	0.557	0.2832		
			Faith's PD	-0.9121	1.7366	0.5995		
			Evenness	0.6871	0.3785	0.0695		
			Ses.pd	0.3517	0.58	0.5442		
Prior experimental lab acclimation	categorical	short (newly collected host and maintained in lab for a few days, ref), long (acclimated in lab for years)	Richness	0.9284	0.3193	0.0036	Warming	study, exposure time
			Shannon	0.0457	0.2364	0.8467		
			Faith's PD	0.3781	0.3517	0.2823		
			Evenness	-0.0949	0.2192	0.665		
			Ses.pd	0.0063	0.4976	0.9899		
			Richness	0.6922	1.1924	0.5616	Cooling	study, host_taxa, host habitat, exposure_time
			Shannon	-0.0874	0.4264	0.8376		
			Faith's PD	-0.1095	0.327	0.7377		
			Evenness	-0.22	0.3636	0.5451		
			Ses.pd	-0.3125	0.5568	0.5746		
Host lifespan	categorical	month (ref), year	Richness	-1.0314	0.5963	0.0837	Warming	study, exposure time
			Shannon	0.1585	0.4757	0.7391		
			Faith's PD	-0.2926	0.6449	0.6501		
			Evenness	0.4057	0.4316	0.3472		
			Ses.pd	0.6885	0.539	0.2015		
		day (day), month, year	Richness	NS for all levels, so		0.1157	Cooling	
			Shannon			0.8826		

			Faith's PD Evenness Ses.pd	put the p-value from "Test of Moderators"		0.0025 0.377 0.3005		study, host_taxa, host habitat, exposure_time
Host microbiome beta dispersion								
sample size	continuous	NA	Aitchison	-0.0083	0.0391	0.832	Warming	study
			Euclidean on PhILR	0.0032	0.021	0.8795		
			Weighted Unifrac	-1.2396	0.0047	<.0001		
			Unweighted Unifrac	-2.9697	0.0074	<.0001		
			Aitchison	-0.0666	0.0631	0.2918	Cooling	
			Euclidean on PhILR	-0.011	0.0239	0.6468		
			Weighted Unifrac	0.06	0.0072	<.0001		
			Unweighted Unifrac	0.0173	0.0059	0.0033		
host taxonomy (higher rank)	categorical	algae, amphibians, Arthropoda, birds, corals, dinoflagellates, fishes, mammal, mollusca, plant, reptiles, sponges	Aitchison	NS for all levels, so put the p-value from "Test of Moderators"		0.9929	Warming	study
			Euclidean on PhILR			0.8837		
			Weighted Unifrac			0.491		
			Unweighted Unifrac			0.0715		
			Aitchison			0.2721	Cooling	

		amphibians (ref), Arthropoda, fishes, mammal, mollusca, plant, rotifers	Euclidean on PhILR	NS for all levels, so put the p-value from "Test of Moderators"	0.5673		
			Weighted Unifrac	rotifer different from ref	0.0007		
			Unweighted Unifrac		< .0001		
host thermal type	categorical	ectotherms, endotherms, plant	Aitchison	NS for all levels	0.9439	Warming	study
			Euclidean on PhILR		0.9831		
			Weighted Unifrac		0.6616		study, sample size
			Unweighted Unifrac		0.9963		
			Aitchison		0.9247	Cooling	study
			Euclidean on PhILR		0.9601		
			Weighted Unifrac		0.7865		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac		0.8029		
Host body site	categorical	internal, external, whole body	Aitchison	NS for all levels	0.9234	Warming	study
			Euclidean on PhILR		0.7175		
			Weighted Unifrac		0.5035		study, sample size
			Unweighted Unifrac		0.9821		
			Aitchison		0.6771	Cooling	study
			Euclidean on PhILR		0.8538		
			Weighted Unifrac		0.0501		study, sample size, host

			Unweighted Unifrac			0.6007		taxanomy (higher rank)
Amplicon region	categorical	full length (ref), V1-V2, V1-V3, V3, V3-V4, V4, V4-V5, V5-V6	Aitchison	NS for all levels		0.9916	Warming	study
			Euclidean on PhILR			0.0006		
			Weighted Unifrac			0.6969		study, sample size
			Unweighted Unifrac			0.5913		
		V1-V3(ref), V3, V3-V4, V4	Aitchison	1 level sig		0.0006	Cooling	study
			Euclidean on PhILR			0.2962		
			Weighted Unifrac	NS for all levels		0.4405		study, sample size, host
			Unweighted Unifrac			0.461		taxanomy (higher rank)
host immune complexity	categorical	innate, adaptive (ref)	Aitchison	-0.1775	1.5356	0.908	Warming	study
			Euclidean on PhILR	1.2995	0.9271	0.161		
			Weighted Unifrac	-0.0491	0.4051	0.9035		study, sample size
			Unweighted Unifrac	0.3969	0.6935	0.5671		
			Aitchison	3.2686	2.4259	0.1779	Cooling	study
			Euclidean on PhILR	0.9504	0.6659	0.1535		
			Weighted Unifrac	0.7837	0.5386	0.1456		study, sample size, host
			Unweighted Unifrac	1.9627	1.4419	0.1734		taxanomy (higher rank)
host habitat	categorical		Aitchison	0.9415	1.5346	0.5395	Warming	study

		terrestrial, aquatic (ref)	Euclidean on PhILR	0.179	0.9666	0.8531		
			Weighted Unifrac	-0.4156	0.4268	0.3301		study, sample size
			Unweighted Unifrac	-0.018	0.7547	0.981		
			Aitchison	2.4686	2.5705	0.3369	Cooling	study
			Euclidean on PhILR	0.9255	0.6778	0.1721		
			Weighted Unifrac	0.2411	0.3187	0.4492		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	0.3982	0.7835	0.6113		
host native region	categorical	tropical, subtropical (ref), temperate	Aitchison	NS for all levels, so put the p-value from "Test of Moderators"		0.1498	Warming	study
			Euclidean on PhILR			0.8741		
			Weighted Unifrac	both temperate and tropical different from subtropical		<.0001		study, sample size
			Unweighted Unifrac	tropical differnet from subtropical		<.0001		
		subtropical (ref), temperate	Aitchison	NS for all levels, so put the p-value from "Test of Moderators"		0.06	Cooling	study
			Euclidean on PhILR			0.2595		
			Weighted Unifrac	0.86	0.052	<.0001		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	0.187	0.0223	<.0001		
Annual mean temperature	continuous	NA	Aitchison	-0.0347	0.1058	0.7427	Warming	study
			Euclidean on PhILR	-0.0461	0.0758	0.5429		
			Weighted Unifrac	-0.1205	0.0023	<.0001		study, sample size

			Unweighted Unifrac	0.0425	0.0058	<.0001		
			Aitchison	0.0606	0.1993	0.7612	Cooling	study
			Euclidean on PhILR	0.0104	0.0814	0.8986		
			Weighted Unifrac	0.0457	0.0009	<.0001		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	-0.0473	0.0017	<.0001		
Annual temperature range	continuous	NA	Aitchison	0.0175	0.0682	0.7978	Warming	study
			Euclidean on PhILR	-0.0021	0.0517	0.9678		
			Weighted Unifrac	0.0975	0.0014	<.0001		study, sample size
			Unweighted Unifrac	-0.0263	0.004	<.0001		
			Aitchison	-0.1534	0.1267	0.2259	Cooling	study
			Euclidean on PhILR	-0.0831	0.0634	0.1897		
			Weighted Unifrac	-0.0656	0.0026	<.0001		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	0.0162	0.001	<.0001		
experimental exposure time	continuous	NA	Aitchison	0	0.0001	0.7852	Warming	study
			Euclidean on PhILR	0	0.0001	0.6721		
			Weighted Unifrac	0	0	0.3107		study, sample size
			Unweighted Unifrac	0	0	0.9024		
			Aitchison	-0.0009	0.0021	0.6707	Cooling	study
			Euclidean on PhILR	0	0.0015	0.9743		

			Weighted Unifrac	0.0006	0.0002	0.0004		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	0.0004	0.0009	0.6569		
experimental temperature span	continuous	NA	Aitchison	0.0004	0.2552	0.9988	Warming	study
			Euclidean on PhILR	-0.038	0.1331	0.7751		
			Weighted Unifrac	0.0215	0.0575	0.7083		study, sample size
			Unweighted Unifrac	-0.0216	0.0391	0.5801		
			Aitchison	-0.0299	0.2418	0.9015	Cooling	study
			Euclidean on PhILR	-0.0671	0.1031	0.5154		
			Weighted Unifrac	-0.0429	0.0254	0.0913		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	-0.0532	0.0671	0.4279		
experimental temperature change rate	categorical	ramping (ref), static	Aitchison	-2.4061	1.2819	0.0605	Warming	study
			Euclidean on PhILR	-1.6806	0.8643	0.0518		
			Weighted Unifrac	-0.4116	0.4098	0.3153		study, sample size
			Unweighted Unifrac	-0.1729	0.72	0.8103		
			Aitchison	-4.7917	3.076	0.1193	Cooling	study
			Euclidean on PhILR	-0.32	0.8485	0.7061		
			Weighted Unifrac	0.1565	0.4273	0.7141		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	-1.3496	0.832	0.1048		
	categorical		Aitchison	0.4773	0.9921	0.6304	Warming	study

prior experimental lab acclimation		short (newly collected host and maintained in lab for a few days, ref), long (acclimated in lab for years)	Euclidean on PhILR	2.7059	0.6954	<.0001		
			Weighted Unifrac	0.6426	0.5109	0.2085		study, sample size
			Unweighted Unifrac	0.4991	1.0762	0.6428		
			Aitchison	4.18	3.9715	0.2926	Cooling	study
			Euclidean on PhILR	0.5254	1.5596	0.7362		
			Weighted Unifrac	0.4612	0.9601	0.631		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	1.9377	2.4949	0.4374		
Host lifespan	categorical	month (ref), year	Aitchison	-1.5196	1.648	0.3565	Warming	study
			Euclidean on PhILR	-2.6455	0.549	<.0001		
			Weighted Unifrac	0.6796	0.6717	0.3117		study, sample size
			Unweighted Unifrac	-0.5176	1.2729	0.6843		
		day (day), month, year	Aitchison	NS for all levels, so put the p-value from "Test of Moderators"		0.1956	Cooling	study
			Euclidean on PhILR	NS for all levels, so put the p-value from "Test of Moderators"		0.1359		
			Weighted Unifrac	all levels different from ref		<.0001		study, sample size, host taxanomy (higher rank)
			Unweighted Unifrac	all levels different from ref		<.0001		

Host microbiome beta diversity (no variance data for the ES, using lmer rather than rma.mv for multilevel models)

Moderator	**Type of moderator**	**Levels**	**Metric**	**estimate**	**se**	**tvalue**	**Group**	**random factor**
sample size	continuous	NA	Aitchison	0.0004641	0.0008297	0.559	Warming	study

			Euclidean on PhILR	-0.00102	0.001367	-0.746		
			Weighted Unifrac	-0.0004764	0.0015604	-0.305		
			Unweighted Unifrac	0.0005002	0.0008994	0.556		
			Aitchison	-0.0005044	0.001146	-0.44	Cooling	
			Euclidean on PhILR	-0.002213	0.002036	-1.087		
			Weighted Unifrac	-0.003881	0.003383	-1.147		
			Unweighted Unifrac	-0.0009867	0.0019888	-0.496		
host taxonomy (higher rank)	categorical	algae, amphibians, Arthropoda, birds, corals, dinoflagellates, fishes, mammal, mollusca, plant, reptiles, sponges	Aitchison	NS for all levels, F=0.7876		(-0.076,1.943)	Warming	study
			Euclidean on PhILR	NS for all levels, F=1.5888		(-0.533,1.555)		
			Weighted Unifrac	NS for all levels, F=1.0088		(-0.061,2.084)		
			Unweighted Unifrac	NS for all levels, F=0.6736		(-0.08,1.92)		
		amphibians (ref), Arthropoda, fishes, mammal, mollusca, plant, rotifers	Aitchison	NS for all levels, F=0.6793		(-0.529,1.46)	Cooling	
			Euclidean on PhILR	NS for all levels, F=1.085		(-0.246,1.137)		
			Weighted Unifrac	NS for all levels, F=0.302		(-0.043,1.045)		

			Unweighted Unifrac	NS for all levels, F=0.4998	(-0.202,1.303)		
					pvalue		
host thermal type	categorical	ectotherms, endotherms, plant	Aitchison	NS for all levels	>0.0591	Warming	study
			Euclidean on PhILR		>0.254		
			Weighted Unifrac	1 level sig	plant:es=0.32521,se=0.15804, pval=0.0455		
			Unweighted Unifrac	NS for all levels	>0.306		
			Aitchison		>0.97924	Cooling	
			Euclidean on PhILR	1 level sig	plant:es=0.15123,se=0.06508, pval=0.0271		
			Weighted Unifrac	NS for all levels	>0.7701		
			Unweighted Unifrac		>0.62		
Host body site	categorical	internal, external, whole body	Aitchison	NS for all levels	>0.84768	Warming	study
			Euclidean on PhILR		>0.33894		
			Weighted Unifrac		>0.58994		
			Unweighted Unifrac		>0.3254		
			Aitchison		>0.606	Cooling	
			Euclidean on PhILR		>0.939		

			Weighted Unifrac			>0.311		
			Unweighted Unifrac			>0.676		
Amplicon region	categorical	full length (ref), V1-V2, V1-V3, V3, V3-V4, V4, V4-V5, V5-V6	Aitchison	NS for all levels		>0.377	Warming	study
			Euclidean on PhILR			>0.515		
			Weighted Unifrac			>0.507		
			Unweighted Unifrac			>0.534		
		V1-V3 (ref), V3, V3-V4, V4	Aitchison			>0.1537	Cooling	
			Euclidean on PhILR			>0.414		
			Weighted Unifrac	V1-V3 es>0		0.00054		
			Unweighted Unifrac	V1-V3 es>0		0.000162		
host immune complexity	categorical	innate, adaptive (ref)	Aitchison	0.03529	0.03087	1.143	Warming	study
			Euclidean on PhILR	0.03581	0.05146	0.696		
			Weighted Unifrac	0.06185	0.05642	1.096		
			Unweighted Unifrac	0.02873	0.03346	0.858		
			Aitchison	0.04215	0.04081	1.033	Cooling	
			Euclidean on PhILR	0.09353	0.06549	1.428		
			Weighted Unifrac	0.1195	0.1377	0.868		
			Unweighted Unifrac	0.07999	0.07674	1.042		
host habitat	categorical		Aitchison	-0.01995	0.03293	-0.606	Warming	study

		terrestrial, aquatic (ref)	Euclidean on PhILR	-0.03813	0.05483	-0.695		
			Weighted Unifrac	0.009184	0.059936	0.153		
			Unweighted Unifrac	-0.01742	0.03545	-0.491		
			Aitchison	-0.01692	0.04585	-0.369	Cooling	
			Euclidean on PhILR	0.06397	0.07359	0.869		
			Weighted Unifrac	0.1904	0.1356	1.404		
			Unweighted Unifrac	-0.0006126	0.0839674	-0.007		
host native region	categorical	tropical, subtropical (ref), temperate	Aitchison	NS for all levels		(-1.563,-1.613)	Warming	study
			Euclidean on PhILR			(-1.119,-0.56)		
			Weighted Unifrac			(-0.501,-0.149)		
			Unweighted Unifrac			(-1.801,-1.705)		
		subtropical (ref), temperate	Aitchison	0.01861	0.04669	0.399	Cooling	
			Euclidean on PhILR	0.0987	0.08498	1.161		
			Weighted Unifrac	0.184	0.1065	1.727		
			Unweighted Unifrac	0.02211	0.06837	0.323		
Annual mean temperature	continuous	NA	Aitchison	-0.0005002	0.0022271	0.8237	Warming	study
			Euclidean on PhILR	0.001054	0.003701	0.7779		
			Weighted Unifrac	-0.001685	0.004363	0.7019		

			Unweighted Unifrac	-0.0009338	0.0024879	0.7098		
			Aitchison	0.002741	0.003616	0.458	Cooling	
			Euclidean on PhILR	-0.0007988	0.0067617	0.908		
			Weighted Unifrac	-0.001935	0.008783	0.828		
			Unweighted Unifrac	0.004731	0.005153	0.369		
Annual temperature range	continuous	NA	Aitchison	6.52E-04	1.40E-03	0.6446	Warming	study
			Euclidean on PhILR	5.08E-04	2.35E-03	0.82996		
			Weighted Unifrac	0.001389	0.002735	0.615		
			Unweighted Unifrac	4.56E-04	1.56E-03	0.7724		
			Aitchison	-0.003523	0.002064	0.10551	Cooling	
			Euclidean on PhILR	-0.001439	0.004207	0.736		
			Weighted Unifrac	-0.003221	0.005426	0.5589		
			Unweighted Unifrac	-0.005641	0.003096	0.08214		
experimental exposure time	continuous	NA	Aitchison	4.72E-06	1.48E-06	3.186	Warming	study
			Euclidean on PhILR	5.59E-06	2.61E-06	2.146		
			Weighted Unifrac	1.13E-05	2.54E-06	4.424		
			Unweighted Unifrac	2.69E-06	1.77E-06	1.526		
			Aitchison	5.16E-05	3.48E-05	1.484	Cooling	
			Euclidean on PhILR	1.98E-05	6.72E-05	0.294		

			Weighted Unifrac	-1.37E-05	1.21E-04	-0.113		
			Unweighted Unifrac	4.28E-05	6.69E-05	0.64		
experimental temperature span	continuous	NA	Aitchison	0.003369	0.005465	0.617	Warming	study
			Euclidean on PhILR	0.005935	0.009052	0.656		
			Weighted Unifrac	-0.002865	0.010495	-0.273		
			Unweighted Unifrac	0.005527	0.005917	0.934		
			Aitchison	-0.000673	0.003732	-0.18	Cooling	
			Euclidean on PhILR	0.005276	0.006729	0.784		
			Weighted Unifrac	-0.001744	0.011593	-0.15		
			Unweighted Unifrac	-0.001838	0.006558	-0.28		
experimental temperature change rate	categorical	ramping (ref), static	Aitchison	0.07528	0.0305	2.468	Warming	study
			Euclidean on PhILR	0.04056	0.05285	0.767		
			Weighted Unifrac	0.0776	0.06258	1.24		
			Unweighted Unifrac	0.05449	0.03537	1.54		
			Aitchison	0.01494	0.05783	0.258	Cooling	
			Euclidean on PhILR	0.12955	0.08678	1.493		
			Weighted Unifrac	-0.2289	0.1942	-1.179		
			Unweighted Unifrac	-0.1668	0.1134	-1.47		
	categorical		Aitchison	0.02523	0.03781	0.667	Warming	study

prior experimental lab acclimation		short (newly collected host and maintained in lab for a few days, ref), long (acclimated in lab for years)	Euclidean on PhILR	0.003796	0.070004	0.054		
			Weighted Unifrac	0.03384	0.07312	0.463		
			Unweighted Unifrac	0.02849	0.03665	0.777		
			Aitchison	-0.00647	0.07161	-0.09	Cooling	
			Euclidean on PhILR	0.01312	0.15105	0.087		
			Weighted Unifrac	0.14571	0.22597	0.645		
			Unweighted Unifrac	0.03795	0.14217	0.267		
Host lifespan	categorical	month (ref), year	Aitchison	0.01096	0.04983	0.22	Warming	study
			Euclidean on PhILR	0.05139	0.08161	0.63		
			Weighted Unifrac	-0.04633	0.09539	-0.486		
			Unweighted Unifrac	-0.002663	0.054654	-0.049		
		day (day), month, year	Aitchison	NS for both levels		(-0.171,0.039)	Cooling	
			Euclidean on PhILR	NS for both levels		(0.224,0.578)		
			Weighted Unifrac	NS for both levels		(0.234,0.907)		
			Unweighted Unifrac	NS for both levels		(0.529,0.661)		

Chapter 3: Dual stressors of infection and warming can destabilize host microbiomes

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Dual stressors of infection and warming can destabilize host microbiomes

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Abstract

Climate change is causing extreme heating events and intensifying infectious disease outbreaks. Animals harbour microbial communities, which are vital for their survival and fitness under stressful conditions. Understanding how microbiome structures change in response to infection and warming may be important for forecasting host performance under global change. Here, we evaluated alterations in the microbiomes of several wild *Caenorhabditis elegans* isolates spanning a range of latitudes, upon warming temperatures and infection by the parasite *Leucobacter musarum*. Using 16S rRNA sequencing, we found that microbiome diversity decreased, and dispersion increased over time, with the former being more prominent in uninfected adults and the latter aggravated by infection. Infection reduced dominance of specific microbial taxa, and increased microbiome dispersion, indicating destabilizing effects on host microbial communities. Exposing infected hosts to warming did not have an additive destabilizing effect on their microbiomes. Moreover, warming during pre-adult development alleviated the destabilizing effects of infection on host microbiomes. These results revealed an opposing interaction between biotic and abiotic factors on microbiome structure. Lastly, we showed that increased microbiome dispersion might be associated with decreased variability in microbial species interaction strength. Overall, these findings improve our understanding of animal microbiome dynamics amidst climate change and epidemics.

Introduction

Global climate change has led to multiple climate hazards including more extreme temperatures, resulting in population decline and biodiversity loss (Alessandro et al. 2018). Shifting global temperatures is also changing the geographic distribution of infectious diseases, leading to intensified disease outbreaks (Jones et al. 2008; Liang & Gong 2017). As temperatures increase, hosts and parasites are experiencing shifts in their thermal environment, driving variation in disease outcomes (Hector et al. 2023; Mora et al. 2022). Thus, projections of species persistence in the changing world will need to account for threats posed by both warming and infection, as well as the interaction of these dual stressors (Lafferty & Mordecai 2016; Maynard et al. 2015).

Accumulating evidence shows that host health in the face of changing temperatures can be mediated by host microbiomes (Henry et al. 2021; Hector et al. 2022), as well as parasite infection (Ford & King 2016; Stevens et al. 2021). Microbiomes are highly sensitive to biotic or abiotic disturbances (Jani & Briggs 2014; Muletz-Wolz et al. 2019; Ribas et al. 2023). Changes in microbiome structure and stability as a result are increasingly recognized as meaningful indicators to altered host health (Jani & Briggs 2014; Muletz-Wolz et al. 2019; Hill et al. 2012). Studying microbiome dynamics under warming and infection scenarios provides important predictions on species persistence under climate change and infectious diseases (Jani & Briggs 2014; Ribas et al. 2023; Astudillo-García et al. 2019).

Temperature and parasite infection can both disrupt host microbiome structure (Muletz-Wolz et al. 2019; Huus & Ley 2021; Li et al. 2023; Chang et al. 2008; Maji et al. 2018; Luo et al. 2017; Wei et al. 2017; Jani & Briggs 2014). Across animal species, experimental warming has been shown to decrease host microbiome phylogenetic diversity and alter microbiome composition (Li et al. 2023). The effects of temperature on host microbiota can vary depending on local

environmental conditions. Hosts adapted to more variable thermal conditions can experience less loss of microbiome diversity under thermal stress (Li et al. 2023). Whilst infection can alter microbiome diversity, the direction of change varies across host and parasite species. For example, *Clostridioides difficile* infection in the human gut can decrease microbiome diversity (Chang et al. 2008), while *Mycobacterium tuberculosis* infection has the opposite effect (Maji et al. 2018; Luo et al. 2017). Higher temperatures (prior to *Batrachochytrium dendrobatidis* exposure) and infection individually decreased skin microbiome richness on red-backed salamanders (*Plethodon*) (Muletz-Wolz et al. 2019). The extent to which warm temperatures and infection might interact to structure host microbiomes more extensively is unclear.

Here, we explored the separate and combined effects of infection and warming (i.e., at different time points across host lifespan) on host microbiome structure and stability. We used *Caenorhabditis elegans* nematodes, representative species of their natural gut microbiome (CeMbio community isolated from temperate nematodes (Dirksen et al. 2020)), and a natural parasite of *Caenorhabditis* spp., *Leucobacter musarum* (Hodgkin et al. 2013). Use of *C. elegans* with a consortium of culturable bacterial associates enabled us to explore metabolic interactions with the parasite. We included a diversity of wild nematode isolates across a range of latitudes to understand the impact of habitat adaptation on these relationships. The fitness levels of *C. elegans* isolates can be dependent on thermal preferences (Anderson et al. 2011), with phenotypes exhibited varying across temperatures and life stages (Maulana et al. 2022). We predicted that warming and infection might individually destabilize host microbiomes, with the extent of disruption dependent on the timing of warming. Destabilization induced by warming and infection could be characterized by increased interindividual variability in microbiome structure (or dispersion). Such a pattern could indicate a loss of host ability to regulate community composition in an otherwise stable state (Zaneveld et al. 2017; Ma 2020; Lesser et al. 2016). We used 16S rRNA sequencing and metabarcoding analysis, to measure

changes in microbiome diversity and dispersion. We established microbial species co-occurrence networks to assess the strength and direction of species associations at different times when warming occurred. We reconstructed species-level genome-scale metabolic models using whole-genome sequences to explore the potential metabolic interactions between microbiome species and the parasite. Overall, we found that host microbiomes were destabilized non-additively by the stressors. The timing of warming and degree of lab adaptation (lab vs. wild isolates) played a role on microbiome responses to both stressors. These results highlight the dynamic nature of host microbiomes in a more thermally variable and infectious world.

Materials and Methods

Nematode, bacterial strains and maintenance

We used the lab-adapted N2 and eight wild *C. elegans* isolates (obtained from the Caenorhabditis Genetics Centre; CGC, Minnesota, USA) originally collected across a range of latitudes (See Supplementary Table 1 for list of isolates and their locations of origins). N2 has been commonly used in biological research since its introduction to the research community by Sydney Brenner in 1974 (Brenner 1974). The wild isolates were chosen from a range of latitudes at similar elevations. At the start of all experiments, *C. elegans* isolates were thawed from frozen stocks and maintained at 20˚C, according to a standard maintenance protocol using nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 as food (Stiernagle 2006). OP50 was grown at 30˚C overnight in Luria-Bertani (LB) broth, with 100 µl of culture then spread onto each NGM plate and incubated at 30˚C overnight. Worm populations were synchronized and made sterile by bleaching (Stiernagle 2006).

For host microbiome colonisation, we used a community of 12 bacterial isolates found naturally associated with *C. elegans* (CeMbio kit) (Dirksen et al. 2020). The CeMbio community is a

simplified natural microbiome derived from a meta-analysis on wild *C. elegans* (Zhang et al. 2017). Each species is readily culturable, with its full genome sequenced (Dirksen et al. 2020). CeMbio strains can colonize the worm gut individually or comprise a robust community during host development and potentially affect nematode life-history (Dirksen et al. 2020). Each CeMbio strain was grown individually in LB broth for 24-48h at 25˚C. Cultures were then standardised to an optical density (OD, 600nm) of 1 for consistent doses within and across experiments. The community inoculum was prepared by mixing equal volumes of each bacterial strain. Microbiome exposure plates were prepared by spreading 400 µl of the mixture inoculum onto 9 cm NGM plates. For comparison, the OP50 feeding plates were prepared by spreading the same amount of culture (standardized to the same OD as microbiome cultures). We ensured individual CeMbio strains could colonize worm gut during host development. We exposed *C. elegans* to each CeMbio strain (the same dose as in the community mixture), collected and crushed pre-adult worms for DNA extraction, and performed standard PCR using both general and species-specific primers to detect the presence of individual strain.

We exposed nematodes to *Leucobacter musarum* sp. nov. subsp. *musarum* subsp. nov strain CBX152T (*L. musarum*), a highly virulent parasite isolated from *Caenorhabditis tropicalis* in Cape Verde (Andrews 2010). *L. musarum* causes severe rectal disease and ultimately death in *C. elegans* (Andrews 2010). Other species of this genus have been found naturally infecting *C. elegans* (Bates & King 2021; Bates et al. 2021). For parasite exposures, *L. musarum* was grown in LB broth at 30˚C overnight, and the culture was standardized to OD(600) 0.3. The infection exposure plates were prepared by streaking 74 µl of inoculum containing 20% *L. musarum* and 80% OP50 (standardized to OD(600) 1) onto 5.5 cm NGM plates and incubating at 25˚C for 24h (Bates et al. 2021). Control plates were prepared by spreading a similar amount of OP50 culture.

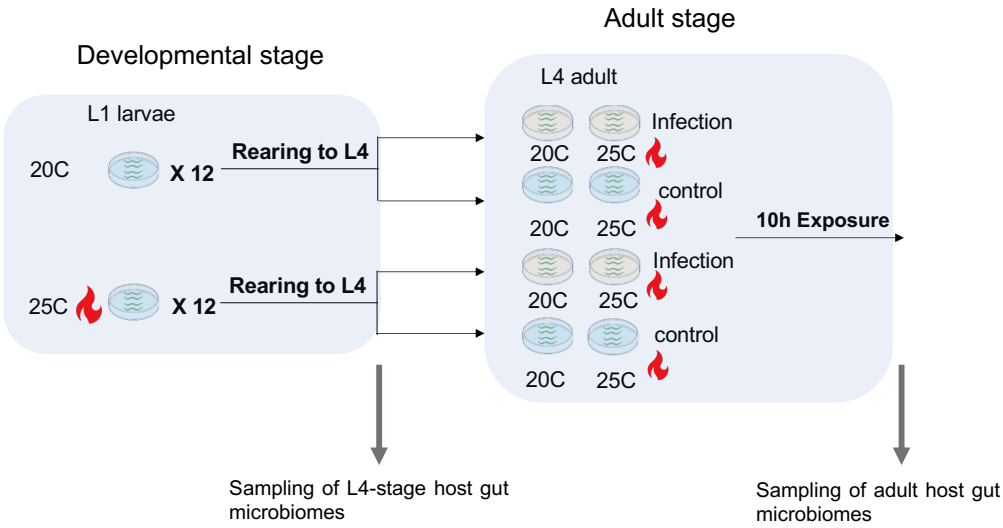

Fig. 1. Schematic of experimental microbiome sampling. In brief, L1 stage lab-adapted N2 worms or wild worm isolates were grown on microbiomes at 20˚C or 25˚C until L4 stage. L4 worms were exposed to parasites (or not) at 20˚C or 25˚C. In total, four temperature regimes were used: 20˚C-20˚C (ambient temperatures during development and adulthood), 20˚C-25˚C, 25˚C-20˚C, and 25˚C-25˚C Host gut microbiomes were sampled pre-adulthood (from L4 young adults) and at adulthood.

Sampling of *C. elegans* gut microbiomes

We manipulated temperature during the worm developmental period (from L1 to L4 young adults) and subsequent parasite exposure (Fig. 1). We used the ambient temperature of 20˚C and a warmer temperature of 25˚C. This higher temperature causes mild heat stress for temperate *C. elegans* isolates with the potential to shorten lifespan and reduce reproductive output (Xiao et al. 2013; Gouvêa et al. 2015).

To study host microbiome dynamics under infection and warming, approx. 1000 L1 nematodes were grown on OP50 or microbiomes feeding plates at either 20˚C or 25˚C for ~48h or ~34h, until they reached L4 (worms develop faster at higher temperatures). L4 young adults were transferred to infection (20% *L. musarum* and 80% OP50) or control (OP50 only) plates, and left for 24h at either 20˚C or 25˚C. Each treatment was replicated six times. Worm populations were sampled before they were transferred to infection plates (labelled “pre-adult”) and after 10h (labelled “adult”) under all temperature treatments. We sampled worm microbiomes at 10h post-parasite exposure to ensure that host microbiomes were sampled from mostly live,

infected hosts (Bates et al. 2021). To collect host gut microbiomes, we washed worms off the NGM plate using M9 buffer. Approx. 700 worms were harvested from each replicate and crushed using the QIAGEN TissueLyser II for 5 min to release gut microbiomes. We collected microbiomes from 60 N2 worm samples and 80 wild isolate samples. Collected bacterial samples were immediately frozen at -20˚C.

Amplicon sequencing and data processing

DNA was extracted from frozen bacterial samples using the ZymoBIOMICS DNA Miniprep kit (Zymo) according to manufacturer's instructions. DNA extractions were conducted in a random order to avoid batch effects on downstream microbiome data. We also extracted DNA from the frozen stock of in-vitro CeMbio community culture that was used for host exposure, as a reference community, to compare with host gut microbiomes. The V3-V4 regions of bacterial 16S rRNA were amplified using the universal primer pair 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R primer (5'-GACTACHVGGGTATCTAATCC-3'). PCR amplicons were sequenced on the Illumina Miseq platform using 2 x 300bp v3 chemistry (Integrated Microbiome Resources, Canada (Comeau et al. 2017)).

FastQC (Andrews 2010) and MultiQC (Ewels et al. 2016) were used for initial visualization of read quality, primers were removed using Cutadapt (Martin 2011). Paired-end reads were joined using vsearch (Rognes et al. 2016). All low-quality reads were then filtered using default quality thresholds before starting the Deblur (Amir et al. 2017) workflow to denoise and classify sequences into ASVs. Trimming length was determined as 400 bp after manually viewing the quality plot. As full-length 16S rRNA sequences for CeMbio strains were well-characterized (Dirksen et al. 2020), we processed the obtained sequencing reads through the closed-reference OTU picking pipeline in QIIME2 (Bolyen et al. 2019). To build the reference, we downloaded full-length 16S sequences for all CeMbio strains and converted sequences to qza formatted reference files for processing by QIIME2. Taxonomy of the resolved ASVs was

assigned by clustering ASVs to the customized reference with 99% similarity thresholds.

Microbiome diversity and compositional analysis

To account for the different number of reads in each sample, we used a normalization method called scaling with ranked subsampling (SRS) (Beule & Karlovsky 2020). This method can better preserve the original microbial community structure and minimize subsampling errors compared with the rarefy approach. After normalization, five samples from the wild isolates assay were discarded because of low sequencing depth. Based on SRS-normalized data, four alpha diversity indices -Shannon's index, Richness, Evenness, and Faith's phylogenetic distance (Faith's PD) - were computed using the R package phyloseq (McMurdie & Holmes 2013). These four alpha diversity metrics individually quantified a different aspect of community diversity (Colwell 2009). Richness is the observed number of different species in the microbial community, which does not consider species abundance. Evenness measures the equity in species abundance in the community, where bigger values represent more evenly distributed species abundance. Shannon's index quantifies the uncertainty in predicting the species identity of an individual taken randomly from the community, which considers both species richness and evenness. Faith's PD is the only metric accounting for species phylogenetic distance in the community. It is measured as the sum of branch lengths between the observed species on a phylogenetic tree.

To quantify the dominance level of microbial communities, three different dominance indices – Berger-Parker, McNaughton's, and Simpson's indices – were calculated using the R package mia (Ernst et al. 2023). The Berger-Parker index is the relative abundance of the most abundant species in the community, and McNaughton's index is the sum of relative abundances of the two most abundant species in the community. Simpson's index is the probability that two randomly chosen species are the same. All three range from 0 to 1, where bigger values represent greater dominance.

For beta diversity, community distance matrices of unweighted and weighted UniFrac, Bray-Curtis and Aitchison dissimilarity were calculated based on either srs-normalized relative abundance or srs-normalized read counts, using the R package phyloseq. Different beta diversity metrics can lead to variable study power, thus consistency across multiple metrics indicates strong and more reliable patterns (Kers & Saccenti 2022). Permutational analysis of variance (PERMANOVA) was conducted with 9999 replications on each distance metric to evaluate differences in the microbiome structure and composition between treatments using the R package vegan (Dixon 2003). To test microbial stability and dispersion at a multivariate level, for each beta diversity metric, we calculated pairwise distance of samples, representing the distance of two samples within the same treatment group. Sets of values were compared between treatment groups using Wilcoxon Rank-sum test.

Bacterial co-occurrence analysis

Microbial co-occurrence networks are commonly built from species abundance data. They are widely applied to explore interactions and co-existence of bacterial species (Ma et al. 2020). In the co-occurrence network, nodes are the bacterial species, and links between nodes usually represent significant species associations (Ma et al. 2020). Links can have different weights which indicate varying association strength. Positive links (i.e. higher abundance of bacterial A associated with higher abundance of bacterial B) indicate facilitative interactions, and negative links (i.e. higher abundance of bacterial A associated with lower abundance of bacterial B) indicate competitive interactions (Ma et al. 2020).

We established microbial co-occurrence networks on the species level for different treatment groups using SparCC (Friedman & Alm 2012) program wrapped in SpiecEasi R package (Kurtz et al. 2015). This program is robust to any distribution of community abundances. One-hundred bootstrap replicates were used to calculate significant levels, the threshold for SparCC

correlation matrix was set at 0.3 and spurious links (absolute correlation coefficient < 0.3) were removed. Links with p-values < .05 were considered significant correlations. The number of positive and negative links were summarized for each treatment group.

Test for differentially abundant species

We performed differential species analysis using ALDEx2 (a clr based method) (Fernandes et al. 2014). Non-parametric Wilcoxon rank-sum test was conducted on each of the species between treatment groups using aldex.ttest in R. Species with a Benjamini & Hochberg (BH) adjusted p-value < 0.05 were considered to be differently enriched between groups. The expected value of group distribution difference (median log2 difference) and pooled group variance (median log2 dispersion) were calculated using the aldex.effect function. So was the standardized effect size on species abundance difference between groups. Species with an effect-size of > 0.3 were considered to have a large difference between groups. Further exploration between species enrichment and their potential metabolic interactions and phylogenetic distance were conducted (SI Methods and Results).

Statistical analysis

Unless specified, all analyses were conducted in R 4.1.0 (RStudio 2023.03.1+446). Data was assessed for normality using the shapiro.test function in R prior to a t-test or non-parametric Wilcoxon rank-sum test. To test differences between more than two levels, an ANOVA or Kruskal Wallis test was used. We compared microbiome alpha, beta diversity and beta dispersion between infected vs. uninfected hosts, as well as between hosts under different warming regimes. Comparison was also made between pre-adult and uninfected adult host microbiomes, which reflected the temporal change of pre-adult host microbiomes after 10h off the environmental microbiomes.

Results

We generated a total of 2,014,429 high-quality sequences (average length 400 bp) from 142 microbiome samples (including 140 in vivo samples, and two in-vitro CeMbio community cultures). We found that CeMbio microbiomes commonly colonised worm guts together, across the lab and wild isolates (see Fig. S1 for pre-adult host microbiome profiles). JUb19, MYb71 and BIGb0170 had high relative abundance while JUb134 was less abundant in the community (Fig. S1), indicating stronger colonization abilities for the former and poorer for the latter, consistent with previous findings (Dirksen et al. 2020). We did not find that bacterial colonisation abilities (the fraction of relative abundance) were dependent on their placement on the phylogenetic tree (Fig. S9a).

Temporal dynamics of infected and uninfected host microbiomes

We evaluated the temporal dynamics of host microbiomes by comparing microbiomes of pre-adult and adult hosts in the presence or absence of parasites. We found that across lab and wild hosts, alpha-diversity (richness and phylogenetic diversity) decreased, and dominance increased, through time in both infected and uninfected hosts (Fig. 2). Uninfected host microbiomes, especially for wild isolates, lost more diversity compared with infected hosts (Fig. 2a, b, see Fig. S2 for other diversity metrics, Supplementary Table 3).

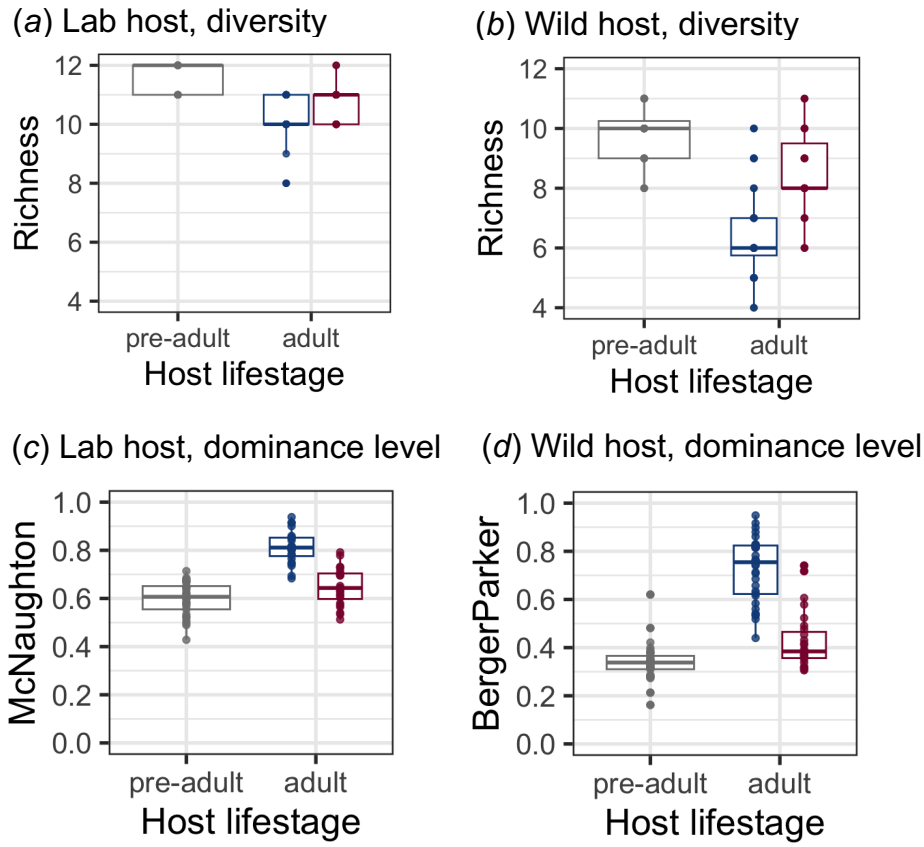

Fig. 2. Temporal dynamics of microbiome diversity and dominance levels, across host lifestages, host type, and infection treatments. (a) Microbiome diversity (Richness) in infected and uninfected lab-adapted hosts over time. (b) Microbiome diversity (Richness) in infected and uninfected wild hosts over time. (c) Microbiome dominance (McNaughton's index) in infected and uninfected lab-adapted hosts. (d) Microbiome dominance (Berger-Parker index) in infected and uninfected wild hosts. Significant differences are detected for all pairwise comparisons shown. Adult microbiome data are shown for infected hosts (red) and for uninfected hosts (blue).

We found that microbiome composition also changed over time in both uninfected and infected hosts (Supplementary Table 3). For uninfected adult hosts, despite being lower in relative abundance early on, MYb10 dominated in lab-adapted host microbiomes, and BIGb0393 dominated in wild host microbiomes (Fig. S10b). BIGb0393 had relatively higher mean metabolic interaction potential with other taxa. This pattern might be associated with an increase in the relative fitness of this species over a temporal scale (SI Methods and Results). For infected hosts, increased dominance was less common over time. We observed the enrichment of BIGb0393 in wild infected adults, and BIGb0172 and BIGb0170 in lab-adapted

infected adults (Fig. S10b), but these enriched species did not widely dominate the microbiome communities (Fig. S1). We found that across lab and wild hosts, microbiome dispersion increased over time, and much more so during infection (Supplementary Table 3).

Infection increased host microbiome diversity and decreased dominance

We found that compared with uninfected adult hosts, infection was associated with significantly higher microbiome diversity and lower dominance, across lab and wild isolates. This pattern was consistent across alpha diversity and dominance metrics (N2: Fig. 2a, b, Table 1; wild: Fig. 2c, d, Table 1, see Fig. S2 and Fig. S3 for other metrics). We did not observe significant associations between wild isolate latitude of origin and microbiome diversity or dominance, in either infected or uninfected controls (Supplementary Table 2: p>0.096 for all correlation tests). Host latitude of origin also did not play a role in pre-adult host microbiome diversity or dominance (Supplementary Table 3: p>0.4 for all correlation tests).

Table 1 Comparisons of microbiome diversity, dominance and dispersion between infected and uninfected adults (uninfected hosts served as the reference in statistical tests)

Alpha-diversity

Factor	Metric	Statistical test	W	Estimate intercept	Significance level	Host
Infected vs. Uninfected						
	Richness	Wilcoxon rank-sum test	187	N/A	*p*=0.021 *	lab-adapted
	Shannon	t test	N/A	0.37	*p*<0.001 ***	lab-adapted
	Evenness	t test	N/A	0.15	*p*<0.001 ***	lab-adapted
	Richness	Wilcoxon rank-sum test	122.5	N/A	*p*<0.001 ***	wild isolates
	Shannon	Wilcoxon rank-sum test	31	N/A	*p*<0.001 ***	wild isolates
	Faith's PD	Wilcoxon rank-sum test	155	N/A	*p*<0.001 ***	wild isolates
	Evenness	Wilcoxon rank-sum test	47	N/A	*p*<0.001 ***	wild isolates

Dominance level

Factor	Metric	Statistical test	W	Estimate intercept	Significance level	Host
Infected vs. Uninfected						

	Berger-Parker Index	Wilcoxon rank-sum test	539.5	N/A	$p<0.001$ ***	lab-adapted
	McNaughton's index	t test	N/A	-0.16	$p<0.001$ ***	lab-adapted
	Simpson's index	Wilcoxon rank-sum test	544	N/A	$p<0.001$ ***	lab-adapted
	Berger-Parker Index	Wilcoxon rank-sum test	817.5	N/A	$p<0.001$ ***	wild isolates
	McNaughton's index	Wilcoxon rank-sum test	834.5	N/A	$p<0.001$ ***	wild isolates
	Simpson's index	Wilcoxon rank-sum test	825	N/A	$p<0.001$ ***	wild isolates
Microbiome dispersion					
Factor	**Metric**	**Statistical test**	**W**	**Significance level**	**Host**
Infected vs. Uninfected					
	Bray-Curtis	Wilcoxon rank-sum test	136	$p<0.001$ ***	lab-adapted
	Weighted Unifrac	Wilcoxon rank-sum test	220	$p<0.001$ ***	lab-adapted
	Aitchison	Wilcoxon rank-sum test	244	*p=0.002* **	lab-adapted
	Bray-Curtis	Wilcoxon rank-sum test	130.5	$p=0.044$ *	wild isolates
	Weighted Unifrac	Wilcoxon rank-sum test	130	$p=0.042$ *	wild isolates
	Aitchison	Wilcoxon rank-sum test	69	$p<0.001$ ***	wild isolates

Warming has a distinct impact on microbiome diversity despite infection

For pre-adult hosts, we found that developmental warming was associated with decreased microbial richness and phylogenetic diversity, compared with ambient developmental temperatures, in wild hosts (Fig. S4, Richness W=51, p=0.032; Faith's PD W=56.5, p=0.009). We did not observe similar effects of developmental temperature on lab-adapted larval host microbiomes (Fig. S5, p>0.08 for all metrics).

Developmental temperature had consistently long-lasting effects on adult host microbiomes, regardless of parasite infection. Developmental warming was shown to increase microbiome richness and phylogenetic diversity for infected N2 hosts (Fig. 3a, Richness W=33.5, p=0.014; Faith's PD W=24.5, p=0.003. See Fig. S5 for other diversity metrics) but did not change

microbiome diversity for infected wild hosts (Fig. S4b, p>0.375 for all metrics). In uninfected N2 hosts, developmental warming increased microbiome phylogenetic diversity (Fig. 3b, Faith's PD W=26.5, p=0.008). In contrast, for uninfected wild hosts, developmental warming decreased microbiome richness (Fig. 3d, Richness W=178.5, p=0.049) and increased dominance (Fig. S6a, McNaughton W=63, p=0.015).

Warming during adulthood also affected microbiome diversity. Compared with ambient temperature, we found that warming increased microbiome evenness during infection in wild adults (Fig. 3c, Evenness W=29, p=0.002). In the absence of parasites, warming temperatures decreased phylogenetic diversity (Fig. S4a, Faith's PD W=185.5, p=0.028). In contrast, for uninfected lab-adapted hosts, warming during the adult stage increased microbiome Shannon diversity and evenness (Fig. S5a, Shannon 25℃ es=0.195, p=0.005; Evenness 25℃ es=0.078, p=0.005), but decreased dominance (Fig. S7a, Berger-Parker Index W=115, p=0.012; McNaughton's index 25℃ es=-0.07, p=0.006; Simpson's index W=120, p=0.005). We did not find significant effects of warming during infection on microbiome diversity or dominance, for infected lab-adapted hosts (Fig. S5b, Fig. S7b).

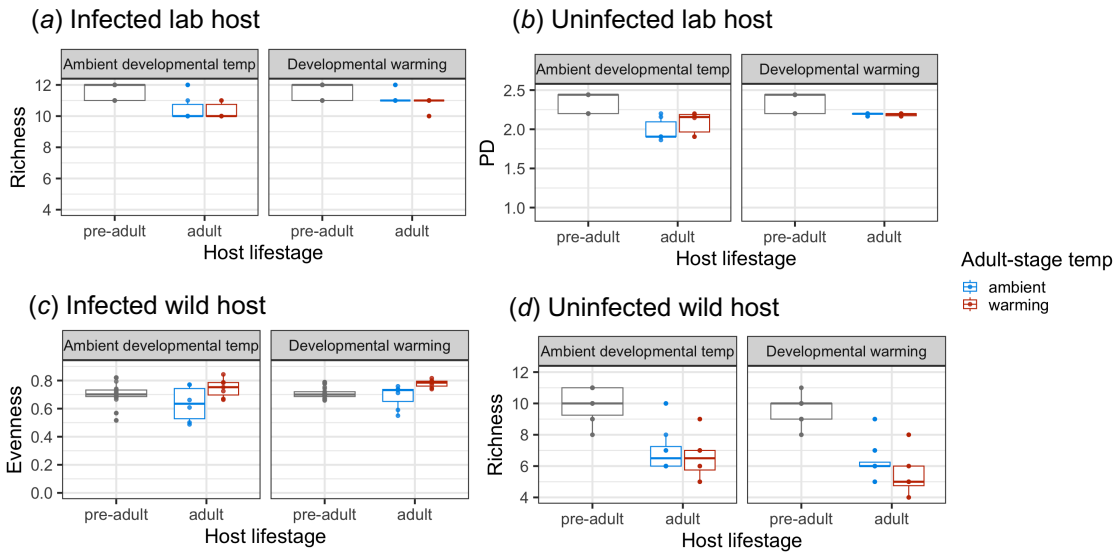

Fig. 3. Microbiome diversity across host lifestages, host type, infection treatments and warming

regimes. (a) Microbiome richness in infected lab-adapted hosts over time. (b) Microbiome phylogenetic diversity in uninfected lab-adapted hosts over time. (c) Microbiome evenness in infected wild hosts. (d) Microbiome richness in uninfected wild hosts. Significant differences are detected for pairwise comparisons made in adult hosts.

Developmental warming alleviated the disruptive effect of parasites

Parasite infection significantly altered host microbiome composition and increased the relative abundance of rare species, such as CEent1 (Supplementary Table 3, Fig. S10a). We investigated inter-individual microbiome variation for infected and uninfected hosts, calculated as the pairwise dissimilarity of microbiome communities. This type of variation can be used to assess microbiome dispersion in hosts under the respective treatment, with more dissimilar microbial communities indicating higher dispersion. We found that under ambient temperatures throughout host development and adult-stage, the pairwise dissimilarity of microbial communities was significantly higher within infected adults than within uninfected adults, across lab-adapted and wild hosts (Fig. 4, Table 1).

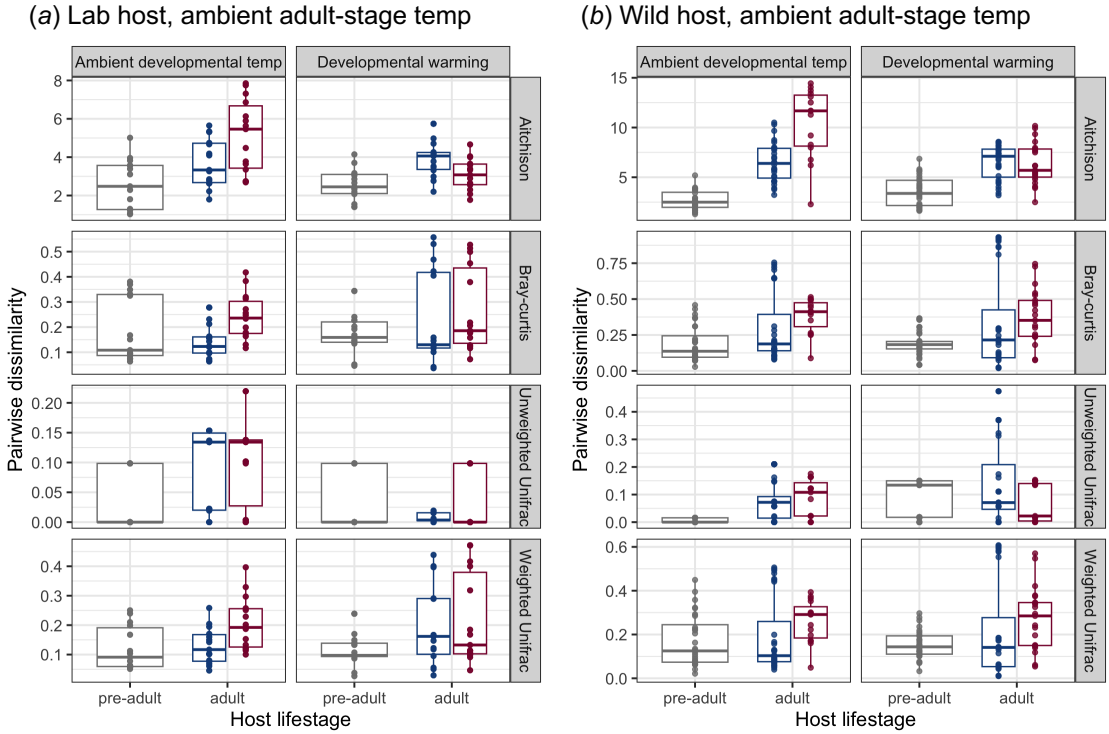

Fig. 4. The impact of infection and developmental temperatures on host microbiome dispersion. (a)

Microbiome dispersion for lab-adapted pre-adults and adults. (b) Microbiome dispersion for wild pre-adult and adult hosts. In both (a) and (b), adult hosts are under ambient adult-stage temperature, and grouped by different infection treatments. Plots were faceted by four beta-diversity metrics, and each data point represents a pairwise distance of two samples within the same treatment group. Adult microbiome data are shown for infected hosts (red) and for uninfected hosts (blue). Significant differences are detected in infected vs. uninfected adults by Aitchison, Bray-Curtis and Weighted Unifrac metrics, at ambient developmental temperatures, across lab and wild hosts.

We found that compared with ambient developmental temperature, developmental warming significantly decreased microbiome dispersion for infected wild hosts, a finding consistent across all dissimilarity metrics (Fig. 4b, Table 2). For uninfected hosts, developmental warming impacted host microbiome dispersion differently for wild and lab-adapted hosts (Fig. 4). These results were inconsistent across different dissimilarity metrics. For example, we found that developmental warming increased microbiome dispersion for uninfected lab-adapted hosts (Fig. 4a, Aitchison W=7052, p=0.0075; Weighted Unifrac W=7144, p=0.0115). Other metrics showed that developmental warming decreased microbiome dispersion for this host type (Fig. 4a, Unweighted Unifrac W=14142, p<0.001). For uninfected wild hosts, developmental warming was shown to significantly decrease microbiome dispersion (Fig. 4b, Aitchison W=9267, p<0.001). Developmental warming also significantly altered lab-adapted host microbiome composition, whereby non-dominant species (BIGb0170) shuffled in relative abundance (Table 2, Fig. S1).

We also assessed the effect of developmental warming on pre-adult host microbiomes. We found that developmental warming significantly altered microbial community composition (PERMANOVA Unweighted Unifrac pseudo-F=10.27, R^2=0.44, p=0.025) and increased microbiome dispersion for wild pre-adults (Fig. 4b, Table 2). We did not observe similar effects of developmental temperature on lab-adapted larval host microbiomes (Fig. 4a).

Table 2 Comparisons of microbiome beta-diversity and dispersion between hosts of different ages under different developmental temperatures

Beta-diversity						
Factor	**Metric**	**Statistical test**	**pseudo-F**	**R2**	**Significance level**	**Host**

Developmental temperature for adult host (20˚C vs. 25˚C)					
Bray-Curtis	PERMANOVA	12.3	0.139	*p*<0.001 ***	lab-adapted
Weighted Unifrac	PERMANOVA	19.83	0.242	*p*<0.001 ***	lab-adapted
Aitchison	PERMANOVA	5.24	0.033	*p*=0.019 *	lab-adapted
Unweighted Unifrac	PERMANOVA	21.58	0.327	*p*<0.001 ***	lab-adapted

Microbiome dispersion

Factor	Metric	Statistical test	W	Significance level	Host
Developmental temperature for pre-adult host (20˚C vs. 25˚C)					
	Aitchison	Wilcoxon rank-sum test	266	*p*=0.039 *	wild isolates
	Unweighted Unifrac	Wilcoxon rank-sum test	126.5	*p*<0.001 ***	wild isolates
Developmental temperature for adult host (20˚C vs. 25˚C)					
Infected					
	Bray-Curtis	Wilcoxon rank-sum test	4429	*p*=0.006 **	wild isolates
	Weighted Unifrac	Wilcoxon rank-sum test	4531	*p*=0.002 **	wild isolates
	Aitchison	Wilcoxon rank-sum test	5264	*p*<0.001 ***	wild isolates
	Unweighted Unifrac	Wilcoxon rank-sum test	4670.5	*p*<0.001 ***	wild isolates

Infection and warming during adulthood increased microbiome dispersion in a non-additive fashion

We found that in the absence of parasites, warming during adulthood significantly increased host microbiome dispersion across lab-adapted and wild adults, compared with ambient adult-stage temperatures (Fig. S8, lab-adapted host: Aitchison W=6264, p<0.001. Wild isolates: Unweighted Unifrac W=3405, p<0.001). This destabilizing effect was similar to that observed for infection. We found that warming during infection did not increase microbiome dispersion additively in lab-adapted hosts (Fig. S8a, p>0.067 for all metrics), but it did reduce dispersion of microbiomes in infected wild hosts (Fig. S8b, Bray-Curtis W=5941.5, p<0.001; Weighted Unifrac W=5484, p<0.001).

Host microbiome dispersion is associated with less variable species interactions

We investigated how warming and infection could influence species interaction strength in host microbiome communities. For lab-adapted adult hosts, we found that parasite infection significantly strengthened both positive and negative interactions but decreased the variation in interaction strength (Fig. 5a, Wilcoxon rank-sum test, positive links: W=68, p=0.01; negative links: W=5, p=0.006; Levene's test: positive links F=5.87, p=0.021). Similar effects of lowered variation in interaction strength were found for wild adult host microbiomes over time, in the absence of infection (Levene's test F=4.14, p=0.046). For wild adult hosts, we found that infection slightly decreased the strength of positive interactions (Fig. 5b, Wilcoxon rank-sum test, W=452, p=0.013). We did not observe significant effects of warming on species interactions strength, in lab-adapted or wild host microbiomes (p>0.05 for all comparisons).

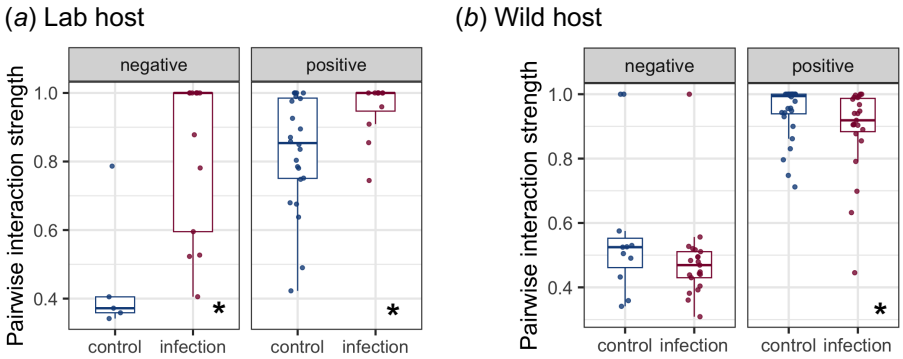

Fig. 5. The impact of parasite infection and host type on the strength and variation of species interactions within microbiomes. (a) Infection impacted the strength of positive and negative interactions, and the variation of positive interaction strength, in the lab-adapted hosts. (b) Infection impacted the strength of positive interactions in wild hosts. The Y axis for both (a) and (b) represents the absolute value of species co-occurrence coefficient. Adult microbiome data are shown for infected hosts (red) and for uninfected hosts (blue). Asterisks indicate significant comparisons between groups on interaction strength.

Discussion

Climate change and infectious diseases have led to population declines of animals and plants, threatening ecosystem biodiversity (Alessandro et al. 2018; Jones et al. 2008; Liang & Gong 2017; Mora et al. 2022). Host-associated microbial communities have the potential to rapidly

respond to biotic and abiotic disturbance, thus providing meaningful early indicators of ecosystem and host health (Ribas et al. 2023; Astudillo-García et al. 2019; Sehnal et al. 2021). Studying the general response of resident microbiomes to temperature changes and infection together could shed light on the persistence of host species in a changing world.

We found that parasite infection significantly altered host microbiome dynamics, with the effects similar to the removal of dominant competitors in the community (Callens et al. 2018). These effects could be driven by parasite-induced alteration in inter-species competition and cooperation (Vonaesch et al. 2018). We observed a gradual loss of microbiome diversity and increased dominance level over time, which were more prominent in uninfected hosts compared with infected hosts. Across temporal scales, we found that some bacterial species (those potentially with stronger competitive abilities) increased in relative abundance and predominantly constituted uninfected host microbiomes. However, parasite infection reduced dominance of these taxa, leading to a more diverse and even community over time. Parasite-induced disturbance has been shown to drive microbial communities to alternative stable states (Mao-Jones et al. 2010), with shifts in the abundance of specific taxa (Gaulke et al. 2019; Vlčková et al. 2018). We showed that, over a short timescale, such a change occurred as rare species increased in abundance and dominant species decreased.

Compared with uninfected hosts, we found that infection was associated with higher microbiome diversity and lower dominance. More widely, the direction in which the parasite alters gut microbiome diversity varies depending on the species and context (Gaulke et al. 2019; Vlčková et al. 2018; Leung et al. 2018), wherein infection is associated with increased microbiome alpha diversity in some studies (Gaulke et al. 2019; Lee et al. 2014; Ramanan et al. 2016) and decreased diversity in others (Holm et al. 2015; Houlden et al. 2015; Cooper et al. 2013; Cahenzli et al. 2013). Whilst the impact of parasites on microbiome dynamics varies across systems (Lee et al. 2014; Ramanan et al. 2016; Holm et al. 2015; Houlden et al. 2015;

Cooper et al. 2013; Cahenzli et al. 2013), increased alpha diversity under infection is hypothesized to be caused by an altered gut immune environment with prominent inflammation (Gaulke et al. 2019; Lee et al. 2014; Broadhurst et al. 2012). We show that warming impacts host microbiome diversity less prominently than infection, with the effects differing by timing of warming as well as the degree of lab adaptation by the hosts.

Here, infection drove larger changes in microbiome composition than warming. Previous study showed that both temperature and infection changed host microbiome structure, and temperature impacted microbiome changes indirectly, through effects on parasite load (Muletz-Wolz et al. 2019). We revealed that parasite infection and warming, independently and simultaneously destabilized host microbiomes via an increase in dispersion (Zaneveld et al. 2017; Byndloss et al. 2018; Foster et al. 2017). Destabilization can suggest that the stressed host is less able to regulate its microbiome community (Zaneveld et al. 2017; Ma 2020; Lesser et al. 2016). This increase in dispersion caused by both abiotic and biotic stressors support the Anna Karenina Principle, adapted and used to predict consequences for animal microbiomes under dysbiosis (Zaneveld et al. 2017). The principal proposes that dispersed microbiome compositions are more likely to occur in stressed individuals than healthy individuals, as found in disease-associated human and animal gut microbiomes (Ma 2020; Lesser et al. 2016; Dey et al. 2013; Giongo et al. 2011). Such infection- or warming-induced Anna Karenina effects have been documented in microbiomes hosted by multiple animal species (Zaneveld et al. 2017; Romer et al. 2022; Walke et al. 2015), as well as in long-term field experiments (Zaneveld et al. 2016).

We found that the level of destabilization caused by infection was the same or lower as when warming was added on top. Stressors can be temporally and spatially variable in nature, but also occur simultaneously, with the synergy between multiple stressors thought to accelerate biodiversity loss (Côté et al. 2016). Previous studies showed that the effects of multiple

stressors (i.e. nutrient pollution (Zaneveld et al. 2016), simulated predation (Maher et al. 2019), overfishing (Zaneveld et al. 2016) and warming (Maher et al. 2019)) on coral microbiomes were acting in an 'opposing', rather than a synergistic or additive fashion (Zaneveld et al. 2016; Maher et al. 2019). Our results from wild nematode microbiomes showed that multiple stressors generate less dispersion compared to single stressors. Multiple stressors can interact opposingly, as revealed in our findings on nematode microbiome structure. However, we showed the timing of those stressors, as well as the degree of lab adaptation by the host, are important factors for shaping the interactive outcome of multiple stressors.

The timing of warming during the host's life-cycle, specifically during development, had a large impact on wild host microbiomes. Developmental warming alone increased microbiome dispersion in wild larval hosts, compared with ambient developmental temperature. However, the timing of warming alleviated the microbiome instability caused by infection. Hosts at different life stages could vary in their sensitivity to warming (Sales et al. 2021). Heat stress during nematode larval stages can activate heat shock transcription and protein production, which is part of the multi-pathogen defense pathways of *C. elegans* (Singh & Aballay 2006; Prithika et al. 2016). A similar protective effect of early-stage heat exposure has been found for broiler chicken (Liew et al. 2003) and plant hosts (e.g. *Arabidopsis thaliana*) (Janda et al. 2019; Lee et al. 2012). Early heat exposure can also protect hosts against heat stress later on in life (Kang et al. 2019). The induced resistance by early environmental stress (e.g. chemical agents (Yassin et al. 2021), physical wounding (Chassot et al. 2008)) is widely applied in plant hosts to increase their basal resistance to future attacks (Perazzolli et al. 2022). Periods of heat stress during climate change should be investigated further as a driver of infection patterns and epidemiology in animals as they age.

We found that compared with uninfected adults, parasite infection induced stronger species interactions (both competitive and facilitative) in lab-adapted host microbiomes. This can be a

signature of poor host control (Foster et al. 2017; Taylor & Vega 2021). Bacterial communities are shaped by inter-species interactions, including competition for shared resources and facilitation by metabolite exchanges (Freilich et al. 2011). Predictions of species competitions can benefit from genome-scale metabolic network construction and inference of species metabolic resource overlap (Zelezniak et al. 2015). We found that species competition strength could also be negatively associated with their phylogenetic relatedness, supported by previous findings (Machado et al. 2021). Microbial species interactions can have large impacts on host fitness (Ludington 2020), highlighting the importance of applying microbial community ecology to understand host-microbe interactions under changing environments. Our results from lab-adapted hosts revealed that increased microbiome dispersion induced by infection was associated with stronger interaction strength in the community, supporting the hypothesis that strong interactions could lead to unstable community dynamics (Allesina & Tang 2012; May 1972; Ratzke et al. 2020; Hu et al. 2022). We also showed that higher microbiome dispersion induced by infection or over time without infection was associated with lower variation in species interaction strength, in lab-adapted and wild nematode hosts, respectively. Higher variation of interaction strength could confer resilience and stability for communities (Kokkoris et al. 2002; Navarrete & Berlow 2006), but this hypothesis remains untested on microbial communities.

Amidst climate change, hosts and their microbiomes are commonly exposed to stressful temperatures. Microbiome changes, particularly patterns of dispersion and instability, have been linked with animal host health (Ribas et al. 2023; Zaneveld et al. 2017; Lesser et al. 2016; Gaulke et al. 2019). Thus, assessing microbiome structure and dynamics in response to multiple stressors (separately and together) across time will likely be important in predicting host population persistence in the wild. This multi-factor approach and focus on dynamics will also help to refine microbiome-based interventions against infection to conserve endangered

species (Santoro et al. 2021; McDevitt-Irwin et al. 2017).

Author contributions

J.L and K.C.K. conceived and designed the study. J.L. conducted the experiment and analysed the data with input from E.J.S. and K.C.K.. J.L. and Y.G. performed DNA extractions. J.L. and K.C.K. wrote the manuscript. All authors contributed to reviewing and editing.

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Conflict of interest

The authors declare that they have no conflict of interest.

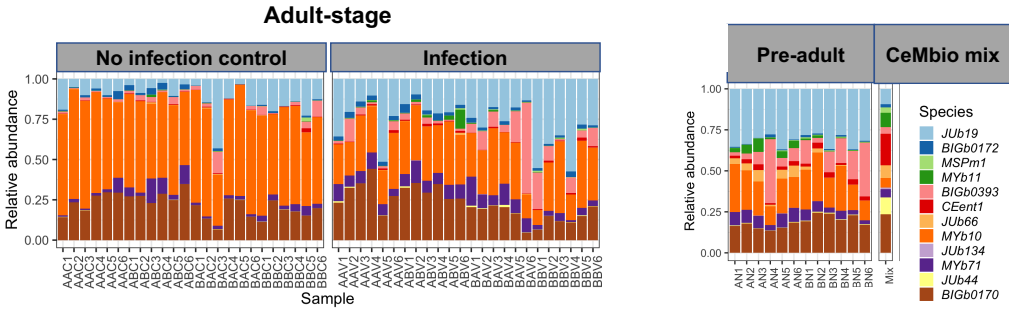
Data accessibility

16S rRNA sequencing data and associated metadata were deposited in the National Center for Biotechnology Information Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under Bioproject ID PRJNA1002096. Electronic supplementary material is available online at figshare (https://doi.org/10.6084/m9.figshare.23850789).

Supplementary Material

Chapter 3

(*a*) Lab host



(*b*) Wild host

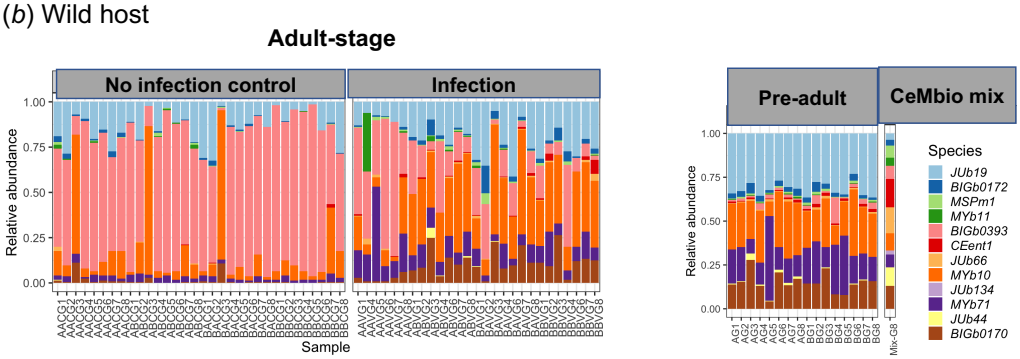
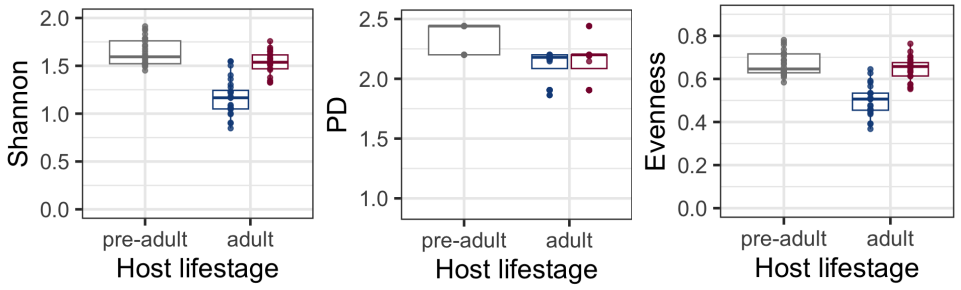


Fig. S1. Relative abundance of taxa in microbiomes of across (a) lab-adapted and (b) wild host isolates. Abundance values are shown for the pre-adult and adult stages of worm development across infection and temperature regimes. Metadata for corresponding sample ID are in Table S1 and Table S2.

(*a*) Lab host, diversity



(*b*) Wild host, diversity

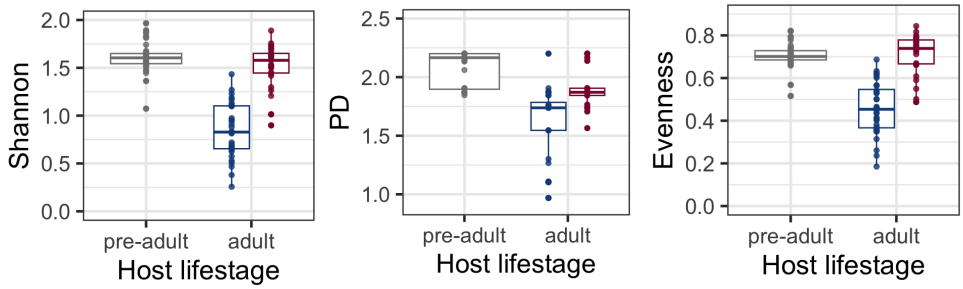


Fig. S2. Temporal dynamics of microbiome diversity across lifestages, host type, and infection status.

(a) Microbiome diversity (Shannon, Faith's PD and Evenness) in infected and uninfected lab-adapted hosts over time. (b) Microbiome diversity (Shannon, Faith's PD and Evenness) in infected and uninfected wild hosts over time. Significant differences are detected in infected vs. uninfected by Shannon and Evenness in (a), and by all three metrics in (b). Adult microbiome data are shown for infected hosts (red) and for uninfected hosts (blue).

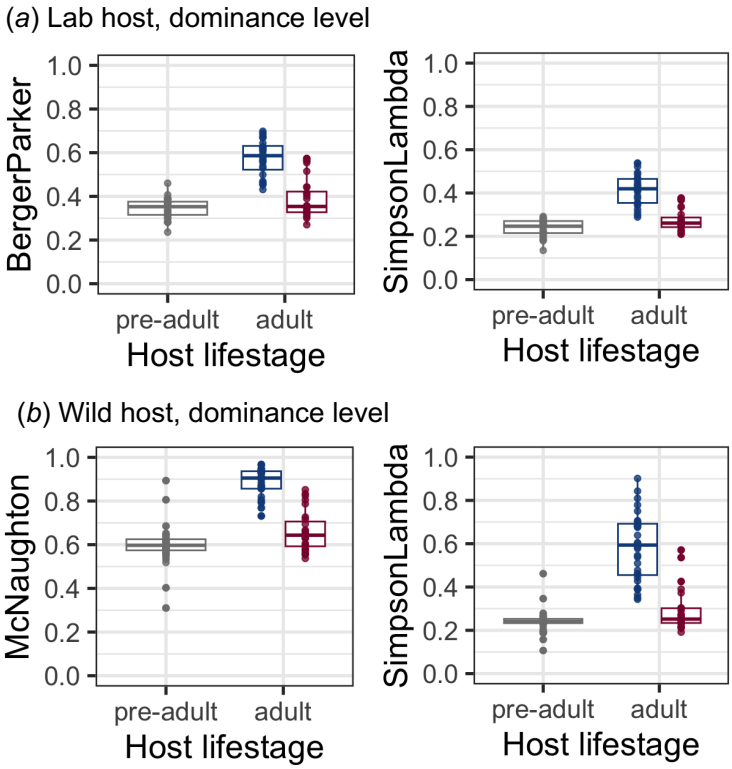


Fig. S3. Temporal dynamics of microbiome dominance levels across lifestages, host type, and infection status. (a) Microbiome dominance level (Berger-Parker's index and SimpsonLambda's index) in infected and uninfected lab-adapted hosts over time. (b) Microbiome dominance level (McNaughton's index and SimpsonLambda's index) in infected and uninfected wild hosts over time. In (a) and (b), significant differences are detected in infected vs. uninfected by all metrics. Adult microbiome data are shown for infected hosts (red) and for uninfected hosts (blue).

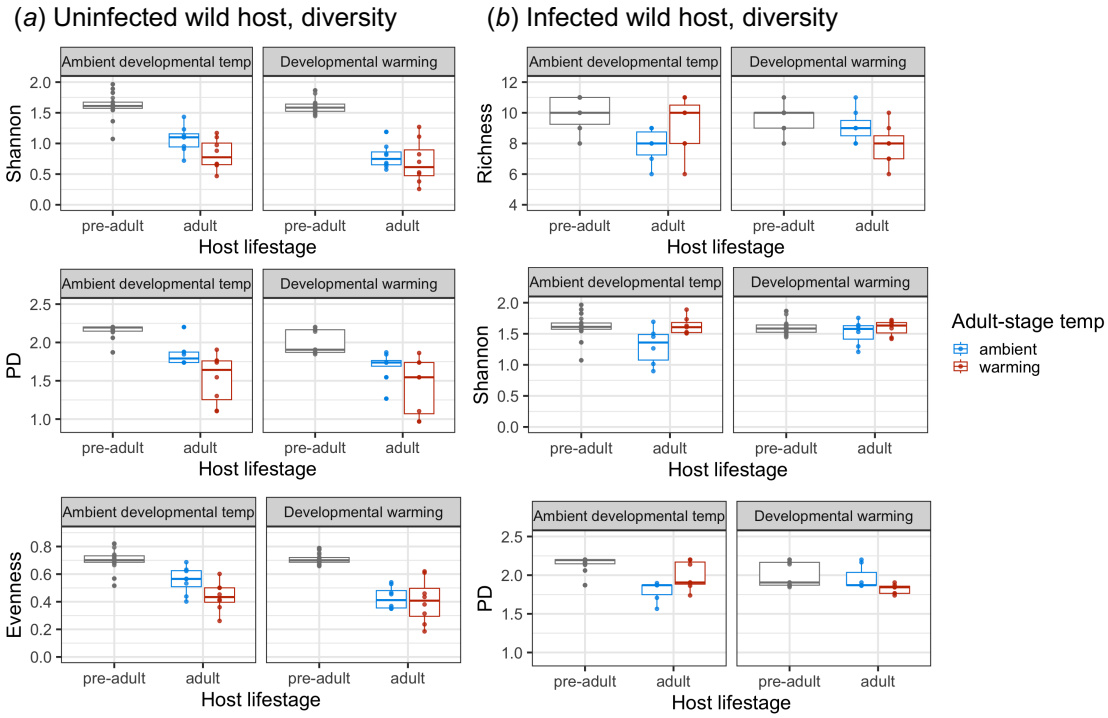


Fig. S4. Microbiome diversity across lifestages, infection status, and warming regimes for wild hosts. (a) Microbiome diversity (Shannon, Faith's PD and Evenness) in uninfected wild hosts over time. (b) Microbiome diversity (Richness, Shannon, Faith's PD) in infected wild hosts over time.

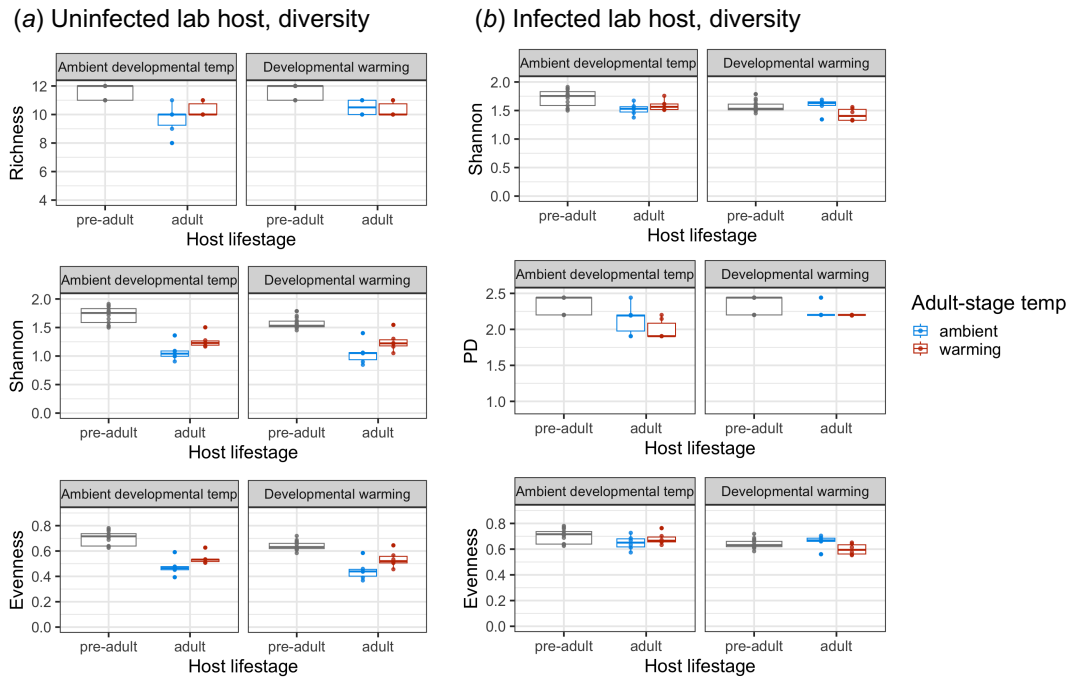


Fig. S5. Microbiome diversity across lifestages, infection status, and warming regimes for lab-adapted hosts. (a) Microbiome diversity (Richness, Shannon and Evenness) in uninfected lab hosts over time. (b) Microbiome diversity (Shannon, Faith's PD and Evenness) in infected lab

hosts over time.

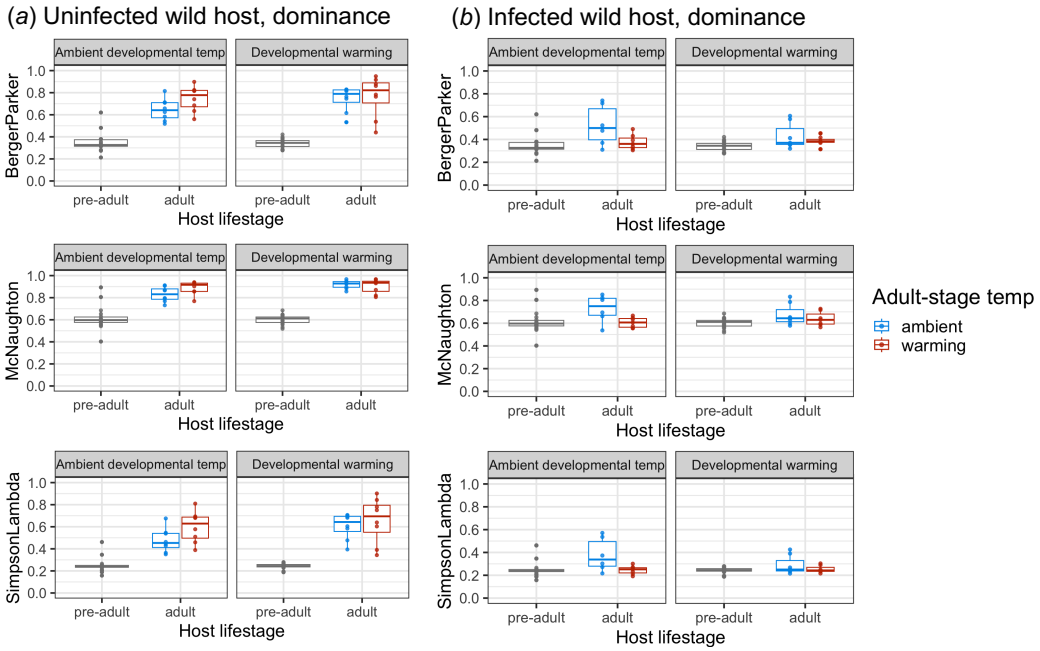


Fig. S6. Microbiome dominance levels across lifestages, infection status, and warming regimes for wild hosts. (a) Microbiome dominance (all three metrics) in uninfected wild hosts over time. (b) Microbiome dominance (all three metrics) in infected wild hosts over time.

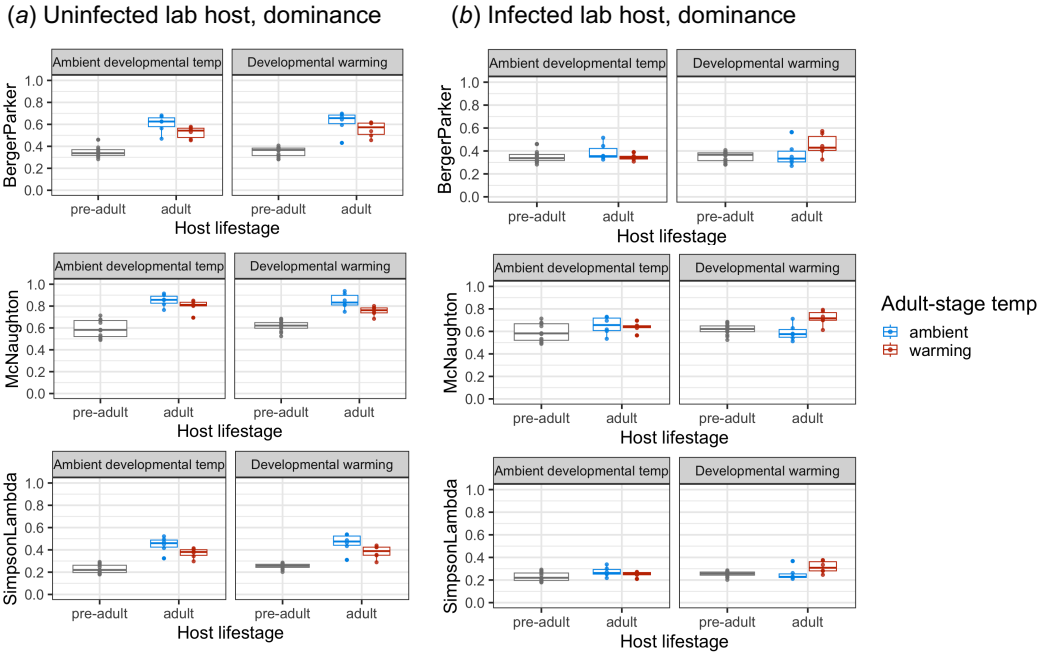


Fig. S7. Microbiome dominance levels across lifestages, infection status, and warming regimes for lab-adapted hosts. (a) Microbiome dominance (all three metrics) in uninfected lab hosts over time. (b) Microbiome dominance (all three metrics) in infected lab hosts over time.

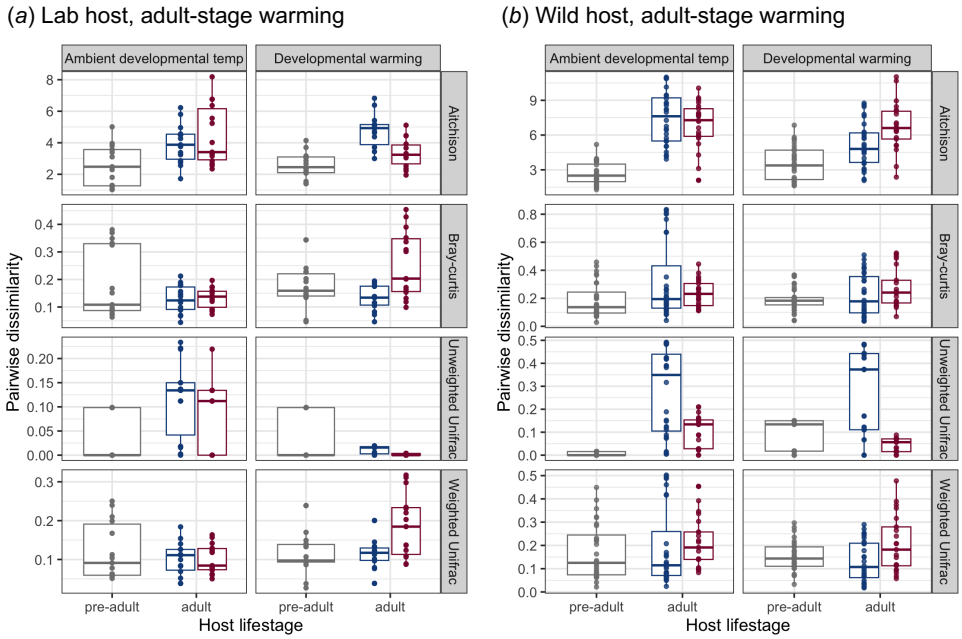


Fig. S8. The impact of infection and developmental temperature on microbiome dispersion. (a) Microbiome dispersion for lab-adapted pre-adults and adults. (b) Microbiome dispersion for wild pre-adult and adult hosts. In both (a) and (b), adult hosts are under adult-stage warming temperature, and grouped by different infection treatments. Plots were faceted by four beta-diversity metrics, and each data point represents a pairwise distance of two samples within the same treatment group. Adult microbiome data shown for infected hosts (red) and for uninfected hosts (blue).

SI Methods and Results

Methods

Phylogenetic analysis

We downloaded full 16S rRNA gene sequences of the 12 CeMbio strains and *L. musarum*. Multiple sequence alignment (MSA) was performed using MUSCLE (Edgar 2004), then the MSA was used to reconstruct a maximum-likelihood tree in IQTREE (v1.6.11) (Nguyen et al. 2015). Tree reconstruction was performed with 1,000 ultrafast bootstrap replicates and the SH-like approximate likelihood ratio test ("-bb 1000 -alrt 1000"). The best-fit model (TIM3e+R3) was selected by ModelFinder (Kalyaanamoorthy et al. 2017) ("-m TIM3+F+I+G4") according to the Bayesian information criterion (BIC). The phylogenetic distance matrix was generated from the consensus tree using the "cophenetic" function (Sneath & Sokal 1973) in R. The eight wild

isolates were grouped into temperate and subtropical/tropical regions rather than by their phylogenetic distance, as whole genome sequences for the isolates were not available.

Genome-scale model reconstruction and analysis

To explore the potential effects of metabolic interactions between the co-occurrent species within microbial communities, we reconstructed genome-scale metabolic models (GEM) for each CeMbio strain. For each CeMbio strain, annotated protein sequences were downloaded from NCBI (Dirksen et al. 2020) and were used for GEM reconstruction based on the top-down carving approach of curated "universal models" using CarveMe. Initial models were then gap-filled using LB media ("—gapfill LB"). Pairwise potential cross-feeding interactions were evaluated using SMETANA score, which can range from 0 (complete independence) and 1 (essentiality) for each metabolite. For CeMbio strain pairs and all co-occurring microbial communities in different treatment groups, the metabolic interaction potential (MIP) and metabolic resource overlap (MRO) were calculated using SMETANA (Zelezniak et al. 2015). MIP calculates how many metabolites the species can share or exchange to decrease their dependency on external resources. This metric can be used to evaluate microbial cooperative metabolism. MRO calculates how much species compete for the same metabolites and can be used to evaluate microbial resource competition. MIP and MRO estimate microbial interactions at their theoretical limit, regardless of the external environment. Spearman and Pearson correlations were calculated for community-level MIP and alpha diversity measures.

Results

Pairwise species MRO was correlated with phylogenetic distance and species competition strength

We found that within CeMbio strains, pairwise species MRO was negatively associated with species phylogenetic distance (Fig. S10a, receiver-donor Pearson cor=-0.425, p=0.000464, Spearman rho=-0.382, p=0.00182; donor-receiver Pearson cor=-0.491, p=3.824e-05,

Spearman rho=-0.398, p=0.00111). BIGb0393 had higher MIP as receiver and donor, and BIGb0172 has higher MRO as receiver and donor (Fig. S10b). Besides, species pairwise mean smetana score was positively associated with phylogenetic distance (donor-to-receiver direction, Pearson cor=0.621, p=0.00267, Spearman rho=0.760, p=6.349e-05, Table S11). These results showed that phylogenetically distant species might have more cooperative metabolic changes. While closer related species could have more competitive interactions as they need to compete for similar nutritional resources. What we observed here was consistent with the findings that species participating in cooperative communities were phylogenetically more distant whereas those in the competitive communities were closer (Machado et al. 2021).

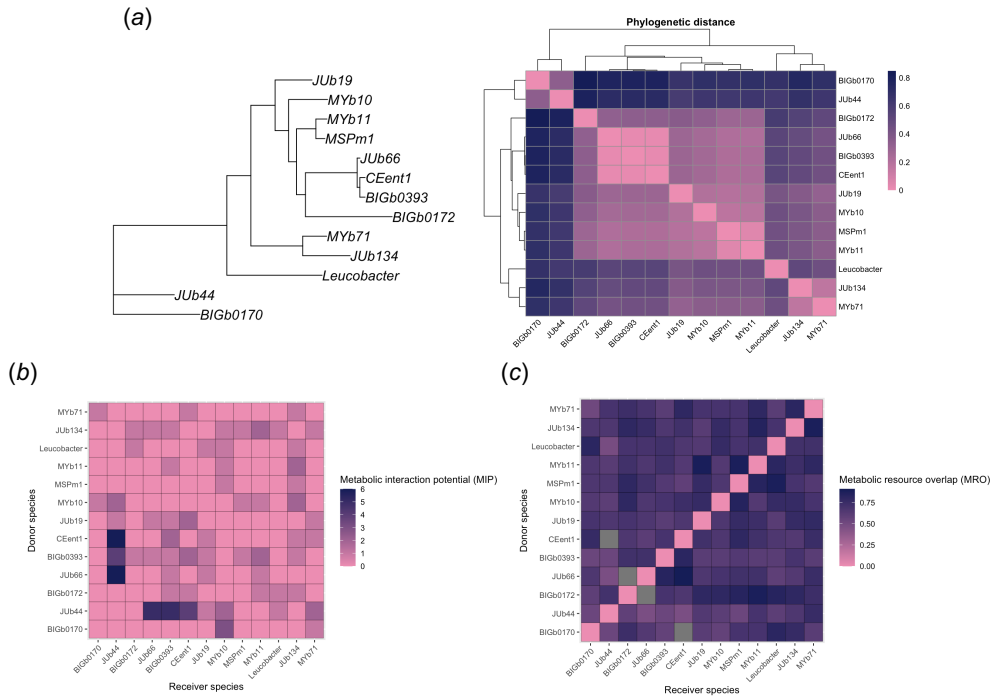


Fig. S9. (a) Phylogenetic tree for the 12 CeMbio strains and the parasite, with species phylogenetic distance heatmap, established using species 16S rRNA genes. (b) Species pairwise MIP. (c) Species pairwise MRO.

By combining results from microbial co-occurrence networks with species pairwise MRO and MIP, we found that the mean and median strength of negative interactions was positively associated with species pairwise MRO (Table S11, lab-adapted host: mean: Spearman rho=0.475, p=0.0297; median: Spearman rho=-0.475, p=0.0297). This outcome indicated that

species having higher nutritional requirement overlap might experience stronger competitive interactions, which fit the hypothesis that MRO can be used for evaluating species resource competition (Zelezniak et al. 2015). The standard deviation of negative interaction strength was strongly correlated with species pairwise phylogenetic distance (wild host: Pearson cor=0.971, p=0.0059). Phylogenetically distant species are less likely to compete (Machado et al. 2021). However, our results suggest that when they do compete, the competitive interactions have more variable interaction strength.

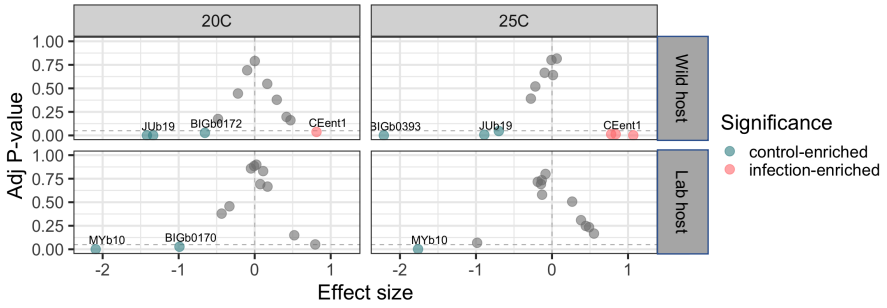
Community-level MRO and MIP were negatively correlated

Across all microbiome samples, community-level MIP and MRO were negatively correlated (Pearson cor=-0.642, p<2.2e-16; Spearman rho=-0.558, p=1.863e-15). We found that community-level MIP and alpha-diversity were positively associated (Table S11, p<0.003 for all the four metrics). These results indicated that more diverse microbiomes could potentially produce or share more metabolites within the community.

Species enrichment might be explained by their metabolic potential

By comparing microbiomes of pre-adult and adult (uninfected control) lab hosts, we found that CEent1 significantly decreased in relative abundance over time (Fig. S11, Table S10), whereas MYb10 and JUb19 increased in relative abundance (Fig. S11, Table S10). CEent1 and JUb66 had high mean smetana scores as donors but not as receivers (Fig. S12). Thus, exclusive metabolic contributors in microbial communities could easily be outcompeted by other species. BIGb0393 had a relatively higher mean MIP and was enriched in adult host microbiomes, increasing in relative abundance over time (Fig S11, Table S10). However, infection reversed this pattern, reducing the dominance of BIGb0393 in microbiome communities. Conversely, we found that CEent1, which was less abundant in uninfected host microbiomes, showed significant enrichment in host microbiomes under infection (Fig. S11, Table S10), however the mechanisms underlying its enrichment need to be explored.

(*a*)



(*b*)

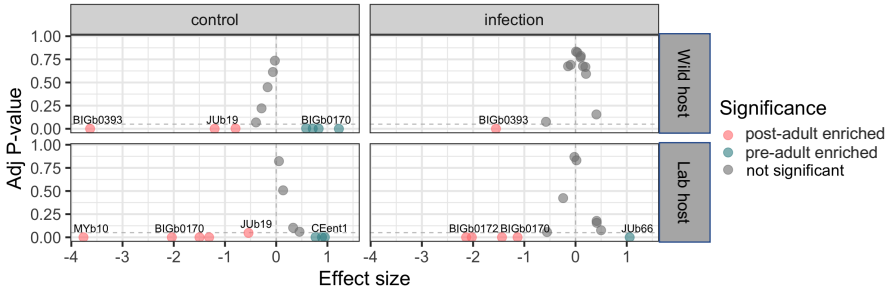


Fig. S10. Differentially enriched bacterial species across infection and warming regimes. (a) Differential species in infection and uninfected controls, grouped by developmental temperature and host type. (b) Differential species over temporal scales, grouped by infection treatment and host type. X axis means enrichment effect for each species, y axis represents adjusted p-value, with < 0.05 indicating significant differential enrichment. Different color showed enrichment in different groups. Non-significant species were represented by grey points.

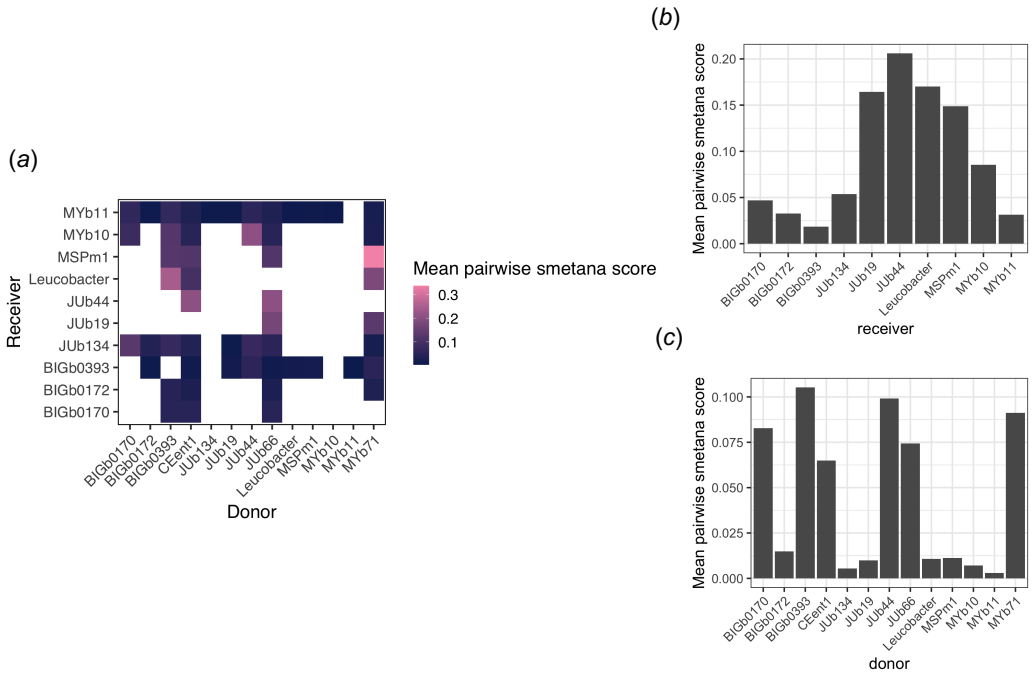


Fig. S11. Species pairwise smetana scores. (a) Heatmap of mean pairwise smetana score of each microbiome species and the parasite. (b) Mean smetana score for individual receiver species. (c) Mean smetana score for individual donor species.

Supplementary Table 1. Location of origin for C. elegans wild isolates used in study

Genotype_ ID	Host_isolate	City, Nation, or State	Lat	Long	Elevation	Isolation time
1	ED3005	Edinburgh, United Kingdom	55.94	-3.36	41	2004
2	ED3012	Edinburgh, United Kingdom	55.92	-3.19	87	2004
3	CB4856	Oahu, Hawaii	21.33	-157.86	64	1972
4	CB4857	Claremont, USA	34.096	-117.719	353	1972
5	CB4858	Pasadena, USA	34.156113	-118.13194	267	1971
6	JU363	Franconville, France	48.98	2.23	93	2002
7	JU1580	Orsay, France	48.7015	2.1725	63	2012
8	CX11262	Los Angeles, USA	34.12946	-118.10987	203	2012

Supplementary Table 2. Correlation between host latitude and microbiome alpha diversity and dominance, for infected and uninfected wild hosts.

Alpha-diversity			
Factor	**Metric**	**Statistical test**	**P**
Wild host latitude of origin			
Uninfected			
	Richness	Spearman's test	*p*=0.313 NS
	Shannon	Pearson's test	*p*=0.096 NS
	Faith's PD	Spearman's test	*P*=0.138 NS
	Evenness	Pearson's test	*p*=0.177 NS
Infected			
	Richness	Pearson's test	*p*=0.383 NS
	Shannon	Spearman's test	*p*=0.710 NS
	Faith's PD	Spearman's test	*p*=0.229 NS
	Evenness	Spearman's test	*p*=0.637 NS
Dominance level			
Factor	**Metric**	**Statistical test**	**P**
Wild host latitude of origin			
Uninfected			
	Berger-Parker Index	Pearson's test	*p*=0.050 NS
	McNaughton's index	Spearman's test	*p*=0.451 NS
	Simpson's index	Pearson's test	*p*=0.074 NS
Infected			
	Berger-Parker Index	Spearman's test	*p*=0.532 NS
	McNaughton's index	Spearman's test	*p*=0.378 NS
	Simpson's index	Spearman's test	*p*=0.584 NS

Supplementary Table 3 Comparisons of microbiome diversity and dominance across host lifestages, location of origin, and infection status

Alpha-diversity							
Factor	**Metric**	**Statistical test**	**W**	**Estimate intercept**	**Significance level**	**Reference level**	**Host**
Uninfected adult vs. pre-adult host							
	Richness	Wilcoxon rank-sum test	8	N/A	*p*<0.001 ***	adult	lab-adapted
	Shannon	t test	N/A	0.553	*p*<0.001 ***		
	Faith's PD	Wilcoxon rank-sum test	7	N/A	*p*<0.001 ***		
	Evenness	t test	N/A	0.194	*p*<0.001 ***		
	Richness	Wilcoxon rank-sum test	8	N/A	*p*<0.001 ***		wild isolates
	Shannon	Wilcoxon rank-sum test	1	N/A	*p*<0.001 ***		
	Faith's PD	Wilcoxon rank-sum test	16.5	N/A	*p*<0.001 ***		
	Evenness	Wilcoxon rank-sum test	9	N/A	*p*<0.001 ***		
Infected adult vs. pre-adult host							
	Richness	Wilcoxon rank-sum test	27	N/A	*p*<0.001 ***	adult	lab-adapted
	Shannon	t test	N/A	0.181	*p*<0.001 ***		
	Faith's PD	Wilcoxon rank-sum test	26	N/A	*p*<0.001 ***		
	Evenness	t test	N/A	0.046	*p*=0.016 *		
	Richness	Wilcoxon rank-sum test	69	N/A	*p*<0.001 ***		wild isolates
	Shannon	Wilcoxon rank-sum test	152	N/A	*p*=0.111 NS		
	Faith's PD	Wilcoxon rank-sum test	69	N/A	*p*<0.001 ***		
	Evenness	Wilcoxon rank-sum test	288	N/A	*p*=0.072 NS		
Wild host latitude of origin							
Pre-adult host							
	Richness	Spearman's test	N/A	N/A	*p*=0.719 NS	N/A	wild isolates
	Shannon	Pearson's test	N/A	N/A	*p*=0.442 NS	N/A	
	Faith's PD	Spearman's test	N/A	N/A	*P*=0.928 NS	N/A	
	Evenness	Pearson's test	N/A	N/A	*p*=0.402 NS	N/A	
Dominance level							
Factor	**Metric**	**Statistical test**	**W**	**Estimate intercept**	**Significance level**	**Reference level**	**Host**

Uninfected adult vs. pre-adult host							
	Berger-Parker Index	Wilcoxon rank-sum test	288	N/A	p<0.001 ***	adult	lab
	McNaughton's index	t test	N/A	-0.23	p<0.001 ***	adult	lab
	Simpson's index	Wilcoxon rank-sum test	288	N/A	p<0.001 ***	adult	lab
	Berger-Parker Index	Wilcoxon rank-sum test	511	N/A	p<0.001 ***	adult	wild
	McNaughton's index	Wilcoxon rank-sum test	506.5	N/A	p<0.001 ***	adult	wild
	Simpson's index	Wilcoxon rank-sum test	511	N/A	p<0.001 ***	adult	wild
Infected adult vs. pre-adult host							
	Berger-Parker Index	Wilcoxon rank-sum test	197	N/A	p=0.078 NS	adult	lab
	McNaughton's index	t test	N/A	-0.069	p=0.010 **	adult	lab
	Simpson's index	Wilcoxon rank-sum test	232	N/A	p=0.002 **	adult	lab
	Berger-Parker Index	Wilcoxon rank-sum test	334.5	N/A	p=0.003 **	adult	wild
	McNaughton's index	Wilcoxon rank-sum test	279.5	N/A	p=0.113 NS	adult	wild
	Simpson's index	Wilcoxon rank-sum test	281	N/A	p=0.106 NS	adult	wild
Wild host latitude of origin							
Pre-adult host							
	Berger-Parker Index	Spearman's test	N/A	N/A	p=0.525 NS	N/A	wild
	McNaughton's index	Spearman's test	N/A	N/A	p=0.585 NS	N/A	wild
	Simpson's index	Spearman's test	N/A	N/A	p=0.420 NS	N/A	wild

Beta-diversity

Factor	Metric	Statistical test	pseudo-F	R2	Significance level	Host
Uninfected adult vs. pre-adult host						
	Bray-Curtis	PERMANOVA	59.8	0.625	p<0.001 ***	lab
	Weighted Unifrac	PERMANOVA	32.87	0.483	p<0.001 ***	lab
	Aitchison	PERMANOVA	37.73	0.522	p<0.001 ***	lab
	Unweighted Unifrac	PERMANOVA	27.83	0.399	p<0.001 ***	lab
	Bray-Curtis	PERMANOVA	76.51	0.626	p<0.001 ***	wild

	Weighted Unifrac	PERMANOVA	118.59	0.722	*p*<0.001 ***	wild
	Aitchison	PERMANOVA	31.09	0.408	*p*<0.001 ***	wild
	Unweighted Unifrac	PERMANOVA	14.8	0.228	*p*<0.001 ***	wild
Infected adult vs. pre-adult host						
	Bray-Curtis	PERMANOVA	6.39	0.142	*p*=0.001 **	lab
	Weighted Unifrac	PERMANOVA	4.65	0.105	*p*=0.013 *	lab
	Aitchison	PERMANOVA	36.10	0.507	*p*<0.001 ***	lab
	Unweighted Unifrac	PERMANOVA	20.17	0.334	*p*<0.001 ***	lab
	Bray-Curtis	PERMANOVA	10.97	0.203	*p*<0.001 ***	wild
	Weighted Unifrac	PERMANOVA	14.58	0.255	*p*<0.001 ***	wild
	Aitchison	PERMANOVA	67.34	0.623	*p*<0.001 ***	wild
	Unweighted Unifrac	PERMANOVA	14.13	0.245	*p*<0.001 ***	wild
Infected vs. Uninfected						
	Bray-Curtis	PERMANOVA	32.13	0.362	*p*<0.001 ***	lab
	Weighted Unifrac	PERMANOVA	18.06	0.22	*p*<0.001 ***	lab
	Aitchison	PERMANOVA	109.37	0.683	*p*<0.001 ***	lab
	Bray-Curtis	PERMANOVA	32.14	0.363	*p*<0.001 ***	wild
	Weighted Unifrac	PERMANOVA	39.72	0.412	*p*<0.001 ***	wild
	Aitchison	PERMANOVA	36.52	0.392	*p*<0.001 ***	wild
	Unweighted Unifrac	PERMANOVA	8.07	0.119	*p*=0.0013 **	wild

Microbiome dispersion

Factor	Metric	Statistical test	W	Significance level	Reference level	Host
Uninfected adult vs. pre-adult host						
	Bray-Curtis	Wilcoxon rank-sum test	36232	*p*<0.001 ***	adult	lab
	Weighted Unifrac	Wilcoxon rank-sum test	38768	*p*<0.001 ***	adult	lab
	Aitchison	Wilcoxon rank-sum test	44180	*p*<0.001 ***	adult	lab
	Unweighted Unifrac	Wilcoxon rank-sum test	34540	*p*<0.001 ***	adult	lab
	Bray-Curtis	Wilcoxon rank-sum test	6380	*p*<0.001 ***	adult	wild
	Weighted Unifrac	Wilcoxon rank-sum test	5837	*p*<0.001 ***	adult	wild

Aitchison	Wilcoxon rank-sum test	7545	$p<0.001$ ***	adult	wild
Unweighted Unifrac	Wilcoxon rank-sum test	6058	$p<0.001$ ***	adult	wild
Infected adult vs. pre-adult host					
Bray-Curtis	Wilcoxon rank-sum test	39078	$p<0.001$ ***	adult	lab
Weighted Unifrac	Wilcoxon rank-sum test	40724	$p<0.001$ ***	adult	lab
Aitchison	Wilcoxon rank-sum test	43504	$p<0.001$ ***	adult	lab
Unweighted Unifrac	Wilcoxon rank-sum test	33460	$p<0.001$ ***	adult	lab
Bray-Curtis	Wilcoxon rank-sum test	30192	$p<0.001$ ***	adult	wild
Weighted Unifrac	Wilcoxon rank-sum test	28515	$p<0.001$ ***	adult	wild
Aitchison	Wilcoxon rank-sum test	32945	$p<0.001$ ***	adult	wild
Unweighted Unifrac	Wilcoxon rank-sum test	26103	$p<0.001$ ***	adult	wild

Chapter 4:

Warming-induced excess deaths of infected animals depend on pathogen traits

Title: Warming-induced excess deaths of infected animals depend on pathogen traits

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Data accessibility statement: The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.22060646.v3.

Main Manuscript for

Warming-induced excess deaths of infected animals depend on pathogen traits

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Keywords: Meta-analysis, global warming, infectious disease, virulence, host-pathogen interactions, species persistence

Abstract

Climate change is causing extreme heating events. Simultaneously, climate change and human activities are leading to more prolonged and intense infectious disease outbreaks. The extent to which warming and infection may together impact host species persistence is, however, unclear. Using a meta-analysis of >190 effect sizes representing 101 animal host-pathogen systems, we provide broad evidence that experimentally increased temperatures drove higher pathogen virulence, specifically pathogen-induced host mortality. Importantly, larger temperature increases were associated with more host deaths hinting at the escalating threat for animal species as the world continues to warm. We further found that hosts with bacterial infections were at the highest risk of death under hotter conditions. This was particularly the case if pathogenic bacteria were naturally established within the host species, although novel infections (emerging, zoonotic, or opportunistic) were more lethal at lower temperatures. The virulence of fungal pathogens increased when temperatures were shifted upwards towards their thermal optimum. The magnitude of these effects was not impacted by host life-stage, immune complexity, or variable experimental protocols. Overall, our findings suggest that global warming could lead to excess deaths caused by infectious diseases in animal populations.

Introduction

Climate change is resulting in more extreme heating events (Dosio et al. 2018). Temperature affects all levels of biology, directly or indirectly impacting the physiology and life-history for all organisms (Lagerspetz 1987). Typically for ectotherms, environmental warming changes their body temperatures, thus influencing their physiological sensitivity and fitness (Huey et al. 2012). Shifting global temperature patterns are also changing the geographic distribution of infectious diseases, generating novel transmission opportunities for the pathogens (Williams et al. 2002; Roberts et al. 2018). Transmission for a pathogen to become established in a new host species can lead to intensified disease outbreaks (Roberts et al. 2018).

Positive relationships between warmer temperatures and disease severity (or pathogen virulence) have been detected in diverse host-pathogen systems (Vezzulli et al. 2010; Jiang et al. 2013; Paull & Johnson 2014; Mayers et al. 2016; Brand et al. 2016; Bugeme et al. 2009). Rising temperature can alter animal immune responses, leading to reduced host resistance to pathogen infections (Moriyama & Ichinohe 2019; Regnier & Kelley 1981; Lecchi et al. 2016; Lacetera et al. 2005). By causing stress and disrupting homeostasis, pathogen infection can reduce the upper temperatures tolerated by hosts, contributing to worsened infection outcomes under warming (Hector et al. 2022). Temperature also directly mediates pathogen virulence (Laine 2007; Vale & Little 2009; Allen & Little 2011; Kimes et al. 2012). For example, higher virulent temperature can increase the virulence of coral pathogen *Vibrio coralliilyticus* by upregulating the expression of virulence factors (Kimes et al. 2012). Moreover, higher ambient temperature may enhance pathogen metabolism,

resulting in faster within-host growth and greater harm. Especially for bacterial agents, as their population growth rates can be increased by warmer temperatures exponentially (Qiu et al. 2022).

Warming can conversely lessen or have neutral impacts on virulence in some host-pathogen systems, emphasising the importance of the biology of different diseases (Lafferty & Mordecai 2016; Karvonen et al. 2010; Delisle et al. 2018; De Silva et al. 2017; Kerchev et al. 2017; López et al. 2017). Both host and the pathogen's response to temperature changes can depend on their thermal tolerance, which is characterized by its critical thermal maximum, minimum and optimum - the upper, lower and optimal temperatures at which it cannot function (Huey et al. 2012; Chown et al. 2010; Hoffmann et al. 2003). Thus, a mismatch in host and pathogen thermal optima can change infection outcomes, such that cold-adapted pathogens might be less harmful in a hot-adapted host at higher temperatures (Cohen et al. 2017; Cohen et al. 2020). Also, virulence of different pathogen types, such as eukaryotic pathogenic nematodes versus prokaryotic pathogens (i.e. bacteria), might be impacted by temperature in distinct ways. Not all host-pathogen interactions will be affected equally by climate change, and it is unclear which outcome will apply to which type of infection (Lafferty & Mordecai 2016; Karvonen et al. 2010).

Understanding the broad ecological consequences of elevated temperature on pathogen virulence (or pathogen-induced host death herein), is crucial for projections of future wildlife disease dynamics and species persistence. If heating generally exacerbates the severity of infection, climate change could worsen disease outcomes for wildlife. Projections of species

persistence in a changing world might need to account for rates of infection and disease severity (Paull & Johnson 2014; Pounds et al. 2006). However, if virulence is broadly unaffected or context-dependent in certain types of infection (Lafferty & Mordecai 2016), the health risks posed by warming and those infectious disease could be considered independently or on a case-by-case basis. Any broad relationship between warming and virulence would have significant consequences for animal conservation (Pounds et al. 2006; Boyd et al. 2013) and potentially human health (Jones et al. 2008; Patz et al. 2005). However, the broad-scale trends for this interaction are currently unknown.

In this study, we conducted a meta-analysis to investigate the generality of the relationship between elevated temperature and pathogen virulence. We searched the published literature for experimental studies and included a breadth of ectothermic animal host-pathogen systems. We used the most widely reported quantitative form of virulence: pathogen-induced host death (Cressler et al. 2016). We focused on infected host death under warming as this measure was relevant for consideration in projections of species extinction and biodiversity loss (Fisher et al. 2012). We calculated effect sizes via relative risk ratios of death at experimentally high and low temperatures. We separately collected thermal optima data for all the pathogens included, and tested whether biological traits (e.g. host type, pathogen type, host-pathogen evolutionary history, pathogen thermal optimum), the scale of temperature increase and experimental variables (pathogen inoculation method, pathogen dosage, infection exposure/measurement time) could explain variation in the temperature-virulence relationship.

Materials and Methods

Literature search and data collection

A literature search was performed on Web of Science Core Collection using keywords 'parasit*' OR 'pathogen*', paired with 'temperature' AND 'virulen*', to identify studies reporting the effect of temperature on pathogen virulence. We used host mortality to quantify pathogen virulence, as it is a commonly used and relevant component of virulence. Our main inclusion criteria were: (i) the pathogen was tested in living animal hosts rather than on cells or dead host tissues; (ii) more than one temperature was used for host infection experiments, and temperature was the only changing variable in the infection experiments; (iii) pathogen virulence was measured by assessing host mortality rate, under different temperatures. In these cases, studies using parasitoids were excluded as host mortality would always be 100%; (iv) host mortality (with mean mortality rate and sample size, sample size is defined as the number of individual host used in each infection treatment) under different temperatures were available publicly or shared by the author by May 2022.

Following our inclusion criteria, we retrieved data from a total of 60 research articles (PRISMA flow-chart see Fig. S1, with PRISMA-EcoEvo Checklist included), published between 1996 and 2021 (summary of studies and moderator variables are provided in Supplementary Table 1). To maximise the ecological relevance, we imposed a series of restrictions to exclude data from extreme high or low temperatures and pathogen dosages unlikely to happen in nature. Firstly, if more than five temperatures were tested, data were extracted from the second lowest and second highest temperatures (when between two to

four temperatures were tested, data were extracted from the highest and lowest temperatures). Secondly, if multiple pathogen dosages were used, data of the middle value (or middle minus one value, in case of even number) was extracted. Thirdly, host mortality data was extracted from the end time point of the assay. However, if mortality began to appear in controls, data were extracted from the timepoint with control mortality close to or at zero. This prevented us incorporating host mortality due to temperature alone.

We ensured that all host mortality in the pathogen treatment groups was due to infection rather than other reasons. Hosts not exposed to pathogens had no mortality (e.g. Jiang et al. 2013), very low mortality (<10%, e.g. Brand et al. 2016) or host mortality in the infection treatment was corrected for control mortality following Abbott's (1952) formula (e.g. Bugeme et al. 2008). Data on host mortality in individual articles were extracted manually from text and tables in the main text or supplementary information, figures by using WebPlotDigitizer (Rohatgi 2015), or raw data made available from the authors by request. Fifteen effect sizes from six studies were discarded due to large host mortality in control groups.

When host mortality was expressed as a percentage, we estimated the number of alive and dead in both temperature groups by multiplying percentage of host mortality by the number of host individuals tested. We otherwise obtained the number of alive and dead hosts from the raw data sent by authors. These data were used to calculate the risk ratio (RR) effect size, using *escalc* function in 'metafor' R package (Viechtbauer 2010). RRs represent the relative risk of warming on infected hosts' survival. An overall total of 192 effect sizes from

60 studies were used across 56 pathogens and 50 host species (101 host-pathogen combinations, Fig. S2).

Moderator variables

Host, pathogen and experimental variables were included to assess their impact on pathogen virulence when temperature rose. To explore whether pathogen type could predict effect size estimates, we recorded whether the pathogen used in the study was bacteria, fungi, nematode, virus, or protist. As numbers of effect sizes in virus and protist were low (5 ESs for virus, 1 ES for protist), our analysis focused on bacteria, fungi and nematode pathogens.

We collected data on the optimal growth temperature (T_{opt}) for well-studied pathogens as virulence can correlate with pathogen growth rates (Anderson & May RM 2019). These data were collected for 39 pathogen species, included in the meta-analysis, from published literature (Supplementary Table 2, T_{opt} for *in vitro* growth/fitness were measured for non-virus pathogens). Using known thermal optimums for these pathogens, we were able to define whether experimental warming was towards or away from their T_{opt}. "Towards T_{opt}" was used when the temperature range in the study was below T_{opt}. "Away from T_{opt}" was used when the study temperature range was above T_{opt}. When the low and high temperatures in the study straddled T_{opt}, we used "Range includes T_{opt}".

To test whether the response to increased temperature depended on the impact of natural selection, we defined a moderator based on whether the interaction was "established" (system originally collected from the wild and with previous evolutionary history (see Bally

& Garrabou 2007 as an example), “semi-established” (host infected by the pathogen in the wild, but combination in the study were not co-isolated (see Wekesa et al. 2010 as an example), and “novel” (no known record of an infection between the pathogen and the host in nature, see Ekesi et al. 1999 as an example). The “novel” associations might infer opportunistic infection or new host shift for the pathogen.

Host traits including host life-stage (“adult”, “larva”, “pupae” and “juvenile”), host source (“collected from wild”, “lab-reared”) and host type with different immune complexity (“vertebrate”, “invertebrate”) were also tested as moderator variables. Lastly, we collected and evaluated experimental variables including temperature span (difference between high temperature and low temperature used in the study), infection exposure time (measured by day), pathogen dosage (within each pathogen type) and pathogen inoculation method (“injection”, “not injection”). These experimental protocols were evaluated given their potential for a bias in extrapolations to nature (Hairston 1989).

Statistical analysis

By fitting sample size or correction method as a moderator, we checked that neither sample size nor the methods used to correct for control mortality influenced the effect sizes (sample size $p=0.9630$, correction method $p>0.52$ for all levels). All categorical moderators were assessed for multicollinearity by calculating the variance inflation factor (VIF, *VIF* function in ‘regclass’ R package) when fitted together in a regression model and no multicollinearity was observed (Supplementary Table 3, GVIF^(1/(2*Df)) <= 2.5 for all moderators included). Correlation of continuous variables were assessed for Pearson correlation (cor.test function

in R) and the possibility of multicollinearity was excluded (Supplementary Table 3, $p>0.07$ for all pairwise correlations).

We coded the data, whereby RR>1 (logRR>0) indicated greater host mortality at higher temperatures (higher risk associated with higher temperatures) and RR<1 (logRR<0) indicated lower host mortality at higher temperatures. A relationship in which temperature has no effect on pathogen virulence was indicated by RR=1 (logRR=0). We fitted a multi-level random-effects model using *rma.mv* function in 'metafor' R package to estimate mean effect sizes across all the studies. We used host species, pathogen species and study as random factors to account for statistical non-independence due to repeated measures within species and studies. We did not include the phylogenic tree of either host species or pathogen species as random factor. For many, we could not resolve species-level phylogeny of hosts or pathogens from published literature, and replacing those with their relatives would introduce more bias.

For categorical moderators, we first conducted sub-group analysis to calculate summary effect size for each sub-group. We then assessed whether there was significant difference between different sub-group levels by fitting the moderator in multilevel random effects meta-regression models (*rma.mv* function in R). For continuous moderators, we fitted the moderator in multilevel random effects meta-regression models (*rma.mv* function in R) with the effect sizes as response variables. Separate tests of interactions between moderators were driven by *a priori* hypotheses (all hypothesis and model results in Supplementary Table 3).

Results and Discussion

More death of infected hosts at hotter temperatures

We firstly tested whether hotter temperatures generally increase host death (i.e. the proportion of dead individuals in the infected host population). We incorporated data from 60 studies which conducted infection experiments at different temperatures. These studies included 50 ectothermic animal host species and 56 pathogen species (resulting in 101 analysed host-pathogen combinations, see Fig. S2), from which we calculated 192 effect sizes (ESs). Our data set contained a variety of pathogen types including bacteria, fungi, nematodes, and viruses from diverse geographic regions (Fig. S3). We included diverse host-pathogen systems spanning terrestrial (42 ESs) and aquatic habitats (150 ESs). Terrestrial hosts mostly consisted of Insecta (125 ESs), and aquatic mostly consisted of fish (17 ESs) and mollusca (14 ESs) (Fig. S2). Overall, bacterial pathogens were mostly studied in aquatic animals, while most fungal pathogens as well as all of the nematoda pathogens were tested in insecta hosts and in novel host-pathogen systems in lab (Fig. S2).

Across studies, the rate of uninfected host mortality under warming remained zero or low (<10%). In contrast, infected hosts suffered greater death rates with increase in temperature (Fig. 1A, logRR>0, RR=1.47, p=0.0046). Previous findings that infection can reduce the ability of hosts to tolerate heat (Hector et al. 2022; Porras et al. 2021; Greenspan et al. 2017) may reflect this increase in pathogen virulence.

We subsequently investigated whether biological factors could explain variation in effect sizes, using sub-group analysis and meta-regressions. We specifically tested whether the

relationship between temperature and virulence differed between (i) pathogen types (bacteria, fungi, nematoda, viruses), (ii) host-pathogen evolutionary history (established vs. novel), (iii) host types and immune complexity (vertebrate vs. invertebrate), (iv) host life-stage (e.g. adult vs. larva), and (v) wild-collected vs. lab-reared hosts.

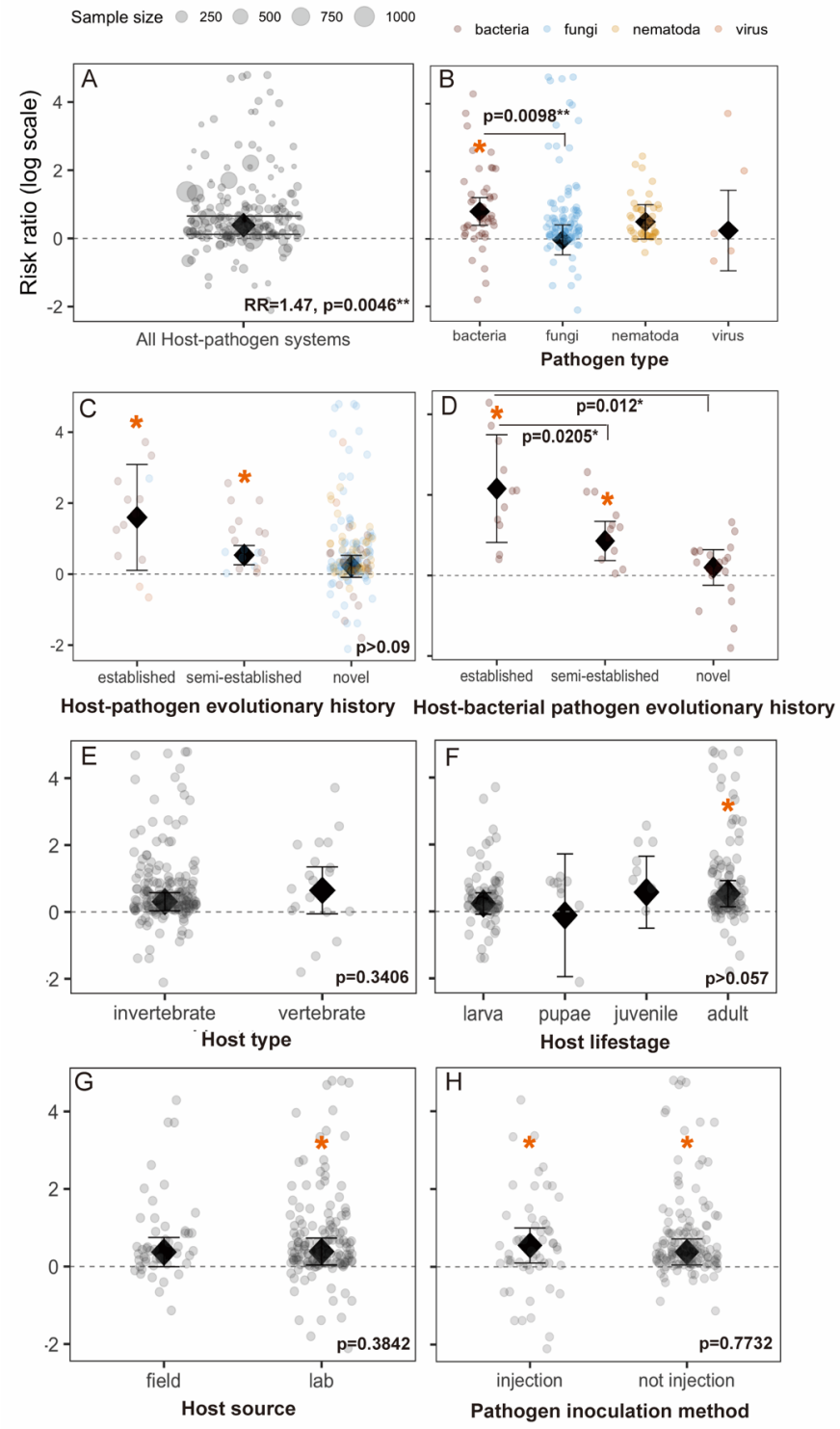

Figure 1. Results of the meta-analysis on the impact of heating on pathogen-induced host death. (A) Summary effect size (Risk ratio, RR) from overall meta-analysis is significantly >1 (logRR>0, indicating positive relationship between temperature and virulence). Bold black diamond with 95% confidence interval represents summary effect size, and individual effect size are displayed as jittered points. Point size indicates sample size (number of host individuals in the infection treatment) used for effect size calculation. The summary RR with the corresponding p-value is shown in the bottom right corner of the plot. The presence of the effect differed by (B) pathogen type and (D) host-

pathogen evolutionary history for bacterial infections, but less pronounced when combined all effect sizes across pathogen types (C). The presence of an effect was not impacted by (E) host type with varying immune complexity, (F) host early or adult lifestage, (G) host source being wild-collected or lab-reared or (H) pathogen was inoculated by injection or other means. Orange star is shown above the subgroups that have summary RR significantly >1. P-values shown on the bottom right corner indicate significance level of subgroup comparisons. In (B-D), jittered points are colored by pathogen type.

We found that the impact of higher temperatures on host death differed among pathogen type. Bacterial pathogens exhibited the strongest and most positive effect sizes (Fig. 1B, bacteria RR=2.25, p=0.0001; fungi RR=0.97, p=0.8930, nematoda RR=1.66, p=0.0523, virus RR=1.28, p=0.6868; bacteria vs. fungi p=0.0098, p>0.09 for all other comparisons). As most of bacterial effect sizes were calculated from aquatic hosts, our results indicate that bacterial pathogens might pose significant greater risk under warming, specifically to aquatic hosts such as fishes. Increase in infectious disease outbreaks for aquatic animals, especially fishes, has been an overwhelming issue for the aquaculture industry, which can cause considerable harm to economy as well as human health (Dayana Senthamarai et al. 2023). Bacterial diseases are common in the pisciculture industry (Hegde et al. 2023), we suggest that future disease management should also consider the aggravating effects of warming on the severity of epidemics.

Our results showed that host death increased with warming in established host-pathogen systems with a shared evolutionary history, but not in novel systems (Fig. 1C, established RR=4.94, p=0.0357; semi-established RR=1.71, p=0.0001, novel RR=1.2, p=0.1639). However, no significant difference was found between “established” and “novel” subgroups (Fig. 1C, p>0.09). Since the interactive effect was detected between pathogen type and evolutionary history (p=0.0014 for “novel”:”virus”), we then assessed the effect of host-

pathogen evolutionary history within each pathogen type. We found that the effects of excess host death in established, but not novel systems were more pronounced in host-bacterial pathogen systems (Fig. 1D, established RR=8.64, p=0.0016; semi-established RR=2.35, p=0.0006; novel RR=1.22, p=0.3794; established vs. novel p=0.0120). These effects were significant regardless of host type (p=0.0664 for “host-pathogen system”:“host type”). We were not able to detect similar effects in non-bacterial pathogen types due to low number of effect sizes in “established” subgroup. We also found that host mortality in novel systems was already higher than that in established associations, at low/baseline temperature (Fig. S4A, C, p=0.0007). This high level of virulence might be due to pathogen maladaptation when it shifts to a new host (Longdon et al. 2015). These results suggest that emerging infections might already be highly virulent at present ambient temperatures, and less likely to be worsened by temperature increases specifically during global climate change.

There was no evidence that the magnitude of the temperature-host death association was influenced by other moderators tested – host life-stage, host type, or host source (Fig. 1E-G, p>0.05 for all comparisons). The overall pattern of increased pathogen virulence under warming was also consistent despite variation in experimental protocols. We tested the impact of these experimental moderators (i) pathogen inoculation method (injection vs. not injection), (ii) pathogen dosage, and (iii) exposure or measurement time points. Host death was increased at hotter temperatures consistently across all protocols investigated (Fig. 1H, pathogen inoculation method: injection: RR=1.73, p=0.0172; not injection: RR=1.46,

p=0.0258; comparison: p=0.7732; Fig. S5, pathogen dosage: p>0.05 for all pathogen types; infection exposure time: p=0.8642).

More host death with larger temperature changes

We tested whether the magnitude of the temperature-death relationship would scale with temperature increases. We fit temperature span as a moderator in a meta-regression. Larger temperature changes in experiments were associated with bigger effect sizes (Fig. 2A, p<.0001), regardless of any biological factors (Supplementary Table 3, p>0.1 for all interactions). We then assessed the robustness of the empirical evidence by re-analysing a single pathogen species - *Beauveria bassiana*, which had the most data across multiple arthropod host species (25 effect sizes). Similarly, higher levels of host death during infection by this fungal species were associated with greater temperature changes (Fig. S6, p=0.0110).

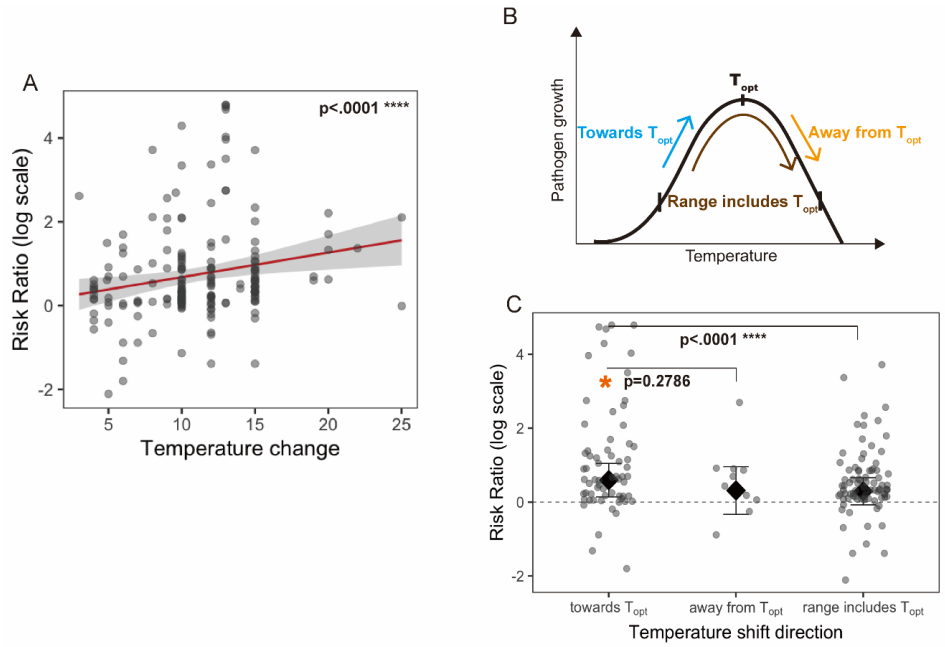

Figure 2. Risk ratio (RR) varied with scale and direction of temperature change. (A) Positive association between scale of temperature change and effect sizes. (B) Schematic illustration of the classification for directions of temperature shift related to pathogen thermal optima (T_{opt}). The curve represents a generic thermal performance curve with T_{opt} as the optimal growth temperature. The

direction of a temperature shift is represented by arrows. “Towards T_{opt}”: the temperature range in the study was below T_{opt}. “Away from T_{opt}”: the temperature range was above T_{opt}. “Range includes T_{opt}”: the low and high temperatures in the study straddled T_{opt}. (C) Summary RR for subgroups of temperature shift directions. Black diamond with 95% confidence interval represents summary effect size, and individual effect sizes are displayed as jittered points. Asterisk indicates RR significantly > 1 (logRR>0) for a subgroup. P-values between subgroups show level of significance.

Experimental temperature increases included in our analysis ranged from 3°C to 25°C. This range is relevant to the upper boundary of the predicted temperature rise on Earth: 4°C increase in annual mean surface temperature by 2100 and 14.1°C increase by 2300 (Arias et al. 2021). If no actions are taken to alleviate climate change, our findings suggest that the outcomes of infectious disease could worsen to a greater extent in regions of more intense warming and more frequent extreme thermal events (Rangwala et al. 2013). More studies are needed to investigate whether host responses (i.e., evolutionary adaptation in shorter-lived species, acclimatization in longer-lived species, and migration in both) to warming could help mitigate these negative effects (Bennett et al. 2021).

Pathogen thermal optima may hint at a mechanism

We hypothesized that the increase in host death may be the result of faster pathogen growth or higher within-host loads at higher temperatures. We estimated pathogen thermal optima (T_{opt}) using *in vitro* growth rate data for 39 well-studied species (Supplementary Table 2). We found that warming increased virulence when temperature was elevated in the direction of pathogen optima, using sub-group analysis on temperature changes relative to T_{opt} (Fig. 2B, Fig. 2C, subgroup “towards T_{opt}”: RR=1.81, p=0.0104). This indicated that warmer and more optimal temperature can promote pathogen growth, leading to heightened pathogen load and greater host harm. The extent of change in host death was

significantly different between subgroup “towards T_{opt}” and “range includes T_{opt}” (Fig. 2C, p<.0001), but not different between subgroup “towards T_{opt}” and “away from T_{opt}” (Fig. 2C, p=0.2786).

We then compared effect sizes of these subgroups within the same pathogen type. Compared with other types of pathogens (i.e. bacteria, nematode), we found that the direction of the temperature shift relative to T_{opt} was more vital for outcomes of fungal infections. Unlike that for bacterial infections, hosts with fungal pathogens did not result in higher average host death under warmer temperatures (Fig. 1B, RR=0.97, p=0.8930), supporting the observation that fungi are heat sensitive compared to bacteria (Alster et al. 2018). However, effect sizes for host death caused by fungi increased when the temperature was shifted towards fungal T_{opt} (“towards T_{opt}”:“fungi” es>0, p=0.0181, Fig. S7). As 92% of these effect sizes were calculated from novel host-fungi systems, this result might adapt to predict patterns of emerging fungal diseases. Most fungal species are limited in their capability to grow at elevated temperatures which limits their potential to infect mammals (Garcia-Solache & Casadevall 2010). Our finding suggests that fungal pathogen species could be deadlier if temperatures shifted towards their T_{opt}. It has previously been shown that *Batrachochytrium dendrobatidis* (Bd) – a deadly fungal pathogen driving the extinction of amphibian species – is more prevalent at highland localities where the temperatures are shifted towards the pathogen’s T_{opt} (Bosch et al. 2007). At higher temperate latitudes, the persistently warmer temperatures caused by climate change are contributing to the ongoing expansion in the ranges of fungal pathogens such as *Coccidioides*, *Blastomyces*, *Histoplasma*, and *Sporothrix* (Friedman & Schwartz 2019;

Gadre et al. 2022). With continued warming in these regions, emerging fungal infections could spread faster and be more severe, posing a greater threat to susceptible and endangered hosts, such as amphibians and reptiles (Cohen et al. 2019).

Apart from the direction of temperature shift, host thermal tolerance and immune response might also play important roles in shaping such infection outcomes. For example, host immunopathology induced by infection can be heightened under warmer conditions, causing host cell and tissue damage and worsening infection outcomes (Dittmar et al. 2014; Evans et al. 2015). It can be difficult to distinguish the adverse effects of host immunopathology from direct effects of pathogens under warming (Graham et al. 2005). Further experiments on the impact of temperature on immune activation and immunopathology are needed. Tackling the impact of global climate change on wildlife health requires an understanding the mechanisms that might increase host mortality during infection when periods of extreme warming hit.

Conclusions and future perspective

A greater diversity of host-pathogen systems should be included in future experimental tests of the temperature-virulence relationship. While invertebrate hosts included in our analysis ranged from terrestrial (e.g., insects, mite, etc.; 78.1% of the effect sizes) to aquatic (e.g., corals, oysters, etc.; 12% of the effect sizes), there were fewer experimental studies with aquatic vertebrate hosts (fishes, amphibians, 9.9% of the effect sizes) and none with terrestrial vertebrates. Bias towards this limited set of systems could be due to most of our included studies stemming from the field of agriculture (e.g., used Arthropoda hosts) or

aquaculture (e.g., used fish hosts). Our study focused on ectotherms in which both the host and pathogen are directly exposed to warming, while more investigation is needed to on endotherms with more complex thermal regulation mechanisms. Nevertheless, most emerging human pathogens originate in other vertebrates, especially mammals (Cleaveland 2001; Woolhouse & Gowtage-Sequeria 2005; Taylor et al. 2001). Further research on the impact of higher temperatures in vertebrate-pathogen systems is essential for robust predictions on the outcomes of infections with zoonotic potential under climate change.

Both ecological and evolutionary consequences of elevated temperatures are important for understanding the impacts of warming on ecosystems. Our study revealed temperature's effect on infection outcomes on an ecological timescale, for which pathogen and warming exposure were not carried across multiple host generations. Over longer evolutionary timescales, the difference between the host's and pathogen's ability to acclimate or adapt with thermal changes may be important. Due to their generation times and faster metabolisms, pathogens might adapt more rapidly to temperature shifts than their hosts, leading to increased pathogen growth within hosts (Gillooly et al. 2001). To test whether the pattern on ecological timescales will remain the same across evolutionary timescales, longitudinal studies (perhaps using experimental evolution) could be used to track the potential for host and pathogen adaptation under temperatures relevant to climate change projections. Slow temperature increases may facilitate host acclimation or adaptation thereby mitigate the negative effect of warming on infection outcomes (Schaum et al. 2022).

Parasites and pathogens are ubiquitous. Thus, understanding the impact of warming on the harm caused by infection is critical for projections of animal species persistence under global climate change (Garamszegi 2011; Altizer et al. 2013; Smith et al. 2009; Rohr & Cohen 2020; Harvell et al. 2002). Our meta-analysis provides evidence that warming, and the degree and direction of temperature increase, can potentially lead to excess mortality in infected animal populations. These patterns show distinct consequences of warming on bacterial and fungal infections, and for hosts in different habitats. More intense warming, if concomitant with epidemics caused by bacterial pathogens, could exacerbate aquatic animal population declines. While emerging fungal diseases for terrestrial animals are more of a concern when heat extremes are more optimal for pathogen growth. There are important consequences in this temperature-virulence relationship for species at risk, ecosystems sensitive to biodiversity loss. Human activities are warming the Earth at an unprecedented rate (IPCC 2021) and leading to an increased frequency and intensity of temperature extremes (Arias et al. 2021). The current global effort to restrict warming to 1.5°C above pre-industrial levels by 2050 (Rogelj et al. 2018) may also have the unintended benefit of limiting the lethal impacts of infectious diseases.

Acknowledgments

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Supplementary Material

Chapter 4

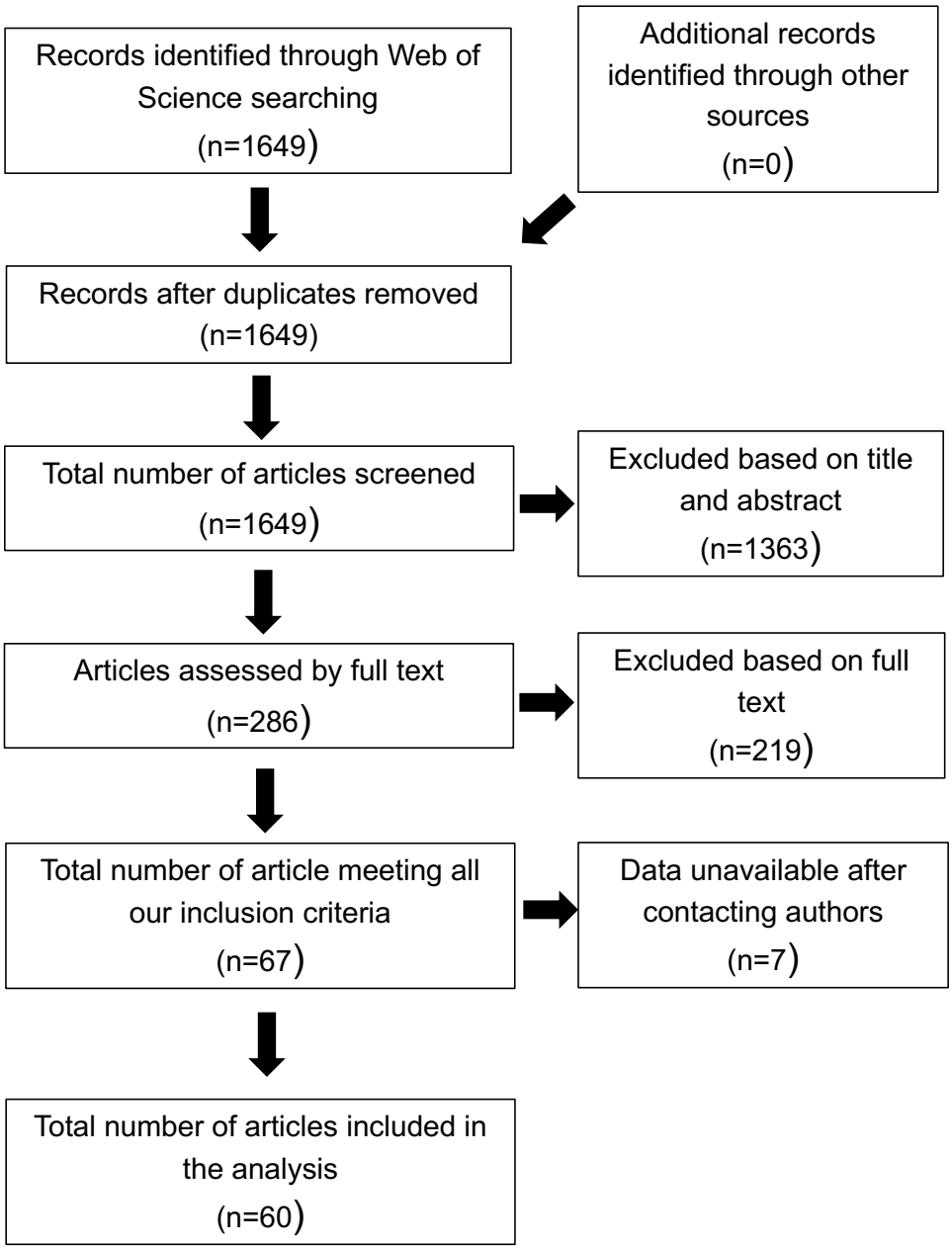

Fig. S1. PRISMA-style flow diagram of screening and inclusion of journal articles. The 1649 records from a Web of Science search were screened based on our criteria, resulting in the 60 total articles included in the meta-analysis. n represents the number of articles.

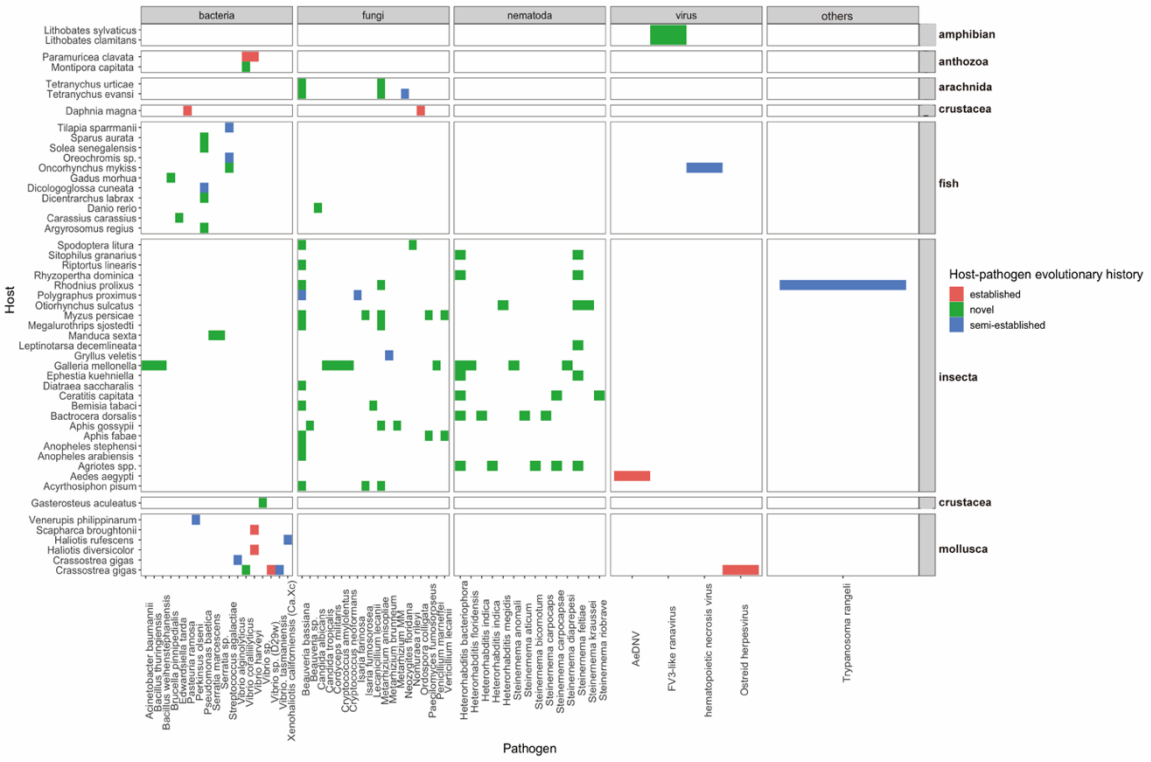

Fig. S2. The evolutionary history of the 101 host-pathogen combinations included in the meta-analysis. Each is colored by the evolutionary history of the host-pathogen system (established, semi-established, and novel as defined in the Main Text).

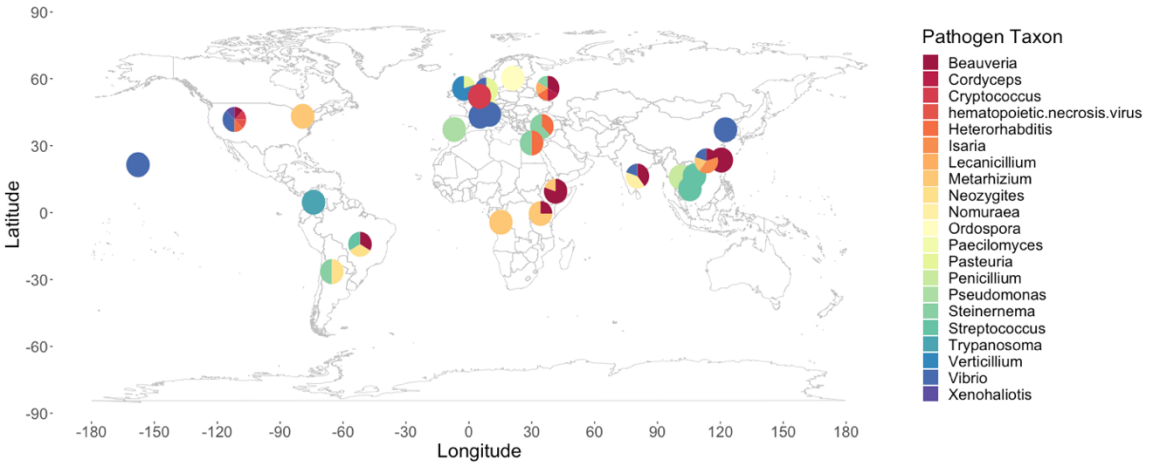

Fig. S3. Geographical locations of pathogens with known collection sites. Some pathogens were collected years ago and may have adapted to lab environment. Different colors represent pathogen taxa (shown on genus-level when available). Collection sites data were variable in their geographic specificity, ranging from precise coordinates to broader regions such as cities and countries, depending on details reported in the original literature. The proportions in each piechart represents proportion of pathogen taxa from each collection site. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

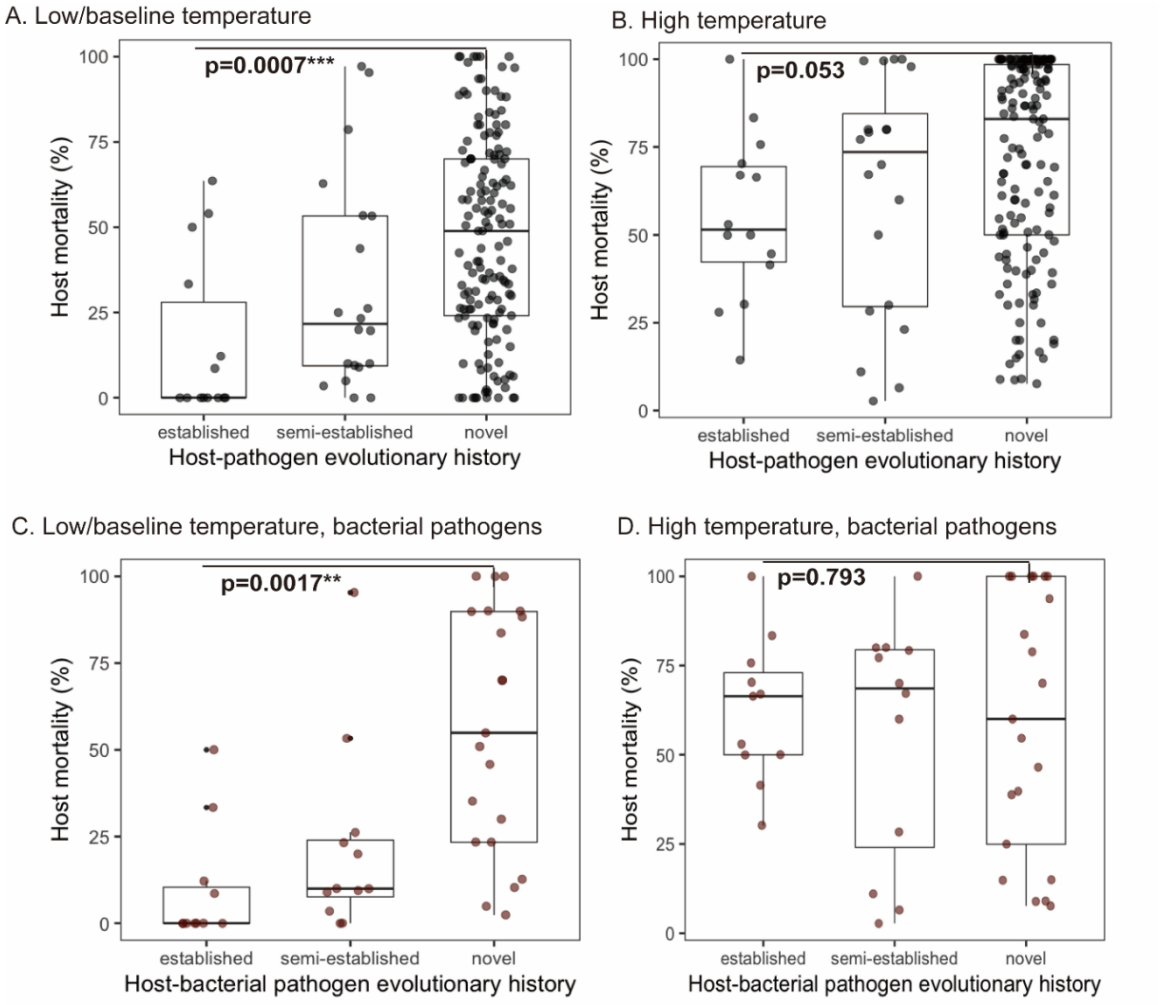

Fig. S4. Host killing at low/baseline (A, C) and high temperature (B, D), for host-pathogen systems with different evolutionary histories. Host mortality at low or high temperatures is showed as combining all effect sizes across pathogen taxa (A, B), and for bacterial pathogens specifically (C, D). Under low temperatures, significantly higher host killing is observed in “novel” compared with that in “established” systems.

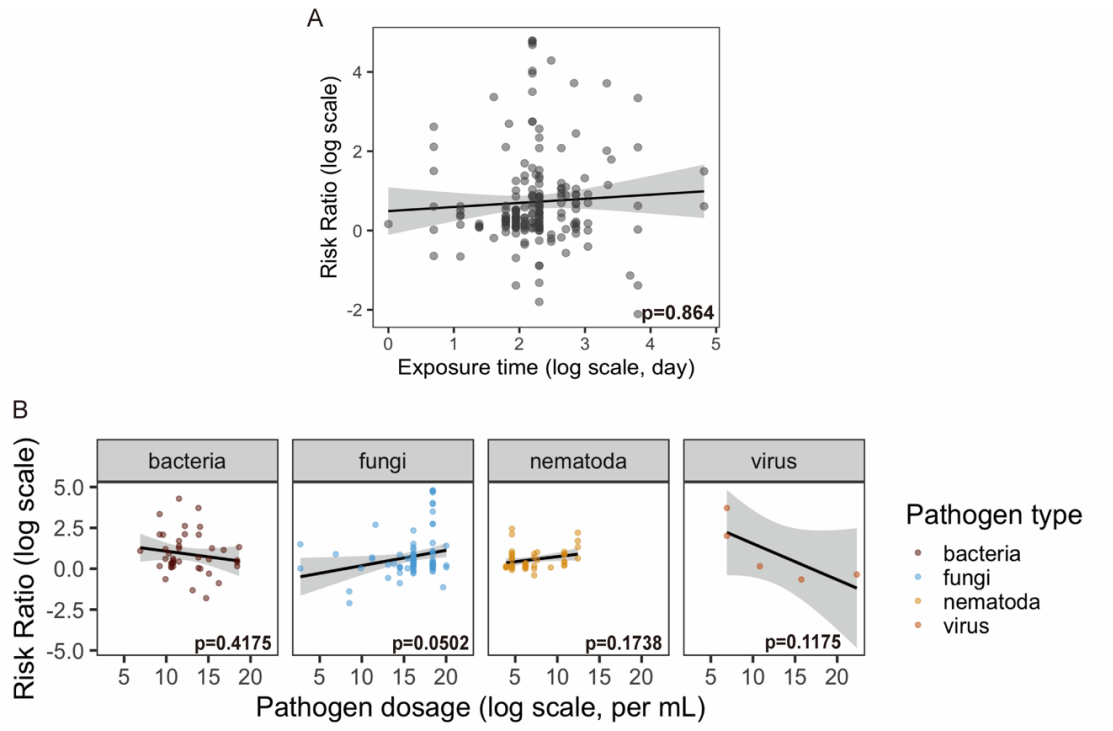

Fig. S5. The impacts of experimental protocols on effect sizes. Increased host killing under warmer temperatures was not impacted by (A) time over which host mortality data was collected, namely exposure time, or (B) pathogen dosage used in the experiments. The significance level is shown on the bottom right of each plot.

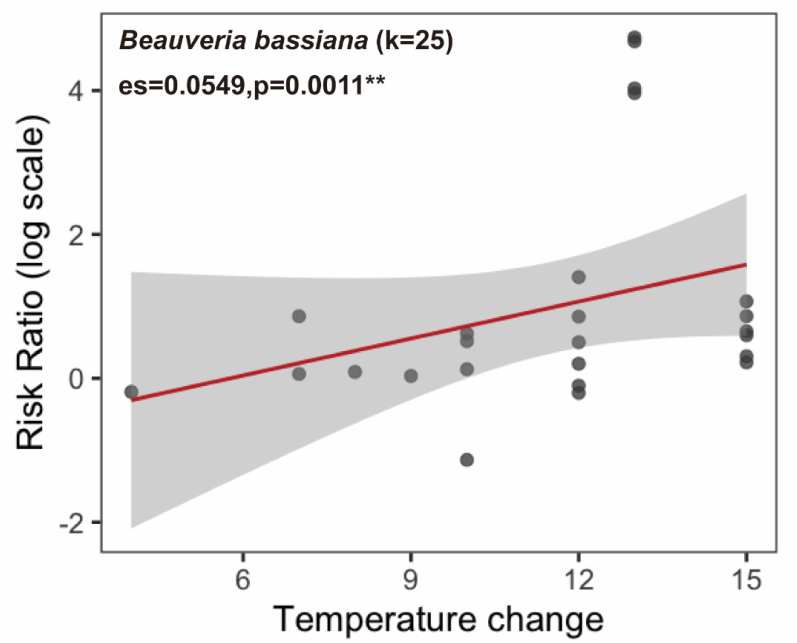

Fig. S6. Association between the scale of temperature change and effect sizes across studies of the pathogen Beauveria bassiana (k: 25 effect sizes across multiple hosts). The significance level is shown on the top left of each faceted plot.

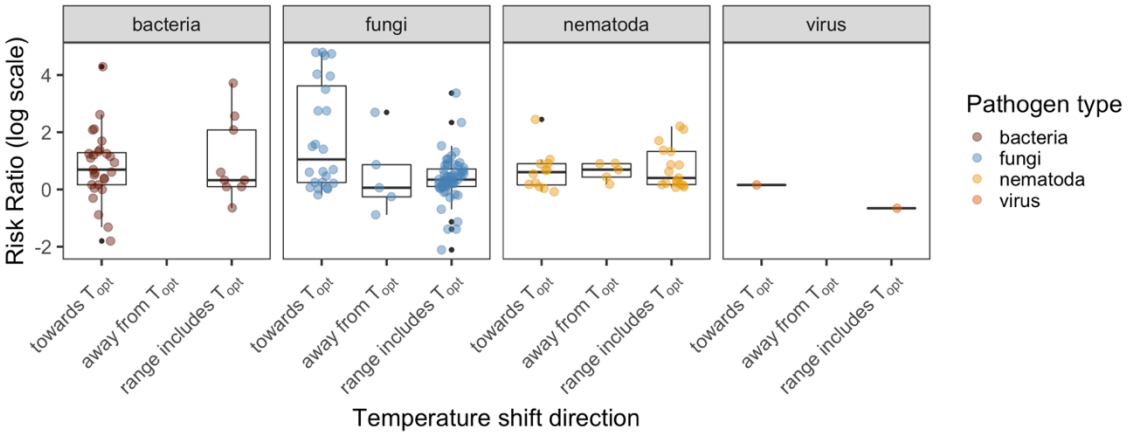

Fig. S7. Comparisons between effect sizes across temperature shift directions, within each pathogen type. Effect sizes for fungal pathogens have significant larger increase from “away from Topt” to “towards Topt”.

Supplementary Table 1 Summary for included host-pathogen systems and calculated effect sizes for meta-analysis

Study	host_name	pathogen_name	host_lifestage	host_taxa	habitat	host_field_or_lab	injected	dosage	exposure_time_day	temp_range_optimal	temp_span	ES	variance
Bally and Garrabou, 2007	Paramuricea_clavata	Vibrio coralliilyticus	adult	anthozoa	aquatic	field	no	2.00E+05	2	colder	3	2.61780451	1.9920796
Bally and Garrabou, 2007	Paramuricea_clavata	Vibrio coralliilyticus	adult	anthozoa	aquatic	field	no	2.00E+05	2	colder	8	2.11084 33	2.088425 38
Vale et al., 2008	Daphnia_magna	Pasteuria ramosa	adult	crustacea	aquatic	lab	yes	10000	45	unknown	10	3.34116425	1.96552829
Vale and Little, 2009	Daphnia_magna	Pasteuria ramosa	adult	crustacea	aquatic	lab	yes	10000	45	unknown	10	2.09840218	0.91865067
Vezzulli et al., 2010	Paramuricea_clavata	Vibrio harveyi	adult	anthozoa	aquatic	field	no	1.00E+05	8	colder	6	1.38524381	2.00052555
Vezzulli et al., 2010	Paramuricea_clavata	Vibrio harveyi	adult	anthozoa	aquatic	field	no	1.00E+05	8	colder	6	1.24921382	2.07346026
Vezzulli et al., 2010	Paramuricea_clavata	Vibrio harveyi	adult	anthozoa	aquatic	field	no	1.00E+05	8	colder	6	1.69477986	2.56997443
Jiang et al., 2013	Haliotis_diversicolor	Vibrio harveyi	adult	mollusca	aquatic	field	no	1.00E+06	17	covered	8	3.71843826	1.98402756

Schade et al., 2014	Gasterosteus_aculeatus	Vibrio sp.	adult	marine fish	aquatic	field	yes	1.00E+05	10	unknown	4	0.4389878	0.5344821 8
Ushijima et al., 2014	Montipora_capitata	Vibrio coralliilyticus	adult	anthozoa	aquatic	field	no	1.00E+08	7	colder	4	0.16393169	0.0493832 3
Lokmer and Wegner, 2015	Crassostrea_gigas	Vibrio sp.	adult	mollusca	aquatic	field	yes	1.00E+08	7	unknown	14	0.40546511	0.2499166 8
Lokmer and Wegner, 2015	Crassostrea_gigas	Vibrio sp.	adult	mollusca	aquatic	field	yes	1.00E+08	7	unknown	14	0.51070564	0.0999586 7
Waki and Yoshinaga, 2015	Venerupis_philippinarum	Perkinsus olseni	adult	mollusca	aquatic	lab	no	2.00E+07	28.5	optimal	10	1.15003564	0.0845206 6
Lopez et al., 2017	Sparus_aurata	Pseudomonas baetica	adult	fish	aquatic	lab	yes	2500000	10	optimal	6	-1.8010442	0.2928275
Lopez et al., 2017	Dicologoglossa_cuneata	Pseudomonas baetica	adult	fish	aquatic	lab	yes	1.00E+06	10	optimal	6	0.04693822	0.0046671 4
Lopez et al., 2017	Solea_senegalensis	Pseudomonas baetica	adult	fish	aquatic	lab	yes	1250000	10	optimal	6	0	0.0023228 8

Lopez et al., 2017	Dicentrarchus_labrax	Pseudomonas baetica	adult	fish	aquatic	lab	yes	5.00E+05	10	optimal	6	-1.3185901	0.1358910 4
Lopez et al., 2017	Argyrosomus_regius	Pseudomonas baetica	adult	fish	aquatic	lab	yes	1.1E+07	10	optimal	6	-0.8860218	0.0718575 8
Vater et al., 2018	Haliotis_rufescens	Xenohaliotis californiensis	adult	mollusca	aquatic	lab	no	NA	123.5	unknown	4.9	1.4942515	2.4180638
Vater et al., 2018	Haliotis_rufescens	Xenohaliotis californiensis	adult	mollusca	aquatic	lab	no	NA	123.5	unknown	4.9	0.6115600 7	0.6554059 2
Larsen et al., 2018	Gadus_morhua	Brucella pinnipedialis	adult	fish	aquatic	lab	yes	1.25E+08	20	colder	9	1.3217558 4	0.8462963
De Silva et al., 2018	Galleria_mellonella	Acinetobacter baumannii	adult	insecta	terrestrial	lab	yes	10000	1	optimal	9	0.1633466 6	0.7865224 4
Wei et al., 2019	Scapharca_broughtonii	Vibrio harveyi	adult	mollusca	aquatic	field	yes	1.00E+05	12	colder	10	4.2904594 4	1.9733432 1
Jiang et al., 2019	Carassius_carassius	Edwardsiella tarda	adult	fish	aquatic	lab	yes	1000	15	optimal	10	1.0957592 2	0.1234025 2

Chideroli et al., 2017	Tilapia_sparrmanii	Streptococcus agalactiae	juvenile	fish	aquatic	lab	yes	1210000	10	covered	9	2.5649493 6	1.9720279 7
Chideroli et al., 2017	Tilapia_sparrmanii	Streptococcus agalactiae	juvenile	fish	aquatic	lab	yes	1040000	10	covered	9	2.0794415 4	0.925
Petersen and Tisa, 2012	Manduca_sexta	Serratia marcescens	larva	insecta	terrestrial	lab	yes	40000	7	covered	15	0.0968498 3	0.0376233 9
Petersen and Tisa, 2012	Manduca_sexta	Serratia marcescens	larva	insecta	terrestrial	lab	yes	40000	7	covered	15	0.0968498 3	0.0376233 9
Petersen and Tisa, 2012	Manduca_sexta	Serratia marcescens	larva	insecta	terrestrial	lab	yes	40000	7	covered	15	0.3242396 7	0.0808978 3
Petersen and Tisa, 2012	Manduca_sexta	Serratia marcescens	larva	insecta	terrestrial	lab	yes	40000	7	covered	15	0.3242396 7	0.0808978 3
Rejasse et al., 2012	Galleria_mellonella	Bacillus weihenstephanensis	larva	insecta	terrestrial	lab	no	3500000	8	optimal	15	0.5819215 5	0.4925855 4
Rejasse et al., 2012	Galleria_mellonella	Bacillus weihenstephanensis	larva	insecta	terrestrial	lab	no	3500000	8	optimal	15	-0.3033074	0.3468880 3

Ushijima et al., 2018	Crassostrea_gigas	Vibrio coralliilyticus	larva	mollusca	aquatic	lab	no	50000	3	colder	4	0.39603997	0.02372498
Ushijima et al., 2018	Crassostrea_gigas	Vibrio tasmaniensis	larva	mollusca	aquatic	lab	no	50000	3	unknown	4	0.14660347	0.36702552
Ushijima et al., 2018	Crassostrea_gigas	Vibrio coralliilyticus	larva	mollusca	aquatic	lab	no	50000	3	colder	4	0.50568422	0.10105883
Ushijima et al., 2018	Crassostrea_gigas	Vibrio coralliilyticus	larva	mollusca	aquatic	lab	no	50000	3	colder	4	0.61023527	0.02149734
Ushijima et al., 2018	Crassostrea_gigas	Vibrio coralliilyticus	larva	mollusca	aquatic	lab	no	50000	3	colder	4	0.36139965	0.02271936
Buisson et al., 2019	Galleria_mellonella	Bacillus thuringiensis	larva	insecta	terrestrial	lab	yes	20000	2	covered	12	0.60230008	0.01835627
Buisson et al., 2019	Galleria_mellonella	Bacillus thuringiensis	larva	insecta	terrestrial	lab	yes	20000	2	covered	12	-0.6422729	0.01712059
Yang et al. 2021	Crassostrea gigas	Vibrio alginolyticus	adult	mollusca	aquatic	field	yes	5.00E+06	7	optimal	10	1.25276297	0.14761905

Sepahi et al. 2013	rainbow trout	Streptococcus agalactiae	juvenile	fish	aquatic	lab	yes	1.00E+06	14	colder	6	0.69314718	0.1
Phuoc et al. 2021	Oreochromis sp.	Streptococcus agalactiae	juvenile	fish	aquatic	lab	yes	15000	14	optimal	10	2.07944154	0.30833333
Phuoc et al. 2021	Oreochromis sp.	Streptococcus agalactiae	juvenile	fish	aquatic	lab	yes	20000	14	optimal	10	1.19815696	0.11972363
Phuoc et al. 2021	Oreochromis sp.	Streptococcus agalactiae	juvenile	fish	aquatic	lab	yes	30000	14	optimal	10	0.94155126	0.11024848
Rodrigues et al., 2016	Rhodnius_prolixus	Trypanosoma rangeli	larva	insecta	terrestrial	lab	yes	500	30	covered	9	1.79175947	1.06666667
Ekesi et al., 1999	Megalurothrips_sjostedti	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	14	covered	15	0.86538154	0.03982167
Ekesi et al., 1999	Megalurothrips_sjostedti	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	14	covered	15	1.06971704	0.02393665
Ekesi et al., 1999	Megalurothrips_sjostedti	Metarhizium anisopliae	adult	insecta	terrestrial	lab	no	1.00E+08	14	covered	15	0.32471553	0.00491904

Ekesi et al., 1999	Megalurothrips_sjostedti	Metarhizium anisopliae	adult	insecta	terrestrial	lab	no	1.00E+08	14	covered	15	-0.1740321	0.00579434
Ekesi et al., 1999	Megalurothrips_sjostedti	Metarhizium anisopliae	adult	insecta	terrestrial	lab	no	1.00E+08	14	covered	15	1.05248761	0.02331819
Ekesi et al., 1999	Megalurothrips_sjostedti	Metarhizium anisopliae	adult	insecta	terrestrial	lab	no	1.00E+08	14	covered	15	0.4427994	0.00707336
Yeo et al., 2003	Aphis_fabae	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	9	colder	13	4.73970108	1.98469563
Yeo et al., 2003	Myzus_persicae	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	9	colder	13	4.68350066	1.98570629
Yeo et al., 2003	Aphis_fabae	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	9	colder	13	4.02917092	0.97637805
Yeo et al., 2003	Myzus_persicae	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	9	colder	13	3.96443472	0.9775583
Yeo et al., 2003	Myzus_persicae	Paecilomyces fumosoroseus	adult	insecta	terrestrial	lab	no	1.00E+08	9	optimal	13	4.79012161	1.98383601
Yeo et al., 2003	Aphis_fabae	Paecilomyces fumosoroseus	adult	insecta	terrestrial	lab	no	1.00E+08	9	optimal	13	4.79012161	1.98383601

Yeo et al., 2003	Aphis_fabae	Paecilomyces fumosoroseus	adult	insecta	terrestrial	lab	no	1.00E+08	9	optimal	13	2.74654037	0.24729679
Yeo et al., 2003	Myzus_persicae	Paecilomyces fumosoroseus	adult	insecta	terrestrial	lab	no	1.00E+08	9	optimal	13	2.74654037	0.24729679
Yeo et al., 2003	Myzus_persicae	Lecanicillium lecanii	adult	insecta	terrestrial	lab	no	1.00E+08	9	optimal	13	3.50084159	0.53898443
Yeo et al., 2003	Aphis_fabae	Lecanicillium lecanii	adult	insecta	terrestrial	lab	no	1.00E+08	9	optimal	13	1.57691472	0.06374204
Yeo et al., 2003	Aphis_fabae	Lecanicillium lecanii	adult	insecta	terrestrial	lab	no	1.00E+08	9	optimal	13	0.06695245	0.00141551
Yeo et al., 2003	Myzus_persicae	Lecanicillium lecanii	adult	insecta	terrestrial	lab	no	1.00E+08	9	optimal	13	0.05261904	0.00141474
Bugeme et al., 2008	Tetranychus_evansi	Beauveria bassiana	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.65376804	0.0126258
Bugeme et al., 2008	Tetranychus_evansi	Beauveria bassiana	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.30646999	0.00493173
Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	2.34158725	0.13070666

Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	1.5237732	0.0447454
Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	1.3072017	0.0353886
Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	1.1766074	0.0280209
Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.8312975	0.0224697
Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.8472978	0.0167165
Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.7615310	0.0168650
Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.6693361	0.0119907
Bugeme et al., 2008	Tetranychus_evansi	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.3439398	0.0079662

Bugeme et al., 2009	Tetranychus_urticae	Beauveria bassiana	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.59817585	0.0103247
Bugeme et al., 2009	Tetranychus_urticae	Beauveria bassiana	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.22159196	0.0032348
Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.9370006	0.0194367
Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.61090908	0.0106143
Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.43948199	0.0105616
Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.45496903	0.0103474
Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.53853521	0.0090166
Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.45488999	0.0076903

Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.34993697	0.00610826
Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.22159994	0.00394016
Bugeme et al., 2009	Tetranychus_urticae	Metarhizium anisopliae	adult	arachnida	terrestrial	lab	no	1.00E+07	10	covered	15	0.14357579	0.00270569
Tesfaye and Seyoum, 2010	Aphis_gossypii	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	6	unknown	10	0.27872209	0.01829887
Tesfaye and Seyoum, 2010	Aphis_gossypii	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+06	6	unknown	10	0.29552635	0.01006078
Tesfaye and Seyoum, 2010	Aphis_gossypii	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	6	unknown	10	0.22040007	0.00589702
Tesfaye and Seyoum, 2010	Aphis_gossypii	Beauveria bassiana	adult	insecta	terrestrial	lab	no	1.00E+08	6	unknown	10	0.07800107	0.007583

Tesfaye and Seyoum, 2010	Aphis_gossypii	Metarhizium anisopliae	adult	insecta	terrestrial	lab	no	1.00E+08	6	unknown	10	0.34169666	0.01890977
Tesfaye and Seyoum, 2010	Aphis_gossypii	Metarhizium anisopliae	adult	insecta	terrestrial	lab	no	1.00E+08	6	covered	10	0.22959677	0.01345314
Kikankie et al., 2010	Anopheles_arabiensis	Beauveria bassiana	adult	insecta	terrestrial	field	no	NA	5	optimal	4	-0.18975	0.8462958 3
Wekesa et al., 2010	Tetranychus_evansi	Neozygites floridana	adult	arachnida	terrestrial	field	no	NA	7	optimal	12	0.25034564	0.0010446 2
Wekesa et al., 2010	Tetranychus_evansi	Neozygites floridana	adult	arachnida	terrestrial	field	no	NA	7	optimal	12	0.46422998	0.0019770 7
Wekesa et al., 2010	Tetranychus_evansi	Neozygites floridana	adult	arachnida	terrestrial	field	no	NA	7	optimal	12	0.21934548	0.0009786
Heinig et al., 2015	Anopheles_stephensi	Beauveria bassiana	adult	insecta	terrestrial	lab	no	5.00E+08	9.5	optimal	12	1.40651962	0.0341736 3
Heinig et al., 2015	Anopheles_stephensi	Beauveria bassiana	adult	insecta	terrestrial	lab	no	5.00E+08	9.5	covered	8	0.08913569	0.0019069 4

Kerchev et al., 2017	Polygraphus_proximus	Beauveria bassiana	adult	insecta	terrestrial	field	no	5.00E+06	45	colder	10	0.62228764	0.0219032
Kerchev et al., 2017	Polygraphus_proximus	Isaria farinosa	adult	insecta	terrestrial	field	no	5.00E+06	45	colder	10	0.02481567	0.00085701
Kirk et al., 2018	Daphnia_magna	Microsporidia	adult	crustacea	aquatic	lab	no	112000	6.3	warmer	9.6	2.6943028	2.0943625
Saif-Ur-Rehman et al., 2019	Acyrthosiphon_pisum	Beauveria bassiana	adult	insecta	terrestrial	field	no	1.00E+08	7	covered	10	0.51622767	0.0295361
Saif-Ur-Rehman et al., 2019	Myzus_persicae	Beauveria bassiana	adult	insecta	terrestrial	field	no	1.00E+08	7	covered	10	0.12613201	0.0286691
Saif-Ur-Rehman et al., 2019	Acyrthosiphon_pisum	Paecilomyces fumosoroseus	adult	insecta	terrestrial	field	no	1.00E+08	7	covered	10	0.1952162	0.0248328
Saif-Ur-Rehman et al., 2019	Acyrthosiphon_pisum	Paecilomyces fumosoroseus	adult	insecta	terrestrial	field	no	1.00E+08	7	covered	10	0.32331027	0.0225801
Saif-Ur-Rehman	Myzus_persicae	Paecilomyces	adult	insecta	terrestrial	field	no	1.00E+08	7	covered	10	0.28702586	0.0216105

et al., 2019		fumosoroseus											
Saif-Ur-Rehman et al., 2019	Myzus_persicae	Paecilomyces fumosoroseus	adult	insecta	terrestrial	field	no	1.00E+08	7	covered	10	-0.0719331	0.01450008
Saif-Ur-Rehman et al., 2019	Myzus_persicae	Metarhizium anisopliae	adult	insecta	terrestrial	field	no	1.00E+08	7	covered	10	0.06312201	0.03962918
Saif-Ur-Rehman et al., 2019	Acyrthosiphon_pisum	Metarhizium anisopliae	adult	insecta	terrestrial	field	no	1.00E+08	7	covered	10	0.34069068	0.02920229
Keerio et al., 2020	Bemisia_tabaci	Beauveria bassiana	adult	insecta	terrestrial	field	no	1.00E+07	12	covered	12	-0.2031693	0.02235401
Keerio et al., 2020	Bemisia_tabaci	Beauveria bassiana	adult	insecta	terrestrial	field	no	1.00E+07	12	covered	12	-0.1008472	0.01471415
Keerio et al., 2020	Bemisia_tabaci	Lecanicillium lecanii	adult	insecta	terrestrial	field	no	1.00E+07	12	covered	12	-0.2819926	0.03238218
Seman et al., 2018	Danio_rerio	Candida albicans	juvenile	fish	aquatic	lab	yes	15	2	colder	12	1.5018527	0.25004432

Seman et al., 2018	Danio_rerio	Candida albicans	juvenile	fish	aquatic	lab	yes	15	2	colder	12	0.01798898	0.00053716
Hu et al., 1996	Riptortus_linearis	Beauveria bassiana	larva	insecta	terrestrial	field	no	3.40E+08	40	covered	10	-1.113428	0.0351295
Rao et al., 2006	Spodoptera_litura	Beauveria bassiana	larva	insecta	terrestrial	lab	no	2.00E+06	10	reverse_optimal	7	0.86374352	0.19154478
Rao et al., 2006	Spodoptera_litura	Beauveria bassiana	larva	insecta	terrestrial	lab	no	2.00E+06	10	reverse_optimal	7	0.06093133	0.02819687
Rao et al., 2006	Spodoptera_litura	Nomuraea rileyi	larva	insecta	terrestrial	lab	no	2.00E+06	10	reverse_optimal	7	-0.254495	0.1139845
Rao et al., 2006	Spodoptera_litura	Nomuraea rileyi	larva	insecta	terrestrial	lab	no	2.00E+06	10	reverse_optimal	7	-0.8872296	0.0153479
Garcia-Solache et al., 2013	Galleria_mellonella	Cryptococcus neoformans	larva	insecta	terrestrial	lab	yes	5.00E+05	6	optimal	4	0.2489796	0.00624998
Garcia-Solache et al., 2013	Galleria_mellonella	Cryptococcus amylolentus	larva	insecta	terrestrial	lab	yes	5.00E+05	15	unknown	4	-0.566854	0.0161468
Svedese et al., 2013	Diatraea_saccharalis	Beauveria bassiana	larva	insecta	terrestrial	field	no	NA	7	covered	12	0.85755024	0.0942951

Svedese et al., 2013	Diatraea_saccharalis	Beauveria bassiana	larva	insecta	terrestrial	field	no	NA	7	covered	12	0.50087529	0.0660066
Mesa-Arango et al., 2013	Galleria_mellonella	Candida tropicalis	larva	insecta	terrestrial	lab	yes	2.00E+06	10	covered	7	0.08518086	0.05478023
Mesa-Arango et al., 2013	Galleria_mellonella	Candida tropicalis	larva	insecta	terrestrial	lab	yes	2.00E+06	10	covered	7	0.11669826	0.00836094
Huang et al., 2015	Galleria_mellonella	Penicillium marneffei	larva	insecta	terrestrial	lab	yes	NA	5	covered	12	3.36729583	1.97372742
Huang et al., 2015	Galleria_mellonella	Penicillium marneffei	larva	insecta	terrestrial	lab	yes	NA	7	covered	12	-0.6931472	0.275
Huang et al., 2015	Galleria_mellonella	Penicillium marneffei	larva	insecta	terrestrial	lab	yes	NA	7	covered	12	-1.3862944	0.2125
Garcia et al., 2016	Rhodnius_prolixus	Beauveria bassiana	larva	insecta	terrestrial	lab	no	20000	15	covered	9	0.03289584	0.00215413
Garcia et al., 2016	Rhodnius_prolixus	Metarhizium anisopliae	larva	insecta	terrestrial	lab	no	1000	15	covered	9	0.89199804	0.04827076
Kryukov et al., 2018	Galleria_mellonella	Cordyceps militaris	larva	insecta	terrestrial	lab	yes	5000	45	covered	15	-1.3861444	0.18329084

Kryukov et al., 2018	Galleria_mellonella	Cordyceps militaris	pupae	insecta	terrestrial	lab	yes	5000	45	covered	5	-2.1091677	0.27222383
Heinig et al., 2015	Anopheles_stephensi	Beauveria bassiana	adult	insecta	terrestrial	lab	no	5.00E+08	9.5	covered	12	0.20225335	0.00301963
Ferguson and Sinclair 2020	Gryllus_veletis	Metarhizium anisopliae	larva	insecta	terrestrial	lab	yes	75000	9	optimal	19	0.60353502	0.10969388
Ferguson and Sinclair 2020	Gryllus_veletis	Metarhizium anisopliae	larva	insecta	terrestrial	lab	yes	75000	9	optimal	19	0.69314718	0.28571429
Tesfaye et al. 2014	Aphis gossypii	Beauveria (DLCO105)	adult	insecta	terrestrial	lab	no	1.00E+07	6	covered	10	0.52312315	0.05508478
Tesfaye et al. 2014	Aphis gossypii	Beauveria (DLCO105)	adult	insecta	terrestrial	lab	no	1.00E+07	6	unknown	10	0.6242186	0.05794445
Tesfaye et al. 2014	Aphis gossypii	Beauveria (DLCO105)	adult	insecta	terrestrial	lab	no	1.00E+07	6	unknown	10	0.16706982	0.03946913
Tesfaye et al. 2014	Aphis gossypii	Beauveria (DLCO105)	adult	insecta	terrestrial	lab	no	1.00E+07	6	unknown	10	0.3773853	0.02579544
Tesfaye et al. 2014	Aphis gossypii	Beauveria (DLCO105)	adult	insecta	terrestrial	lab	no	1.00E+07	6	unknown	10	-0.0407845	0.03722883
Tesfaye et al. 2014	Aphis gossypii	Beauveria (DLCO105)	adult	insecta	terrestrial	lab	no	1.00E+07	6	unknown	10	0.12771064	0.04101307

Trdan et al., 2009	Leptinotarsa_decemlineata	Steinernema feltiae	adult	insecta	terrestrial	field	no	10000	7	covered	5	0.07625644	0.01392461
Yuksel et al., 2019	Rhyzopertha_dominica	Heterorhabditis bacteriophora	adult	insecta	terrestrial	lab	no	500	8	unknown	10	0.3254224	0.11559829
Yuksel et al., 2019	Rhyzopertha_dominica	Heterorhabditis bacteriophora	adult	insecta	terrestrial	lab	no	500	8	unknown	10	0	0.10151515
Yuksel et al., 2019	Sitophilus_granarius	Heterorhabditis bacteriophora	adult	insecta	terrestrial	lab	no	500	8	unknown	10	0.04879016	0.03134921
Yuksel et al., 2019	Sitophilus_granarius	Heterorhabditis bacteriophora	adult	insecta	terrestrial	lab	no	500	8	unknown	10	0.10536052	0.02539683
Yuksel et al., 2019	Rhyzopertha_dominica	Steinernema feltiae	adult	insecta	terrestrial	lab	no	500	8	covered	10	0.23841102	0.12191142
Yuksel et al., 2019	Rhyzopertha_dominica	Steinernema feltiae	adult	insecta	terrestrial	lab	no	500	8	covered	10	0.3254224	0.11559829
Yuksel et al., 2019	Sitophilus_granarius	Steinernema feltiae	adult	insecta	terrestrial	lab	no	500	8	covered	10	0.14732471	0.02392912

Yuksel et al., 2019	Sitophilus_granarius	Steinernema feltiae	adult	insecta	terrestrial	lab	no	500	8	covered	10	0.17034537	0.01583477
Grewal et al., 1996	Galleria_mellonella	Heterorhabditis bacteriophora	larva	insecta	terrestrial	lab	no	NA	10	covered	22	1.36888481	0.00436746
Grewal et al., 1996	Galleria_mellonella	Steinernema anomali	larva	insecta	terrestrial	lab	no	NA	10	unknown	25	-0.0086232	0.00087444
Long et al., 2000	Otiorhynchus_sulcatus	Heterorhabditis megidis	larva	insecta	terrestrial	lab	yes	100	17.5	colder	12	0.66783142	1.00025639
Long et al., 2000	Otiorhynchus_sulcatus	Heterorhabditis megidis	larva	insecta	terrestrial	lab	yes	100	17.5	colder	12	0.09517052	0.03613478
Long et al., 2000	Otiorhynchus_sulcatus	Steinernema feltiae	larva	insecta	terrestrial	lab	yes	100	17.5	optimal	12	0.89567144	0.1712585
Long et al., 2000	Otiorhynchus_sulcatus	Steinernema feltiae	larva	insecta	terrestrial	lab	yes	100	17.5	optimal	12	0.03950244	0.00270426
Long et al., 2000	Otiorhynchus_sulcatus	Steinernema kraussei	larva	insecta	terrestrial	lab	yes	100	17.5	colder	12	2.44809268	0.91711847
Long et al., 2000	Otiorhynchus_sulcatus	Steinernema kraussei	larva	insecta	terrestrial	lab	yes	100	17.5	colder	12	1.05098424	0.1678011
Long et al., 2000	Otiorhynchus_sulcatus	Steinernema kraussei	larva	insecta	terrestrial	lab	yes	100	17.5	colder	12	0.22391545	0.01067301

Long et al., 2000	Otiorhynchus_sulcatus	Steinernema kraussei	larva	insecta	terrestrial	lab	yes	100	17.5	colder	12	-0.0778193	0.00173562
Del Valle et al., 2014	Galleria_mellonella	Steinernema diaprepesi	larva	insecta	terrestrial	lab	yes	100	6	covered	25	2.10413415	1.91056911
Shapiro-Ilan et al., 2014	Galleria_mellonella	Heterorhabditis floridensis	larva	insecta	terrestrial	lab	no	2000	7	unknown	8	1.00865652	0.05924661
Shapiro-Ilan et al., 2014	Galleria_mellonella	Heterorhabditis floridensis	larva	insecta	terrestrial	lab	no	2000	7	unknown	8	0.53111918	0.02706982
Shaurub et al., 2015	Ceratitis_capitata	Heterorhabditis bacteriophora	larva	insecta	terrestrial	lab	no	250000	NA	covered	20	1.70474809	0.00867701
Shaurub et al., 2015	Ceratitis_capitata	Heterorhabditis bacteriophora	larva	insecta	terrestrial	lab	no	250000	NA	covered	20	1.32890968	0.00477414
Shaurub et al., 2015	Ceratitis_capitata	Steinernema carpocaps	larva	insecta	terrestrial	lab	no	250000	NA	covered	20	0.62497264	0.00399845
Shaurub et al., 2015	Ceratitis_capitata	Steinernema riobrave	larva	insecta	terrestrial	lab	no	250000	NA	covered	20	2.20532179	0.01923201

Yuksel et al., 2019	Ephestia_kuehniella	Heterorhabditis bacteriophora	larva	insecta	terrestrial	lab	no	50	4	unknown	10	0.07232066	0.01031977
Yuksel et al., 2019	Ephestia_kuehniella	Heterorhabditis bacteriophora	larva	insecta	terrestrial	lab	no	50	4	unknown	10	0.11375889	0.0070022
Yuksel et al., 2019	Ephestia_kuehniella	Steinernema feltiae	larva	insecta	terrestrial	lab	no	50	4	covered	10	0.17034537	0.01583477
Yuksel et al., 2019	Ephestia_kuehniella	Steinernema feltiae	larva	insecta	terrestrial	lab	no	50	4	covered	10	0.09156719	0.01549658
Aatif et al., 2020	Bactrocera_dorsalis	Heterorhabditis bacteriophora	larva	insecta	terrestrial	field	no	50000	9	covered	10	0.21599419	0.00833278
Aatif et al., 2020	Bactrocera_dorsalis	Heterorhabditis indica	larva	insecta	terrestrial	field	no	50000	9	warmer	10	0.43409203	0.01508695
Aatif et al., 2020	Bactrocera_dorsalis	Steinernema carpocaps	larva	insecta	terrestrial	field	no	50000	9	unknown	10	0.19599385	0.01156387
Aatif et al., 2020	Bactrocera_dorsalis	Steinernema carpocaps	larva	insecta	terrestrial	field	no	50000	9	covered	10	0.40224264	0.01825586

Ogretmen et al., 2020	Agriotes	Heterorhabditis bacteriophora	larva	insecta	terrestrial	field	no	1400	21	unknown	5	-0.4054651	0.1583333
Ogretmen et al., 2020	Agriotes	Heterorhabditis indica	larva	insecta	terrestrial	field	no	1400	21	warmer	5	0.91629073	0.3
Ogretmen et al., 2020	Agriotes	Steinernema bicornotum	larva	insecta	terrestrial	field	no	1400	21	unknown	5	0	0.05
Ogretmen et al., 2020	Agriotes	Steinernema carpocaps	larva	insecta	terrestrial	field	no	1400	21	reverse_optimal	5	0.18232156	0.1333333
Ogretmen et al., 2020	Agriotes	Steinernema feltiae	larva	insecta	terrestrial	field	no	1400	21	warmer	5	0.69314718	0.2
Long et al., 2000	Otiorhynchus_sulcatus	Heterorhabditis megidis	pupae	insecta	terrestrial	lab	yes	100	17.5	colder	12	0.68787012	1.0000556
Long et al., 2000	Otiorhynchus_sulcatus	Steinernema feltiae	pupae	insecta	terrestrial	lab	yes	100	17.5	optimal	12	0.17645644	0.0895252
Long et al., 2000	Otiorhynchus_sulcatus	Steinernema kraussei	pupae	insecta	terrestrial	lab	yes	100	17.5	colder	12	0.54361545	0.0669802
Long et al., 2000	Otiorhynchus_sulcatus	Steinernema kraussei	pupae	insecta	terrestrial	lab	yes	100	17.5	colder	12	0.90895492	0.0222456

Aatif et al., 2020	Bactrocera_dorsalis	Heterorhabditis bacteriophora	pupae	insecta	terrestrial	field	no	50000	9	covered	10	0.85434119	0.061815
Aatif et al., 2020	Bactrocera_dorsalis	Heterorhabditis indica	pupae	insecta	terrestrial	field	no	50000	9	warmer	10	0.89891213	0.08031733
Aatif et al., 2020	Bactrocera_dorsalis	Steinernema carpocaps	pupae	insecta	terrestrial	field	no	50000	9	unknown	10	1.03539333	0.08837006
Aatif et al., 2020	Bactrocera_dorsalis	Steinernema carpocaps	pupae	insecta	terrestrial	field	no	50000	9	covered	10	0.86244868	0.09480339
Delisle et al., 2018	Crassostrea_gigas	Ostreid herpesvirus	adult	mollusca	aquatic	field	yes	6900000	3	covered	8	-0.6567795	0.01369312
Páez et al., 2021	Oncorhynchus_mykiss	hematopoietic necrosis virus	larva	fish	aquatic	lab	no	50000	17	optimal	9	0.15704713	0.03707268
Suchman et al. 2006	Aedes aegypti	AeDNV	larva	insecta	terrestrial	lab	no	5.00E+09	8	unknown	4	-0.3551039	0.00604994
Brand et al. 2016	Wood frog	FV3-like ranavirus	larva	amphibian	aquatic	field	no	1000	28	unknown	15	3.71357207	1.95354239
Brand et al. 2016	Green frog	FV3-like ranavirus	larva	amphibian	aquatic	field	no	1000	28	unknown	15	2.01440907	0.94944371

Supplementary Table 2 Pathogen in vitro T_{opt} data collected from published literatures

Species	**taxa**	**pathogen_optimal_temp**	**pathogen_growth_upper**	**pathogen_growth_lower**	**trait_measured**	**Reference**
Acinetobacter baumannii	bacteria	37	25	45	in vitro growth	Antunes L. C. S., Imperi F., Carattoli A. & Visca P. 2011 Deciphering the multifactorial nature of Acinetobacter baumannii pathogenicity. PLoS One 6 (8), e22674.
AeDNV	virus	unknown	unknown	unknown	NA	NA
Bacillus thuringiensis	bacteria	30	15	40	in vitro growth and biomass profuction	Dagga, A., Aziz, M.E., Elmanama, A.A., Al-Sharif, M., jubb, A.A., & Hindi, M.W. (2016). Isolation, characterization & optimization of cultural condition for bacillus thuringinsis bt from soils of gaza strip.
Bacillus weihenstephanensis	bacteria	30	7	30	in vitro growth	Logan N.A. De Vos P. (2009) Genus I. Bacillus Cohn 1872, 174AL. Bergey's Manual of Systematic Bacteriology3 (De Vos P. Guarrity G.M. Jones D. Krieg N.R. Ludwig W. Rainey F.A. Schleifer K-H. Whitman W.B., eds), pp. 21–128. Springer, New York, NY.
Beauveria bassiana	fungi	25-28	8	35	in vitro vegetative growth	Fargues J, Goettel MS, Smits N et al. (1997) Effect of temperature on vegetative growth of Beauveria bassianaisolates from different origins. Mycologia 89:389–392
Brucella pinnipedialis	bacteria	37	20	40	in vitro growth	Johansson, Karl-Erik (2014): VetBact - culturing bacteriological knowledge for

						veterinarians. Veterinary Record 174: 162-164 doi: 10.1136/vr.g162
Candida albicans	fungi	37	unknown	42	in vitro germ tube formation	S. Nadeem, A. Shafiq, S. Hakim, Y. Anjum and S. U. Kazm, "Effect of Growth Media, pH and Temperature on Yeast to Hyphal Transition in Candida albicans," Open Journal of Medical Microbiology, Vol. 3 No. 3, 2013, pp. 185-192. doi: 10.4236/ojmm.2013.33028.
Candida tropicalis	fungi	25-35	unknown	unknown	growth	Wilson, C. B.; Nizet, V.; Maldonado, Y.; Remington, J. S.; Klein, J. O. (2015). Remington and Klein's Infectious diseases of the Fetus and Newborn Infant. Philadelphia, US: Saunders. ISBN 9780323241472.
Cordyceps militaris	fungi	20	10	25	in vitro growth	Jiaojiao, Z., Fen, W., Kuanbo, L., Qing, L., Ying, Y., & Caihong, D. (2018). Heat and light stresses affect metabolite production in the fruit body of the medicinal mushroom Cordyceps militaris. Applied microbiology and biotechnology, 102(10), 4523–4533. https://doi.org/10.1007/s00253-018-8899-3
Cryptococcus amylolentus	fungi	unknown	unknown	37	in vivo tolerance	Bloom, A., Jin, R. M., Leipheimer, J., Bard, J. E., Yergeau, D., Wohlfert, E. A., & Panepinto, J. C. (2019). Thermotolerance in the pathogen Cryptococcus neoformans is linked to antigen masking via mRNA decay-dependent reprogramming. Nature

						communications, 10(1), 4950. https://doi.org/10.1038/s41467-019-12907-x
Cryptococcus neoformans	fungi	32-40	unknown	unknown	NA	NA
Edwardsiella tarda	bacteria	30-37	15	40	in vitro growth	Ishihara S, Kusuda R: Growth and survival of Edwardsiella tarda bacteria in environmental water. Bull Jpn Soc Sci Fish. 1982, 48: 483-488.
hematopoietic necrosis virus	virus	15	4	20	in vivo growth, virus	CHINCHAR, V. G. (2000). Ecology of Viruses of Cold-Blooded Vertebrates. Viral Ecology, 413–445. https://doi.org/10.1016/B978-012362675-2/50012-4
Heterorhabditis bacteriophora	nemato da	unknown	12	25	The nematodes are infective when the media temperature ranges from 12 to 25°C.	https://www.bioforce.co.nz/products/nematop.html
Heterorhabditis floridensis	nemato da	unknown	unknown	37	by infection efficacy	Shapiro-Ilan, D. I., Blackburn, D., Duncan, L., El-Borai, F. E., Koppenhöfer, H., Tailliez, P., & Adams, B. J. (2014). Characterization of Biocontrol Traits in Heterorhabditis floridensis: A Species with Broad Temperature Tolerance. Journal of nematology, 46(4), 336–345.

Heterorhabditis indica	nematoda	15	5	unknown	in vitro survival	Strauch, O., Niemann, I., Neumann, A. et al. Storage and formulation of the entomopathogenicnematodes Heterorhabditis indica and H. bacteriophora. BioControl 45, 483–500 (2000). https://doi.org/10.1023/A:1026528727365
Heterorhabditis megidis	nematoda	20-24	unknown	unknown	infection, reproduction, and development were fastest	Saunders, J. E., & Webster, J. M. (1999). Temperature Effects on Heterorhabditis megidis and Steinernema carpocapsae Infectivity to Galleria mellonella. Journal of nematology, 31(3), 299–304.
Isaria farinosa	fungi	20 (variable findings: 20-25, 15-25, 14-18)	unknown	unknown	in vitro culture	Liu, F., Xiang, M., Guo, Y. et al. Culture conditions and nutrition requirements for the mycelial growth of Isaria farinosa (Hypocreales: Cordycipitaceae) and the altitude effect on its growth and metabolome. Sci Rep 8, 15623 (2018). https://doi.org/10.1038/s41598-018-33965-z
Isaria fumosorosea	fungi	25 (20-30)	unknown	unknown	vegetative growth	Vidal, C., Fargues, J., & Lacey, L. A. (1997). Intraspecific variability of Paecilomyces fumosoroseus: Effect of temperature on vegetative growth. Journal of Invertebrate Pathology, 70, 18–26. https://doi.org/10.1006/jipa.1997.4658

Lecanicillium lecanii; Metarhizium anisopliae	fungi	25	15	30	germination, growth and sporulation	Onsongo, S. K., Gichimu, B. M., Akutse, K. S., Dubois, T., & Mohamed, S. A. (2019). Performance of Three Isolates of Metarhizium Anisopliae and Their Virulence against Zeugodacus Cucurbitae under Different Temperature Regimes, with Global Extrapolation of Their Efficiency. Insects, 10(9), 270. https://doi.org/10.3390/insects10090270
Metarhizium brunneum	fungi	25 (25-30)	unknown	unknown	in vitro growth	Couceiro JC, Fatoretto MB, Demétrio CGB, Meyling NV and Delalibera Í Jr (2021) UV-B Radiation Tolerance and Temperature-Dependent Activity Within the Entomopathogenic Fungal Genus Metarhizium in Brazil. Front. Fungal Biol. 2:645737. doi: 10.3389/ffunb.2021.645737
Neozygites floridana	fungi	25, 29	unknown	unknown	germination rates optimal at 25 and sporulation rates optimal at 29	Wekesa, V. W., Moraes, G. J., Ortega, E. M., & Delalibera, I., Jr (2010). Effect of temperature on sporulation of Neozygites floridana isolates from different climates and their virulence against the tomato red spider mite, Tetranychus evansi. Journal of invertebrate pathology, 103(1), 36–42. https://doi.org/10.1016/j.jip.2009.10.003
Nomuraea rileyi	fungi	25	5	35	optimum temperature for	C. M. Ignoffo, C. Garcia, D. L. Hostetter, Effects of Temperature on Growth and

					mycelial growth and sporulation was 25°C	Sporulation of the Entomopathogenic Fungus Nomuraea rileyi, Environmental Entomology, Volume 5, Issue 5, 1 October 1976, Pages 935–936, https://doi.org/10.1093/ee/5.5.935
Ordospora colligata	fungi	19	unknown	unknown	optimal performance (growth, reproduction, infection)	Charlotte Kunze, Pepijn Luijckx, Andrew L Jackson, Ian Donohue (2022) Alternate patterns of temperature variation bring about very different disease outcomes at different mean temperatures eLife 11:e72861 https://doi.org/10.7554/eLife.72861
Ostreid herpesvirus	virus	26	21	unknown	virus, virulence and infectivity	Delisle, L., Petton, B., Burguin, J. F., Morga, B., Corporeau, C., & Pernet, F. (2018). Temperature modulate disease susceptibility of the Pacific oyster Crassostrea gigas and virulence of the Ostreid herpesvirus type 1. Fish & shellfish immunology, 80, 71–79. https://doi.org/10.1016/j.fsi.2018.05.056
Paecilomyces fumosoroseus	fungi	20-30	unknown	unknown	vegetative growth	Vidal, C., Fargues, J., & Lacey, L. A. (1997). Intraspecific Variability ofPaecilomyces fumosoroseus:Effect of Temperature on Vegetative Growth. Journal of Invertebrate Pathology, 70(1), 18–26. https://doi.org/10.1006/JIPA.1997.4658
Penicillium marneffei	fungi	17-28	unknown	unknown	mold growth	Pruksaphon, K., Nosanchuk, J. D., Ratanabanangkoon, K., & Youngchim, S.

						(2022). Talaromyces marneffei Infection: Virulence, Intracellular Lifestyle and Host Defense Mechanisms. Journal of Fungi 2022, Vol. 8, Page 200, 8(2), 200. https://doi.org/10.3390/JOF8020200
Perkinsus olseni	bacteria	15-32	unknown	unknown	sporulation	La Peyre, M., Casas, S., & La Peyre, J. (2006). Salinity effects on viability, metabolic activity and proliferation of three Perkinsus species. Diseases of aquatic organisms, 71(1), 59–74. https://doi.org/10.3354/dao071059
Pseudomonas baetica	bacteria	20-25	4	30	in vitro growth	https://bacdive.dsmz.de/strain/13189
Serratia marcescens; Serratia sp.	bacteria	25-37	5	40	growth	Petersen, L. M., & Tisa, L. S. (2012). Influence of temperature on the physiology and virulence of the insect pathogen Serratia sp. Strain SCBI. Applied and environmental microbiology, 78(24), 8840–8844. https://doi.org/10.1128/AEM.02580-12
Steinernema carpocaps	nemato da	25	unknown	unknown	in vitro maximum yields	Hirao, A., & Ehlers, R. U. (2009). Effect of temperature on the development of Steinernema carpocapsae and Steinernema feltiae (Nematoda: Rhabditida) in liquid culture. Applied microbiology and biotechnology, 84(6), 1061–1067. https://doi.org/10.1007/s00253-009-2035-3

Steinernema diaprepesi	nematoda	20-25	10	40	optimum temperature for reproduction	del Valle, E. E., Balbi, E. I., Lax, P., Rondán Dueñas, J., & Doucet, M. E. (2014). Ecological aspects of an isolate of Steinernema diaprepesi (Rhabditida: Steinernematidae) from Argentina. Biocontrol Science and Technology, 24(6), 690–704. https://doi.org/10.1080/09583157.2014.890171
Steinernema feltiae	nematoda	14-26	unknown	unknown	performance	https://www.koppert.com/news/2021/extended-season-for-nematode-biocontrol-with-entonem/
Steinernema kraussei; Steinernema kraussei	nematoda	25	5	30	performance	https://www.biobestgroup.com/en/biobest/products/biological-pest-control-4463/beneficial-nematodes-4487/kraussei-system-4623/; Hirao, A., & Ehlers, R. U. (2009). Effect of temperature on the development of Steinernema carpocapsae and Steinernema feltiae (Nematoda: Rhabditida) in liquid culture. Applied microbiology and biotechnology, 84(6), 1061–1067. https://doi.org/10.1007/s00253-009-2035-3
Steinernema riobrave	nematoda	25	15	35	development and infection	life cycle of Steinernema abbasi and S. riobrave in Galleria mellonella. (n.d.). Retrieved October 3, 2022, from https://agris.fao.org/agris-

						search/search.do?recordID=US201302943572
Streptococcus agalactiae	bacteria	30	5	45	in vitro growth	Laith, A. A., Ambak, M. A., Hassan, M., Sheriff, S. M., Nadirah, M., Draman, A. S., Wahab, W., Ibrahim, W. N., Aznan, A. S., Jabar, A., & Najiah, M. (2017). Molecular identification and histopathological study of natural Streptococcus agalactiae infection in hybrid tilapia (Oreochromis niloticus). Veterinary world, 10(1), 101–111. https://doi.org/10.14202/vetworld.2017.101-111
Trypanosoma rangeli	euglenozoa	27	unknown	unknown	in vitro growth	Ferreira, R.C., Teixeira, C.F., de Sousa, V.F.A. et al. Effect of temperature and vector nutrition on the development and multiplication of Trypanosoma rangeli in Rhodnius prolixus. Parasitol Res 117, 1737–1744 (2018). https://doi.org/10.1007/s00436-018-5854-2
Verticillium lecanii	fungi	20-25	unknown	unknown	in vitro growth	Davidson, G., Phelps, K., Sunderland, K. D., Pell, J. K., Ball, B. V., Shaw, K. E., & Chandler, D. (2003). Study of temperature-growth interactions of entomopathogenic fungi with potential for control of Varroa destructor (Acari: Mesostigmata) using a nonlinear model of poikilotherm development. Journal of

						applied microbiology, 94(5), 816–825. https://doi.org/10.1046/j.1365-2672.2003.01871.x
Vibrio alginolyticus	bacteria	22-37	unknown	unknown	in vitro growth	Gu, D., Guo, M., Yang, M., Zhang, Y., Zhou, X., & Wang, Q. (2016). A σE-Mediated Temperature Gauge Controls a Switch from LuxR-Mediated Virulence Gene Expression to Thermal Stress Adaptation in Vibrio alginolyticus. PLoS pathogens, 12(6), e1005645. https://doi.org/10.1371/journal.ppat.1005645
Vibrio coralliilyticus	bacteria	>27	unknown	unknown	infective above 27	Kimes, N. E., Grim, C. J., Johnson, W. R., Hasan, N. A., Tall, B. D., Kothary, M. H., Kiss, H., Munk, A. C., Tapia, R., Green, L., Detter, C., Bruce, D. C., Brettin, T. S., Colwell, R. R., & Morris, P. J. (2012). Temperature regulation of virulence factors in the pathogen Vibrio coralliilyticus. The ISME journal, 6(4), 835–846. https://doi.org/10.1038/ismej.2011.154
Vibrio harveyi	bacteria	26	unknown	unknown	reproduction	Brehm, T.T., Berneking, L., Rohde, H. et al. Wound infection with Vibrio harveyi following a traumatic leg amputation after a motorboat propeller injury in Mallorca, Spain: a case report and review of literature. BMC Infect Dis 20, 104 (2020). https://doi.org/10.1186/s12879-020-4789-2

Supplementary Table 3 Results from modelling and statistical analyses.

Test for multicolinearity					
Factorial Moderator	**GVIF^(1/(2*Df))**				
host_lifestage	1.306142				
pathogen_system	1.439664				
host_field_or_lab	1.12698				
pathogen type	1.323923				
toward_optimal	1.105307				
host immunity	2.221351				
inoculation method	1.42379				
Continuous moderator	**Pearson coef**				
	temperature span	exposure_time_day	pathogen dosage		
temperature span	1	0.00920328	-0.1211842		
exposure_time_day	0.009203278	1	-0.0336458		
pathogen dosage	-0.121184198	-0.0336458	1		
Continuous moderator	**p value**				
	temperature span	exposure_time_day	pathogen dosage		
temperature span	0	0.07450622	0.104771		
exposure_time_day	0.07450622	0	0.6584693		
pathogen dosage	0.10477103	0.65846927	0		
Test for publication bias					
Moderator	**levels**	**estimate**	**se**	**tvalue**	**pval**
N_high	NA	-2.56E-05	6.48E-04	-0.039	0.969

N_low	NA	-3.01E-05	6.44E-04	-0.047	0.963
Test for the impact of different correction method for control mortality (p-val=0.6814)					
levels	**estimate**	**se**	**tvalue**	**pval**	
corrected (ref)	NA	NA	NA	NA	
infected_host_only	-0.33	4.71E-01	-7.01E-01	0.4831	
low_in_control	0.2039	3.24E-01	6.30E-01	0.529	
no_in_control	0.0096	2.61E-01	3.68E-02	0.9706	
Check for host and pathogen species	**pval**				
host species	0.0058				
pathogen species	<.0001				
Meta-analysis Summary Effect size (not at log scale)					
pred	**ci.lb**	**ci.ub**	**pval**	**Random factor**	
1.47	**1.13**	**1.93**	**0.0046 ****	Authors, host_species, pathogen_species	
Sub-group analysis					
Sub-group	**pred**	**ci.lb**	**ci.ub**	**pval**	**Random factor**
Host lifestage (adult, jevenile, larva, pupae)					
adult	1.7	1.15	2.51	0.0078 **	Authors, host_species, pathogen_species
jevenile	1.77	0.61	5.2	0.2956	Authors, host_species, pathogen_species
larva	1.27	0.92	1.74	0.1494	Authors, pathogen_species
pupae	0.89	0.14	5.58	0.9022	Authors, host_species, pathogen_species
Pathogen type (fungi, bacteria, nematoda, virus, euglenozoa)					

fungi	0.97	0.62	1.51	0.893	Authors, pathogen_species	host_species,
bacteria	2.25	1.49	3.39	0.0001	Authors, pathogen_species	host_species,
nematoda	1.66	0.99	2.76	0.0523	Authors, pathogen_species	host_species,
virus	1.28	0.39	4.21	0.6868	Authors, pathogen_species	
euglenozoa	NA	NA	NA	NA	Authors, host_species, pathogen_species	
Pathogen system (novel, established, non-local from natural system)						
novel	1.24	0.91	1.69	0.1639	Authors, pathogen_species	host_species,
established	4.94	1.11	21.93	0.0357	Authors, pathogen_species	host_species,
non-local from natural system	1.71	1.3	2.24	0.0001	pathogen_species	
Pathogen system, within invertebrates (novel, established, non-local from natural system)			**established all in invertebrate hosts**			
novel	1.15	0.85	1.56	0.3727	Authors, pathogen_species	host_species,
established	4.94	1.11	21.93	0.0357	Authors, pathogen_species	host_species,
non-local from natural system	1.72	1.3	2.27	0.0001	pathogen_species	
Host habitat (terrestrial, aquatic), removed because of multicolinearity						
terrestrial	1.15	0.86	1.55	0.3423	Authors, pathogen_species	host_species,
aquatic	2.31	1.42	3.74	0.0007	host_species, pathogen_species	

Host collected or field (lab, field)						
lab	1.47	1.04	2.08	0.0296	Authors, pathogen_species	host_species,
field	1.45	0.99	2.11	0.0535	Authors, pathogen_species	host_species,
Host immunity (invertebrate, vertebrate)						
invertebrate	1.36	1.04	1.78	0.0259	Authors, pathogen_species	host_species,
vertebrate	1.91	0.94	3.85	0.0719	host_species, pathogen_species	
Temprature towards pathogen optimal or not (covered, yes, no)						
covered	1.34	0.93	1.93	0.1193	Authors, pathogen_species	host_species,
yes	1.81	1.15	2.85	0.0104	Authors, pathogen_species	host_species,
no	1.37	0.72	2.61	0.3401	Authors, pathogen_species	host_species,
Temprature towards pathogen optimal or not, within invertebrate hosts (covered, yes, no)						
covered	1.25	0.88	1.79	0.2125	Authors, pathogen_species	host_species,
yes	1.86	1.04	3.35	0.0374	Authors, pathogen_species	
no	1.37	0.72	2.61	0.3401	Authors, pathogen_species	host_species,
Pathogen inoculation method (By injection, Not by injection)						
injection	1.730086	1.102184	2.715695	0.0172	Authors, pathogen_species	host_species,

not injection	1.462288	1.047016	2.042266	0.0258	Authors, pathogen_species	host_species,

Moderator analysis -- Continuous moderators

Moderator	**es**	**se**	**zval**	**pval**	**random factor**	
exposure_time_day	-0.0012	0.0072	-0.1711	0.8642	Authors, pathogen_species	host_species,
temperature span	0.0576	0.0131	4.3906	<.0001	Authors, host_species, pathogen_species	
pathogen dosage	-0.0422	0.0173	-2.4398	0.0147	Authors, pathogen_species	host_species,

Chapter 5:

Prolonged warming strengthens selection for host resistance to infection

Prolonged warming strengthens selection for host resistance to infection

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Abstract

Global climate change is causing extreme heating events and intensifying infectious disease outbreaks. The selective impacts of these dual stressors on host organisms in a changing world remain unclear. Using experimental evolution with competing genotypes, we directly tested the selective impacts of warming on the evolutionary trajectory of nematode host resistance to infection by a natural bacterial pathogen. Across 10 host generations, we found that warming during host development, then continuing into maturity during infection selected for genetic-based host resistance. Periodic warming within host lifetime drove the loss of genetic-based resistance, despite pathogen presence. Warming during development resulted in plastic temperature-mediated protection which weakened the selection for more costly genetic-based resistance upon cooling to ambient levels. Host evolutionary trajectories were likely determined by the combination of fitness constraints of genetic-based resistance, host plasticity, condition-dependent pathogen virulence, and dilution of pathogen cells by resistant hosts. These findings improve our understanding of the evolutionary implications for patterns of infection and host persistence during global temperature change.

Introduction

Global climate change is leading to higher average temperatures, as well as more prolonged and extreme heat events (Dosio *et al.,* 2018). Increasing frequency and duration of heat periods result in higher chance for organisms to experience temporary or prolonged warming at some point of their life stages (IPCC 2013; Zhang *et al.,* 2015). Shifting environmental temperatures have the potential to modify host-pathogen dynamics (Rohr *et al.,* 2013; Franke *et al.,* 2019; Kunze *et al.,* 2022), and intensify infectious disease outbreaks (Jones *et al.,* 2008; Liang *et al.,* 2017). The disturbance caused by climate warming can worsen infection outcomes for hosts, with consequently serious declines in wildlife populations (Jones *et al.,* 2008; Mora *et al.,* 2022). Pathogens can become more virulent at warmer temperatures (Oh *et al.,* 2009; Kimes *et al.,* 2012), and hosts more vulnerable to succumbing when the dual stressors of heat and infection are combined (Hector *et al.,* 2021, 2023).

As climate change is ongoing with the annual mean surface temperature rise predicted up to 4°C in 2100 (Arias *et al.,* 2021), there are likely to be multi-generational effects of warming for animals and interactions with their pathogens. For example, higher pathogen virulence under warmer temperatures could more strongly select for gene-based resistance (van 1998; Wendling *et al.,* 2022). However, temperatures experienced during host development can cue plastic responses in resistance, with single-generation studies showing that early-age heat stress could confer resistance to infection later in life (Liew *et al.,* 2003; Singh & Aballay, 2006; Lee *et al.,* 2012; Prithika *et al.,* 2016; Janda *et al.,* 2019). Condition-dependent resistance such as this, if repeated across generations, might weaken

selection for more costly genetic-based host resistance (Dallas *et al.,* 2016). In addition to plasticity, selection for genetic-based resistance could be weakened by the presence of 'dilution effects' (Keesing & Ostfeld, 2021; Civitello *et al.,* 2015). Variability in host resistance in a population can benefit susceptible individuals in populations by reducing pathogen density and transmission in the environment (Keesing & Ostfeld, 2021; Civitello *et al.,* 2015). Dilution effects would reduce the likelihood of pathogens encountering susceptible hosts. Therefore, host plasticity and dilution effects could potentially impact host resistance evolution under warming. However, it is still unclear how host resistance evolutionary dynamics will be shaped by warming and infection.

Here, we directly tested the selective impact of warming across timescales and life-stages on resistance evolution in host populations. We used *Caenorhabditis elegans* and a bacterial pathogen *Leucobacter musarum*, naturally isolated from congeners in rotting banana stems in Cape Verde (Hodgkin *et al.,* 2013). This pathogen causes a lethal infection in the lab-adapted N2 worm isolate, but the mutant *srf-2* is resistant (i.e., host survival upon *L. musarum* exposure) due to altered surface structure. We competed these two nematode genotypes and tracked their frequencies in the host population across 10 generations in response to prolonged or periodic warming regimes, and in the presence or absence of pathogens. We subsequently developed a mathematical model parametrized using empirical data from single-generation experiments, to disentangle the impact of dilution effects on host evolutionary dynamics.

Materials and Methods

Nematode and pathogen maintenance

We used the wild-type *C. elegans* N2 and a mutant strain *srf-2*. The pathogen, *Leucobacter musarum* sp. nov. subsp. *musarum* subsp. nov strain CBX152T (*L. musarum*), caused lethal rectal disease to wild-type worms, while the mutant was resistant to killing by the pathogen, by means of altered host surface glycosylation (Hodgkin *et al.,* 2013), Both worm genotypes were originally obtained from the Caenorhabditis Genetics Centre; CGC, Minnesota, USA. *L. musarum* was obtained from Hodgkin lab (Hodgkin *et al.,* 2013). At the start of all experiments, both strains were thawed from frozen stocks and maintained at 20℃, according to a standard maintenance protocol using nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 (Stiernagle 2006). OP50 was grown at 30℃ overnight in Luria-Bertani (LB) broth, then 100 µl OP50 culture was spread onto each NGM plate and incubated at 30℃ overnight to form bacterial lawn. Worm populations were age-synchronized by bleaching following standard protocols (Stiernagle 2006).

Leucobacter musarum is a virulent pathogen isolated from wild *Caenorhabditis tropicalis* in Cape Verde (Hodgkin *et al.,* 2013). This genus has been shown to naturally infect *C. elegans* (Bates & King, 2021; Bates *et al.,* 2021). The bacterium was grown in LB broth at 30℃ overnight, and the culture was standardized to OD(600)=0.3 throughout the infection experiment. For pathogen exposure, we prepared the infection exposure plates by streaking 74 µl of inoculum containing 20% *L. musarum* and 80% OP50 (standardized to OD(600) 1) onto 5.5 cm NGM plates and incubating at 25℃ for 24h (Bates *et al.,* 2021). Control plates were prepared by spreading a similar amount of OP50 culture.

Nematode population survival and fecundity

We manipulated warming length to occur during worm development (from L1 larvae to L4 young adults) and/or pathogen exposure at the early adult-stage. We used the ambient temperature of 20℃ and a warmer temperature of 25℃. The ambient temperature 20℃ is standard for maintaining *C. elegans* in lab, which is optimal for nematode reproduction (Gouvêa *et al.,* 2015). This warmer temperature causes mild heat stress in *C. elegans* hosts shortening wild-type *C. elegans* lifespan and reducing host reproduction (Xiao *et al.,* 2013; Gouvêa *et al.,* 2015). 25℃ can also result in higher *L. musarum* -induced host mortality (Hodgkin *et al.,* 2013; Bates *et al.,* 2021).

The pathogen exposure assay was conducted following protocols in Chapter 3. Briefly, wild-type or mutant L1 larvae were grown on OP50 food at either 20℃ or 25℃ for ~48h or ~34h, respectively, until they reached stage L4 (as worms had faster development at warmer temperature). L4 young adults were washed off the plate, gravity washed twice, and transferred onto infection or control plates, left for 24h at either 20℃ or 25℃. We transferred approx. 300-400 L4-stage worms onto each 5.5 NGM plate. This number was determined to obtain similar worm density to that in the evolution experiment. Transferred worm populations were either 100% wild-type or mutant, or mixed populations with 50% wild-type and 50% mutant worms. Each treatment was replicated six times. The number of live and dead nematodes were counted on each plate after 24h pathogen exposure. Nematodes were considered dead when they did not respond to a gentle touch with a platinum wire.

To assess population fecundity, all worms and eggs were washed off the plate and

unhatched sterile eggs were collected after bleaching. After 12h incubation in M9 buffer, numbers of the total hatched L1 larvae and *srf-2* mutant L1 larvae were counted in six 5ul drops of M9 buffer. The *srf-2* mutant has a green florescent reporter and can be distinguished from unlabeled wild-type at L1 larva stage. This GFP insertion does not carry a host fitness cost (Hodgkin *et al.,* 2001).

Evolution experiment

Nematode offspring were passaged across ten generations. We competed the susceptible wild-type N2 and the resistant mutant *srf-2* starting from 50:50 ratio and tracked their frequency dynamics under different infection treatments and temperature regimes (Fig. 1). The experiment was started with approximately 500 nematodes of the wild-type N2 and *srf-2* mutant (n per plate = 1000) per replicate population. The population sizes were consistently maintained at approx. 1000 for all generations. The evolution experiment was started with both genotypes thawed from frozen stocks, and all treatments and controls were replicated six times.

We prepared infection exposure plates by streaking 200 µl of inoculum containing 20% *L. musarum* and 80% OP50 (standardized to OD(600) 1) onto 9 cm NGM plates and incubating at 25℃ for 24h (Bates *et al.,* 2021). Control plates were prepared by spreading a similar amount of OP50 culture. Both genotypes were age-synchronized, and L1 worms were developed on OP50 food at 20°C or 25°C for 48h or 36h, respectively, until they reach L4 young adult stage. L4-stage worms were transferred to infection or OP50 plates and incubated at 20°C or 25°C for 24h. After 24h of infection exposure, unhatched sterile eggs

were collected from worm populations after bleaching. After 12h incubation in M9 buffer, frequency estimates for each genotype were determined by counting total hatched L1 worms and mutant L1 worms in six 3ul drops of M9 buffer, under a fluorescent microscope. Approx. 1000 worms from each replicate were then placed onto OP50 plates and raised to L4 stage before being placed back to infection and temperature treatments. For replicates where there were < 1000 worms, individuals were added from either 20℃ or 25℃ uninfected stock plates to keep population sizes constant across replicates. Exposure to pathogens or abiotic environmental stress have been shown to induce nongenetic heritable host resistance or altered fitness in wild-type *C. elegans* (Moore *et al.,* 2019; Palominos *et al.,* 2017; Burton *et al.,* 2020; Wibisono & Sun, 2023; Legüe *et al.,* 2022; Baugh & Day, 2020). Given that adding worms from uninfected stock plates might weaken the epigenetic memory of resistance for replicates in infection treatment (if there was any), the evolutionary frequency data were then analyzed including and excluding these replicates.

Genotype frequency was counted by subsampling. It was thus possible that one genotype at low frequency that was not counted and could bounce back in the next generation. Loss was determined if one genotype was not observed for two consecutive generations. Passages stopped in the replicate where loss was observed.

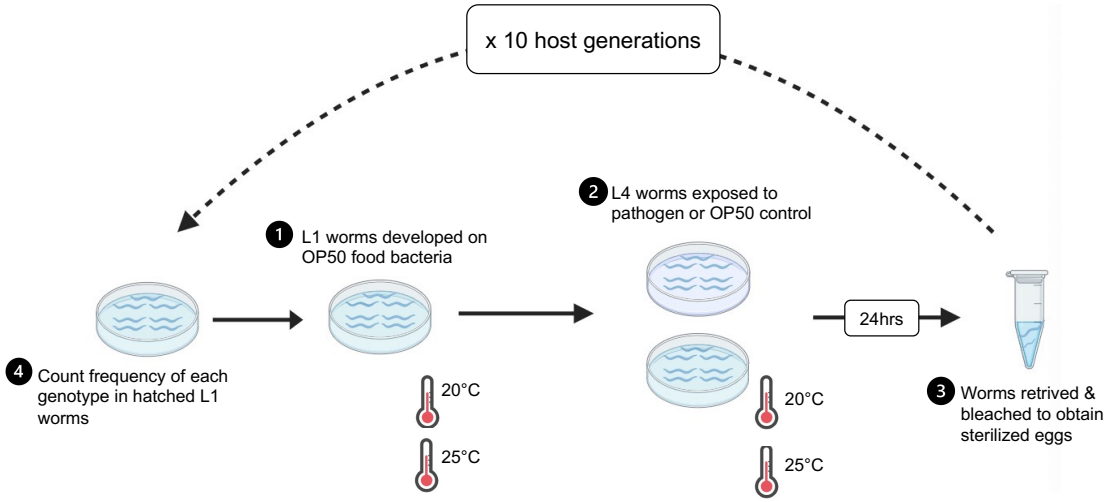

Fig. 1. Schematic illustration of the evolution experiment competing host genotypes. In brief, L1-stage host population consisted of wild-type N2 and mutant *srf-2* were grown on OP50 food bacteria at 20°C or 25°C until they reach L4 stage. L4 worms were exposed to pathogen or OP50 control at 20°C or 25°C. After 24h, worm populations were bleached to obtain sterilized eggs. The frequency of each genotype was determined in the hatched L1. All hatched L1 worms were grown to L4 and returned to the corresponding infection and temperature treatment. Ten passages were made.

Statistical analyses

Unless specified, all analyses were conducted in R 4.1.0 (RStudio 2023.03.1+446). Host mortality data was fitted in generalized linear model (GLM) with quasibinomial distribution. To account for different numbers of susceptible individuals in wild-type and mixed (50% susceptible: 50% mutant) host populations, we scaled the mortality of mixed populations by multiplying the original host mortality by 2. Fecundity data was fitted in GLM with negative binomial distribution. Proportion of mutant in the offspring was fitted in GLM with binomial distribution (with total number of offspring as weights in model). Model performance was evaluated by QQ-plot and dispersion test to ensure they addressed the overdispersion of the data. To test which genotype dominated in the offspring population under different treatments, we used exact binomial tests on each replicate. P-values were pooled to obtain group-level significance using poolr R package (Cinar & Viechtbauer, 2022). We tested the

correlation between host population mortality, fecundity, and the proportion of mutant in the offspring using cor.test function in R.

For the evolution experiment, we calculated the relative fitness of resistant mutant compared to the susceptible genotype. Resistance relative fitness was calculated as a function of the change in genotype frequencies using the equation ln(f resistant /f susceptible) where f represents genotype frequencies (Bates *et al.,* 2021). In cases where one genotype had a frequency of zero, we assumed a frequency of 1/N+1 for this genotype (N is population size). Mean and sd of resistance relative fitness across ten generations were summarized. We used Wilcoxon rank sum test to test for differences in mean relative fitness between treatment groups. To evaluate the trend of genotype frequency change across host generations under different treatments, we fitted the frequency data in a generalized linear mixed model (GLMM) with binomial distribution, as well as Bayesian model with binomial distribution. Genotype dominance at generation ten was assessed using binom.test and poolr R package. Details of all statistical results are in Supplementary Table 1.

Generalized Lotka Volterra model

To disentangle effects that help promote the persistence of the susceptible genotype in host populations, we developed a generalizable mathematical model based on generalized Lotka-Volterra (gLV) equations. Our model assumed that the frequency of each genotype in the host population was determined by its intrinsic fecundity, the reduction in fecundity for infected individuals, the infection-induced host mortality, and intra- and inter-genotype

interactions. Intra-genotype interactions were considered competitive. While inter-genotype interactions might induce both competitive and facilitative effects, specifically dilution effects. Dilution effects occurs when the resistant genotype reduces the intensity and transmission of pathogens among susceptibles.

In this model, we assumed a population (with fixed size N) consisted of genotype *i* and genotype *j*, and the rate of abundance change for genotype *i* (X_i) across generations is given below.

(dXi)/dt was determined by the sum of offspring produced by healthy host individuals and infected host individuals, as shown in the model equation. Individuals for generation t+1 were then randomly sampled from the offspring pool produced at generation t by genotype *i* and *j*.

$$\frac{dX_i}{dt} = r_i(X_i - \beta X_i(1 + \gamma X_j) - \theta X_i)e^{-C_{ii}X_i - C_{ij}X_j} + (1 - \varphi_i)r_i((1 - \sigma_i - \theta)\beta X_i(1 + \gamma X_j))e^{-C_{ii}X_i - C_{ij}X_j}$$

Here, r_i is the reproduction rate of genotype *i*, β is the infection rate, γ is the influence of another genotype *j* on the focal genotype's susceptibility to infection (addressing dilution effects) on individual basis, θ is host natural death rate, C_{ii} is the intra-specific competitive effect for genotype *i*, C_{ij} is the inter-specific competitive effect of genotype *j* on genotype *i*, φ_i is the infection-induced host fecundity reduction for genotype *i*, σ_i is infection-induced death rate for genotype *i*. All parameters were drawn from the random sampling from a distribution inferred from empirical data when possible.

To simplify the gLV model, we firstly set the intra- and inter-genotype interactions coefficients to zero. We inferred normal distribution parameters from the population mortality and fecundity measured by single-generation experiments for each genotype. Then for every iteration, the model parameter was randomly drawn from the inferred normal distribution. We iterated the model for ten simulated host generations.

Results

Mortality and fecundity for susceptible and resistant genotypes

Throughout the experiment, mortality was not observed either in mutant populations exposed to *L. musarum* or uninfected control populations (Fig. 2A). Susceptible wild-type worms and resistant mutants have similar generation times found herein and in a previous study (O'Rourke *et al.,* 2023). We evaluated host mortality in mixed populations and single-genotype populations. We found when susceptible hosts in mixed populations were developed at ambient temperatures, they exhibited lower scaled mortality compared with the susceptible genotype on its own (Fig. 2A, 20℃-20℃: mixture es=-0.43, p=0.031; 20℃-25℃: mixture es=-0.37, p=0.016). This result suggests there might be dilution effects (Keesing & Ostfeld, 2021; Civitello *et al.,* 2015), whereby the resistant genotype is diluting the number of pathogens in the environment resulting in fewer to infect susceptible hosts. Developmental warming eliminated these effects, where similar levels of host mortality are observed in mixed and susceptible populations (Fig. 2A, 25℃-20℃ regime: p=0.787; 25℃-25℃ regime: p=0.129). For susceptibles, developmental warming decreased host mortality during pathogen infection (25℃ es=-1.73, p<0.001), while warming during pathogen

exposure increased host mortality (25℃ es=1.11, p<0.001).

We found that infection reduced fecundity for both susceptible and resistant hosts (Fig. 2B, susceptibles: infection es=-1.47, p<0.001; mixed: infection es=-1.09, p<0.001; mutant: infection es=-0.67, p<0.001). However, this effect was much stronger for susceptible hosts (Fig. 2B, susceptibles: es=0.76, p<0.001).

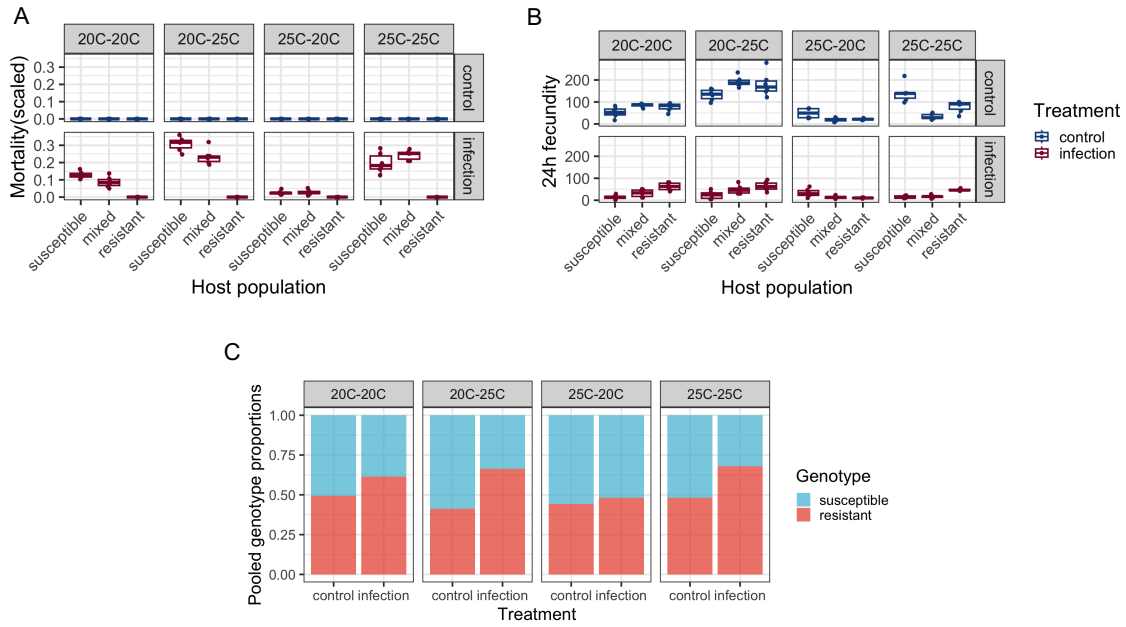

Fig. 2. Mortality and fecundity of host populations consisted of different genotypes. A. Mortality of susceptible, mutant and mixed host populations. Mortality in mixed populations was scaled (by doubling). B. Fecundity of susceptible, mutant, and mixed host populations Number of offspring was collected from host populations after 24h following pathogen exposure or placement in uninfected control. C. Proportion of susceptible and mutant genotype in total offspring produced in mixed host populations, proportions are pooled from six replicates in each treatment group.

Susceptible hosts consistently had lower fecundity at the ambient developmental temperature. Resistant and mixed populations produced more offspring than susceptible populations regardless of infection treatment (Fig. 2B, mutant vs. susceptible: infection 20℃-20℃: es=1.48, p<0.001, 20℃-25℃: es=0.98, p=0.001. Control 20℃-20℃ es=0.39,

p=0.021, 20℃-25℃ es=0.31, p=0.007. mixed vs. susceptible: infection 20℃-20℃: es=0.81, p=0.004; 20℃-25℃: es=0.70, p=0.019; control: 20℃-20℃: es=0.48, p=0.004; 20℃-25℃: es=0.37, p=0.001). However, developmental warming reversed this pattern, where we found that resistant and mixed populations produced fewer offspring compared with susceptible populations, across infection and uninfected controls (Fig. 2B, mutant vs. susceptible: infection 25℃-20℃: es=-1.17, p=7.25e-06. Control 25℃-20℃: es=-0.82, p=9.13e-05; 25℃-25℃: es=-0.58, p=0.001. Mixed vs. susceptible: infection 25℃-20℃: es=-0.90, p=0.0004; control 25℃-20℃: es=-0.94, p=1.03e-05; 25℃-25℃: es=-1.44, p=3.05e-15).

Warming during pathogen infection also impacted fecundity for the two genotypes, but to different extents. With warming throughout development and pathogen infection, resistant populations produced more offspring than susceptible or mixed populations (mutant vs. susceptible es=1.17, p<0.001; mutant vs. mixed es=0.87, p<0.001). The fecundity of mixed populations did not differ from that of susceptible populations during prolonged warming (p=0.408).

When assessing the impact of infection and warming on genotype proportions in total offspring, we found that infection significantly increased the proportion of the resistant genotype (Fig. 2C, infection es=0.76, p<2e-16). This effect was exaggerated when combined with warming during infection (infection:25℃ es=0.64, p=0.0007. Infection 25℃ es=0.3943, p=0.0154). However, in the uninfected control populations, adult-stage warming decreased the proportion of resistant offspring (25℃ es=-0.25, p=0.011). For uninfected

control populations, 20℃-25℃ temperature regime reduced the proportion of resistance in total offspring (es=-0.33, p=0.002). However, infection warming regime (20℃-25℃) increased the proportion of resistance in total offspring to the greatest extent (20℃-25℃:infection es=0.54, p=0.015). The resistant genotype always dominated in total offspring reproduction under infection (Fig. 2C, pooled p-value=0.002 for 20℃-20℃, 20℃-25℃ and 25℃-25℃ regimes), except for that under the developmental warming regime (25℃-20℃) (Fig. 2C, pooled p-value=1). In uninfected control populations, the resistant genotype dominated in total offspring under persistent ambient temperature regime (Fig. 2C, 20℃-20℃, pooled p-value=0.033), while the susceptibles dominated the total offspring reproduction at 20℃-25℃ temperature regime (Fig. 2C, pooled p-value=8.64e-05).

We did not observe a significant trade-off between host survival and fecundity for the susceptible genotype. These traits were positively associated for susceptible-only populations (Spearman rho=-0.74, p<0.001), as well as for mixed populations (Spearman rho=-0.55, p<0.001). Specifically, a decrease in host survival was correlated with decrease in susceptible offspring proportion in mixed populations (Supplementary Table 1, Spearman rho=0.41, p=0.004).

Evolutionary dynamics of host resistance and susceptibility

Coexistence of susceptible and resistant genotypes was common throughout our evolution experiment. We found that in each treatment, both genotypes were able to coexist in at least in one replicate at the end of the evolution experiment (Fig. 3). Similar evolutionary dynamics were found after removing the replicates consisting of worms added from stock

plates (Fig. S3).

The regular loss of resistance, particularly in the uninfected controls, reveals an evolutionary cost to the resistant phenotype. We observed the first loss of resistance in our experiment at generation six, in which the resistant genotype went extinct during evolution in the uninfected developmental warming regime (25℃-20℃) (Fig. 3A, Fig. S1). After eight host passages, we observed the first fixation of resistance (i.e., loss of the susceptible genotype) at prolonged warming regime under pathogen infection (Fig. S1). This replicate was the only one showing fixation of resistance in the evolution experiment. After nine host passages, loss of resistance was observed in most replicates in the developmental warming regime (25℃-20℃), under both infection and uninfected control conditions (Fig. S1).

We found that in the prolonged warming (25℃-25℃) infection group, the resistant genotype dominated host populations (Fig. 3A, pooled p-value<0.001). Otherwise, the susceptible genotype dominated in other treatments (Fig. 3A, susceptible frequency>0.5, pooled p-value<0.033 for all other treatment groups). These results were consistent with the single-generation results that developmental warming reduced the fecundity of the resistant genotype more than that of the susceptibles. The former genotype exhibited higher fecundity than the latter under prolonged warming.

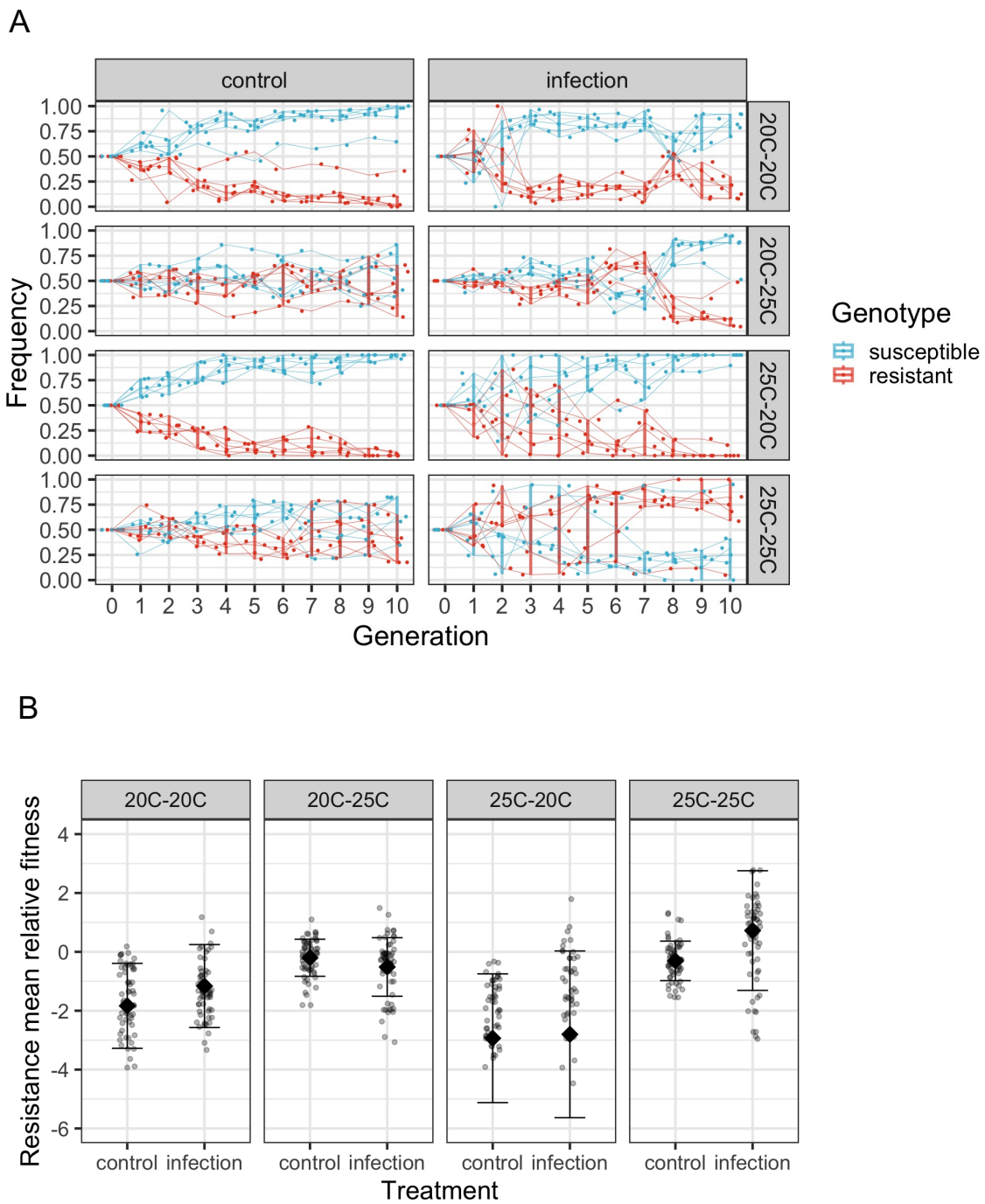

Fig. 3. A. Genotype frequency dynamics for 10 host generations across infection and temperature regimes. Host populations were started at 50:50 ratio of the two genotypes. B. Mean relative fitness of resistant genotype for ten generations. In each facetted plot, the diamond shape represents mean, error bar represents mean ± SD, each point represents relative fitness of resistant genotype in one replicate.

When evaluating the relative fitness of resistance genotype across generations, we found that warming during infection increased the relative fitness of the resistant genotype,

regardless of infection treatment (Fig. 3B. control: $p<0.001$, infection: $p<0.001$). This pattern was significant throughout the evolution experiment (Fig. S2. Generation 1-5: control: $p<0.001$, infection: $p=0.012$. Generation 6-10: control: $p<0.001$, infection: $p=0.002$). Pathogen infection significantly increased resistance relative fitness under prolonged warming (Fig. 3B. Wilcoxon rank sum test, $p=0.003$), but the selective effect for resistance was not significant until later generations (Fig. S2, Generation 1-5: $p>0.222$; Generation 6-10: $p=0.008$). We found that warming increased the variation of resistance relative fitness (Fig. 3B. Levene's Test, adult-stage warming (control): $p<0.001$; developmental warming (infection): $p=0.019$), suggesting warming can increase stochasticity among replicates. This finding was magnified when combined with pathogen infection. During infection warming and prolonged warming treatments, infection significantly increased the variation of relative fitness (Fig. 3B, Supplementary Table 1. Levene's Test, 20℃-25℃: $p=0.018$; 25℃-25℃: $p=0.04$). Selection posed by warming and infection resulted in more stochastic outcomes among replicates.

By fitting data in GLMM (Supplementary Table 1), we found that across host generations, there was a general decrease in the frequency of resistant genotypes in populations (Table 1, Generation es=-0.21, $p<0.001$). We also fitted the data to a Bayesian mixed effect model, and results were consistent between both approaches (Supplementary Table 1). However, pathogen infection across generations favoured higher levels of resistance compared to in the uninfected control (es=0.13, $p<0.001$). Prolonged warming across host generations increased the frequency of resistance in host populations compared with that under persistent ambient temperature regime (es=0.16, $p<0.001$). This pattern was pronounced

during pathogen infection (es=0.089, p=0.003). Conversely, when temperature was lowered to 20℃ after developmental warming, there was a decrease in the frequency of resistance across time (es=-0.082, p=0.005), especially during infection (es=-0.098, p=0.025). The reverse switch, from 20℃-25℃, drove the rapid loss of resistance under infection (es=-0.21, p<0.001).

Table 1. Summary of GLMM, modelling resistance frequency across generations and treatments

	estimate	std.error	p-value
Main effects			
Generation	-0.21	0.016	<0.001***
infection	-0.43	0.346	0.209
20℃-25℃	0.28	0.33	0.399
25℃-20℃	-0.13	0.339	0.691
25℃-25℃	0.39	0.329	0.238
Interaction effects			
Generation:infection	0.13	0.025	<0.001***
Generation:20℃-25℃	0.19	0.019	<0.001***
Generation:25℃-20℃	-0.08	0.029	0.005**
Generation:25℃-25℃	0.16	0.018	<0.001***
infection:20℃-25℃	0.8	0.476	0.091
infection:25℃-20℃	0.92	0.49	0.059
infection:25℃-25℃	-0.08	0.479	0.867
Generation:infection:20℃-25℃	-0.21	0.029	<0.001***
Generation:infection:25℃-20℃	-0.1	0.044	0.025*
Generation:infection:25℃-25℃	0.09	0.03	0.003**

Modelling resistance dynamics with dilution effects

In the absence of genotype competition, the model generated population dynamics showing fixation of resistance at ambient developmental temperature but extinction at developmental warming, in uninfected host populations (Fig. 4). The genotype fixation or extinction rate was slower than that in infected host populations. The simulations showed loss of resistance in infection treatment groups, except for several replicates under prolonged warming regimes (Fig. 4). These trends in infection but not control groups

resembled the evolutionary patterns in the evolution experiment (Fig. 3A). This indicates that infected host population resistance is being shaped mainly by selection from pathogens, while uninfected population dynamics are mainly shaped by competitions. However, loss of resistance was much slower in the experiment, possibly due to intra-genotype competition decreasing the rate of fixation for the susceptible genotype. Dilution effects were detected from single-generation experiments in ambient and infection warming regimes. Removing dilution effects by setting the relevant parameter as 0 in the simulations reversed population dynamics, showing rapid loss of susceptible wild type in the first three generations (Fig. 4).

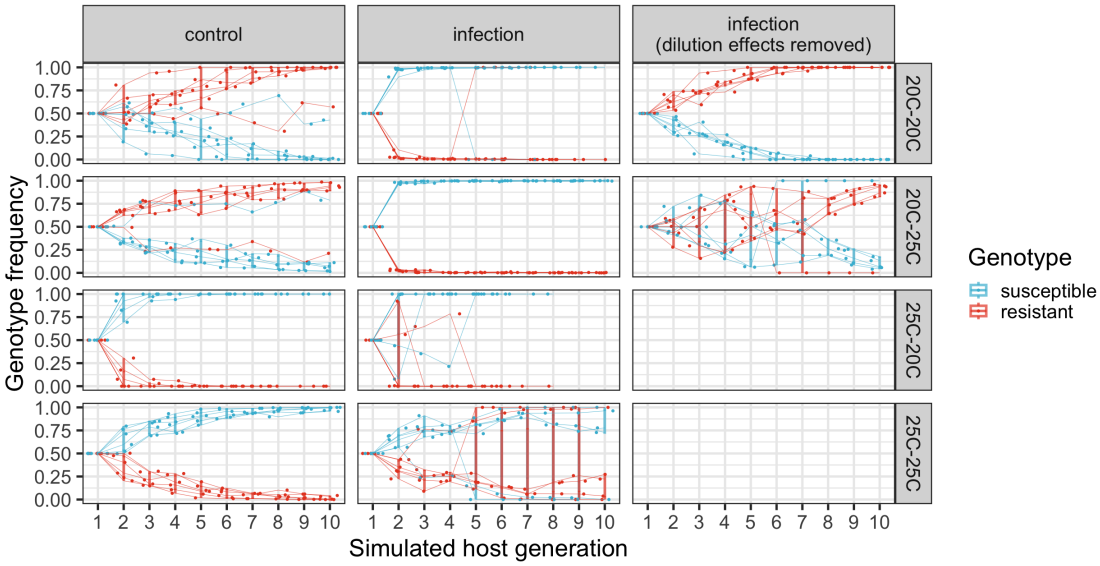

Fig. 4. Genotype frequency dynamics predicted by the gLV model. All parameters were drawn from normal distribution inferred from the experimental population mortality and fecundity data. Dilution effects at persistent ambient (20℃-20℃) and infection warming (20℃-25℃) regimes were inferred from experimental data and were also removed to assess their impact. No host dilution effects were observed under developmental warming (25℃-20℃) and prolonged warming (25℃-25℃) regimes from experiments.

Discussion

Global warming is increasing average temperatures and causing thermal fluctuations,

which could escalate disease threat to biodiversity (Rohr *et al.,* 2013; Kunze *et al.,* 2022; Altizer *et al.,* 2003). Pathogens can impose strong selection for host resistance (Koskella, 2018). Warming could amplify these selective effects by increasing pathogen virulence (Kimes *et al.,* 2012; Oh *et al.,* 2009). Thus, long-term warming across multiple host generations might select for resistance in populations, if the benefits of avoiding infection outweigh costs of resistance (Boots & Haraguchi, 1999; Restif & Koella, 2003; van 1998). Rising temperatures also strengthen (Singh & Aballay, 2006; Liang *et al.,* 2014; Prithika *et al.,* 2016) or weaken (Cheng *et al.,* 2005; Moriyama & Ichinohe, 2019) host immune responses, with effects varying by host life-stage during exposure (Liew *et al.,* 2003; Janda *et al.,* 2019; Lee *et al.,* 2012; Singh & Aballay, 2006; Prithika *et al.,* 2016). Our study focused on a warm-adapted bacterial pathogen with their cool-adapted nematode hosts. By competing resistant and susceptible nematode genotypes across ten generations, we revealed that prolonged (and stressful) warming across development and pathogen exposure strongly selected for resistance in host populations. Resistance was lost or maintained at lower frequencies in populations experiencing periodic warming.

Our results showed a rapid loss of resistant genotypes in host populations, especially in the absence of pathogen attack, indicating an evolutionary cost to genetic-based resistance. The resistant genotype has been shown to have lower mobility than the susceptible wild-type, which makes it inferior in foraging on food bacterial lawn (O'Rourke *et al.,* 2023). That resistance can be physiologically costly and linked to inferior competitive abilities is well-established (Kraaijeveld & Godfray, 1997; Luong & Polak, 2007; Bartlett *et al.,* 2018; Schwenke *et al.,* 2016). This cost was less prominent from single-generation experiments,

where resistant individuals sometimes dominated in offspring production. Environmental factors (i.e. nutritional level) can affect host resistance expression and evolution (Lambrechts *et al.,* 2006), and warming might also influence the genetic basis of host resistance.

In addition to genetic-based resistance, warming during development was shown to mediate plastic resistance for susceptible genotype to infections. In *C. elegans,* this effect is likely due to heat stress activating the heat shock transcription and protein production, part of the host multi-pathogen defense pathways (Singh & Aballay, 2006; Prithika *et al.,* 2016). The protective effect of early-stage heat exposure has been found in other host organisms, from broiler chickens to plants (Liew *et al.,* 2003; Janda *et al.,* 2019; Lee *et al.,* 2012). We found that pathogen-mediated selection for costly genetic-based resistance was weakened by the presence of this induced resistance in the susceptible wild-type host. This pattern diminished when infection occurred at warmer temperature where pathogens became more virulent (Hodgkin *et al.,* 2013). We suggest that temperature-driven plastic responses could confer protection for susceptible hosts against moderate virulent infections, but might be less sufficient to buffer susceptibles against more severe infectious disease outbreaks under climate warming (Kelly, 2019; Jones *et al.,* 2008). Host adaptation (i.e. resistance) is primarily driven by pathogen virulence, while pathogen adaptation (i.e. virulence) can in turn be driven by host resistance (Paterson *et al.,* 2010; Schulte *et al.,* 2010; Kubinak & Potts, 2013). Future studies are needed to disentangle such host-pathogen co-evolutionary dynamics, providing essential insight for predicting the burden of infectious diseases under climate change (Brooks & Boeger, 2019).

We observed the persistence or dominance of the susceptible wild type under infection in constant ambient or periodic warming regimes. This outcome was unexpected given results from single-generation experiments showed that the resistant mutant produced more offspring and had higher survival during infection. As pathogens showed different competencies of infecting susceptible and resistant nematode genotypes, dilution of disease might happen. Dilution effects can be conferred by resistant hosts in our experiment shielding susceptible genotypes from pathogens, and thereby favouring the maintenance of susceptibles in the population (Rudolf & Antonovics, 2005). Model simulations showed that removing dilution effects led to a rapid loss of susceptibles, indicating the importance of dilution effects to the outcome of resistance evolution in our experiment. The impacts on selection were however eliminated when warming coincided with host development. At an ecosystem level, dilution effects might limit disease spread, with the loss of biodiversity worsening epidemics and threatening human and wildlife populations (Civitello *et al.,* 2015). This diversity-disease relationship might be shaped by environmental factors such as warming (Liu *et al.,* 2016), specifically depending on host life-stage of heat exposure (Zhang *et al.,* 2015). Global warming has emerged as a predominant threat to ecosystems in the 21st century, reducing biodiversity by altering species habitat range, interrupt food webs and ecosystem functions (Shivanna, 2022; Wudu *et al.,* 2023). Climate-induced loss of diversity might exaggerate the effects of warming on disease susceptibility, worsening the outlook for plant and animal hosts (Wudu *et al.,* 2023).

Epigenetic inheritance of host resistance might also play a role shaping these evolutionary

trajectories. Multigenerational plasticity in *C. elegans* has been established (Moore *et al.,* 2019; Palominos *et al.,* 2017; Burton *et al.,* 2020; Wibisono & Sun, 2023; Legüe *et al.,* 2022; Baugh & Day, 2020), with environmental stress leading to acquired pathogen avoidance behaviour and resistance transmitted across generations via histone modifications and small RNA regulation in progeny gene expression (Baugh & Day, 2020; Ghildiyal & Zamore, 2009; Fire *et al.,* 1998). Plasticity could enable low-cost exploration of phenotypic space for the susceptible wildtype in response to environmental stress advantageous in the context of environmental heterogeneity (Murren *et al.,* 2015). Temperature also induces epigenetic phenotypic change (Baugh & Day, 2020). Whether prolonged warming could generate or constrain multigenerational plasticity in host resistance has not been directly tested.

Lastly, we found that pathogen-posed selection for resistance can fluctuate, specifically in a negatively frequency-dependent fashion. Whilst pathogens initially selected for costly host resistance, we postulate that once pathogen prevalence decreased due to the spread of resistant genotype (e.g., dilution effect), selection can then act against resistance in host populations (Boots *et al.,* 2009). This dynamic change of host resistance and pathogen prevalence is an example of ecological feedback (Boots *et al.,* 2009). And such feedback might eventually increase the frequency of once rare susceptible genotype in the populations (Sheldon & Verhulst, 1996; Boots *et al.,* 2009).

In conclusion, we showed that host evolutionary trajectories could be shaped by abiotic temperature, with the combination of fitness constraints associated with warming stress. A combination of genetic-based resistance, plasticity, condition-dependent virulence, and

protection conferred by dilution effects all likely played a role in determining the strength and direction of selection acting on host resistance to infection. Assessing these effects on host resistance evolution is essential for forecasting host population persistence and health in a more infectious and warming world (Jones *et al.,* 2008; Dosio *et al.,* 2018; Mora *et al.,* 2022).

Author contributions

J.L and K.C.K. conceived and designed the study. J.L. conducted the experiment and analysed the data, with input from K.C.K., J.L. and J.C. conceived the mathematical model. J.L. and K.C.K. wrote the manuscript. All authors contributed to reviewing and editing.

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Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary Material

Chapter 5

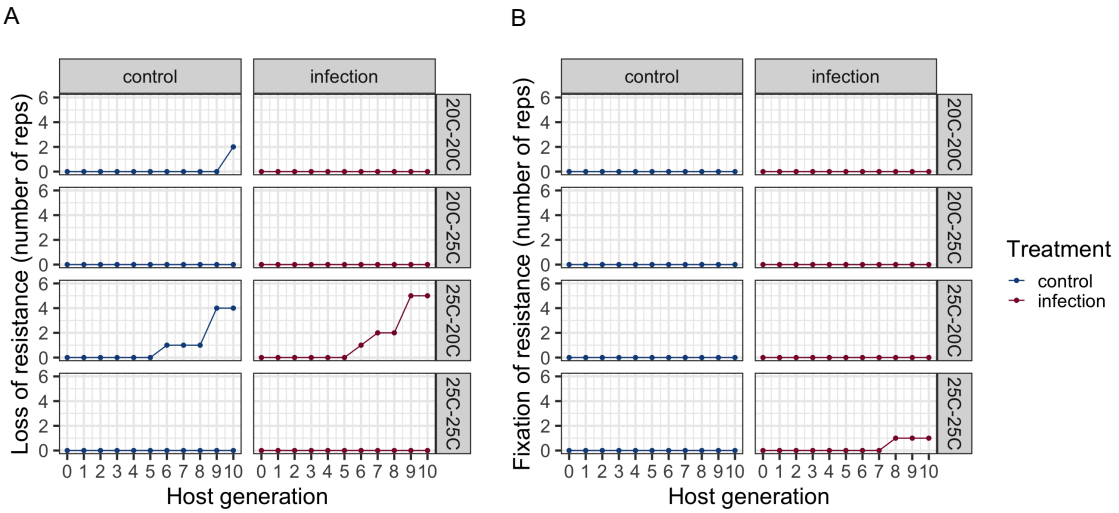

Fig. S1. Loss and fixation of resistance across treatments and host generations. A. Loss of resistance. B. Fixation of resistance. X axis represents host generations, y axis represents number of replicates.

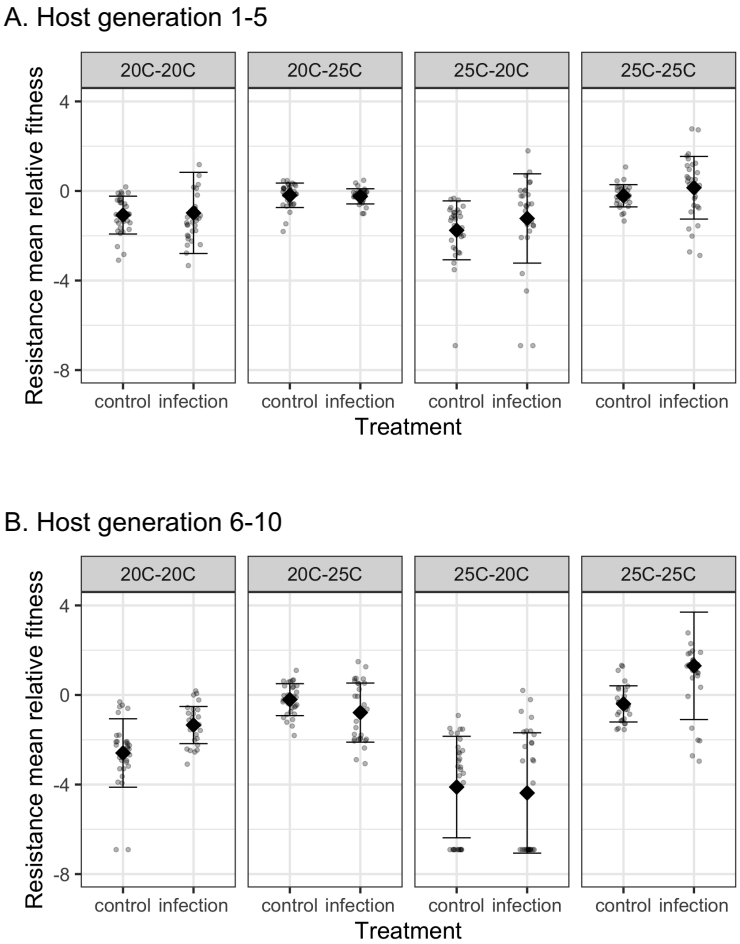

Fig. S2. Mean relative fitness of resistant genotype for 1-5 and 6-10 host generations. A. Mean relative fitness of resistant genotype for 1-5 generations. B. Mean relative fitness of resistant

genotype for 6-10 generations. In each facetted plot, the diamond shape represents mean, error bar represents mean±SD, each point represents relative fitness of resistant genotype in one replicate.

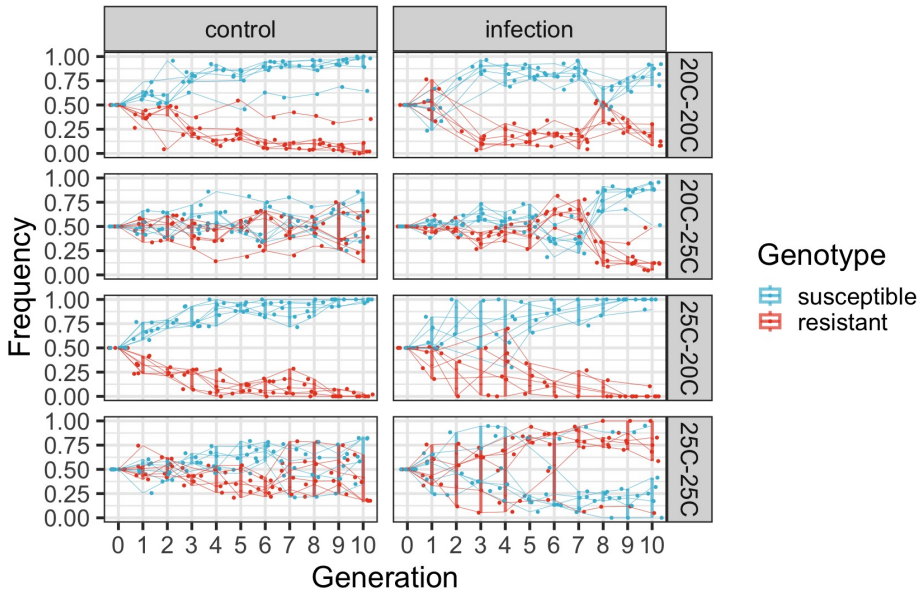

Fig. S3. Genotype frequency dynamics for 10 host generations across infection and temperature regimes, after removing replicated consisting worms added from stock plates. Host populations were started at 50:50 ratio of the two genotypes.

Supplementary Table 1 Modelling results

Relative fitness ratio of susceptible:resistance

	20C-20C	20C-25C	25C-20C	25C-25C
mean ~ Treatment	p=0.190	p=0.579	p=0.992	p=0.003**
sd ~ Treatment	p=0.724	p=0.018*	p=0.471	p=0.040*
Uninfected control group				
	20C-20C	20C-25C	25C-20C	25C-25C
20C-20C	N/A			
20C-25C	p<0.001***			
25C-20C	p=0.215	p<0.001***		
25C-25C	p=0.002**	p=0.365	p<0.001***	N/A
Infected group				
	20C-20C	20C-25C	25C-20C	25C-25C
20C-20C	N/A			
20C-25C	p=0.116			
25C-20C	p=0.126	p=0.014*		
25C-25C	p=0.001**	p=0.029*	p<0.001***	N/A

Baseline population mortality (GLM quasibinomial)

glm(formula = mortality_scaled ~ Genotype * Rear_temp * exposure_temp, family = quasibinomial, data = .)

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-1.91764	0.11947	-16.052	<2.00 E-16
GenotypeN2+srf-2	-0.42691	0.18504	-2.307	0.02631
Rear_temp25C	-1.73116	0.28066	-6.168	2.74E-07
exposure_temp25C	1.10727	0.1475	7.507	3.73E-09
GenotypeN2+srf-2:Rear_temp25C	0.51138	0.39784	1.285	0.20605
GenotypeN2+srf-2:exposure_temp25C	0.05351	0.22497	0.238	0.81322
Rear_temp25C:exposure_temp25C	1.14159	0.31032	3.679	0.00069
GenotypeN2+srf-2:Rear_temp25C:exposure_temp25C	0.12826	0.43972	0.292	0.77204

Baseline population fecundity (GLM negative binomial)

glm.nb(formula = L1_per_30ul ~ Genotype * exposure_temp * Rear_temp * Treatment, data = ., init.theta = 10.04237629, link = log)

(Intercept)	3.9576335	0.1406451	28.139	<2.00E-16
GenotypeN2+srf-2	0.483055	0.1958112	2.467	0.013627
Genotypesrf-2	0.3861719	0.1963209	1.967	0.049178
exposure_temp25C	0.9276905	0.1940022	4.782	1.74E-06
Rear_temp25C	-0.0658132	0.1994461	-0.33	0.741415
Treatmentinfection	-1.3067417	0.2194131	-5.956	2.59E-09
GenotypeN2+srf-2:exposure_temp25C	-0.1108837	0.2714078	-0.409	0.68287
Genotypesrf-2:exposure_temp25C	-0.0757651	0.2718775	-0.279	0.780495
GenotypeN2+srf-2:Rear_temp25C	-1.4217027	0.2891915	-4.916	8.83E-07
Genotypesrf-2:Rear_temp25C	-1.2099393	0.2879025	-4.203	2.64E-05
exposure_temp25C:Rear_temp25C	0.1268823	0.2746192	0.462	0.64406
GenotypeN2+srf-2:Treatmentinfection	0.3317891	0.2975025	1.115	0.264744
Genotypesrf-2:Treatmentinfection	1.0927553	0.2935647	3.722	0.000197
exposure_temp25C:Treatmentinfection	-0.4005284	0.2992301	-1.339	0.180724
Rear_temp25C:Treatmentinfection	0.9214793	0.2995599	3.076	0.002097
GenotypeN2+srf-2:exposure_temp25C:Rear_temp25C	-0.3953164	0.3979378	-0.993	0.320509
Genotypesrf-2:exposure_temp25C:Rear_temp25C	0.3225873	0.3933835	0.82	0.412197
GenotypeN2+srf-2:exposure_temp25C:Treatmentinfection	-0.0004505	0.4091472	-0.001	0.999121
Genotypesrf-2:exposure_temp25C:Treatmentinfection	-0.4223328	0.4053357	-1.042	0.297443
GenotypeN2+srf-2:Rear_temp25C:Treatmentinfection	-0.2970097	0.4293434	-0.692	0.489078

Genotypesrf-2:Rear_temp25C:Treatmentinfection	-1.4401709	0.4299394	-3.35	0.000809
exposure_temp25C:Rear_temp25C:Treatmentinfection	-1.4750248	0.4205637	-3.507	0.000453
GenotypeN2+srf-2:exposure_temp25C:Rear_temp25C:Treatmentinfection	1.5383521	0.5976524	2.574	0.010053
Genotypesrf-2:exposure_temp25C:Rear_temp25C:Treatmentinfection	2.5148037	0.5895267	4.266	1.99E-05

Baseline fecundity for mixed populations -- mutant proportion (GLM binomial with weights)

glm(formula = L1_srf2_prop ~ Temp * Treatment, family = binomial,
data = ., weights = L1_per_30ul)

(Intercept)	-0.02751	0.08866	-0.31	0.75636
Temp20C_25C	-0.32685	0.10697	-3.056	0.00225
Temp25C_20C	-0.19955	0.20759	-0.961	0.33641
Temp25C_25C	-0.04287	0.16729	-0.256	0.79773
Treatmentinfection	0.49413	0.17277	2.86	0.00424
Temp20C_25C:Treatmentinfection	0.53795	0.22094	2.435	0.0149
Temp25C_20C:Treatmentinfection	-0.34118	0.33843	-1.008	0.31339
Temp25C_25C:Treatmentinfection	0.33003	0.30972	1.066	0.28663

Chapter 6:

Thesis discussion

Thesis discussion

This thesis investigates the effects of temperature on microbiome dynamics, infection outcomes and the evolution of host resistance. I also reveal the structuring force of the dual stressors of warming and pathogen infection on host microbiomes. Below I summarise each empirical chapter, followed by potential avenues for future research.

Chapter summaries

Chapter 2: Experimental temperatures shape host microbiome diversity and composition

The microbiome is increasingly regarded as an important indicator of host health. The extent to which microbiomes change in response to higher and lower temperatures may be important for predicting host performance under climate change. In Chapter 2, I broadly investigated whether and how experimental temperatures (warming/cooling) affect host microbiome diversity, composition, and stability across animal and plant species. I tested the effects of a series of moderators including host biological traits (e.g. endothermic or ectothermic), environmental variables (e.g. habitat environments) and experimental factors (e.g. length of lab acclimation) on host microbiomes responses to temperature change from control temperatures. Overall, I detected a decrease in microbiome phylogenetic diversity and alteration of microbiome composition under both experimental warming and cooling. I found that host microbiome diversity and composition were more affected by host habitat and experimental factors, than host biological traits. Aquatic animals in marine habitats, compared to terrestrial animals, experienced more microbiome diversity loss under cooling. This result suggests that aquatic species might be less tolerant to extreme cooling, which

could hinder their ability to expand ranges towards new habitats in polar regions under current climate warming. I found that prior-experimental lab acclimation or ramped warming caused less of a reduction in microbiome diversity, as opposed to sudden static temperature shift. Thus, a gradual and longer time for host acclimation might aid microbiome stability under temperature changes, but whether rapid microbiome adaptation will occur during gradual thermal shifts is unclear. This study highlights the need to consider these factors when predicting species persistence and developing microbe-based interventions to buffer host against thermal stress.

Chapter 3: Dual stressors of infection and warming can destabilize host microbiomes

In current climate scenario, animals are constantly exposed to multiple abiotic and biotic stressors, so are their microbiomes. In Chapter 3, I extended the work of Chapter 2 and included a biotic stressor – pathogen infection, to investigate the dual effects of warming and infection on host microbiomes. Specifically, using the nematode host *Caenorhabditis elegans*, its natural gut microbiome representatives, and a bacterial pathogen *Leucobacter musarum* naturally isolated from *Caenorhabditis spp*, I characterized microbiome changes in a diversity of wild host isolates and the canonical lab strain during warming temperatures and infection. I found a loss of microbiome diversity and increase in dispersion over time. Whilst uninfected hosts experienced microbiome diversity loss, infected hosts had more dispersed microbiota among populations. Pathogen infection was associated with increased microbiome diversity and decreased dominance. I revealed that infection and warming could individually increase microbiome dispersion, indicating the destabilizing effects of both stressors. However, these two stressors did not have additive destabilizing

effects on host microbiomes. The timing of warming and degree of host lab adaptation both played a role in mediating microbiome responses to the dual stressors. Overall, I empirically demonstrated the dynamic nature of host microbiomes under thermally variable and infectious conditions, which has shed light on conservation of endangered species amidst climate warming and epidemics, through microbiome-mediated protection.

Chapter 4: Warming-induced excess deaths of infected animals depend on pathogen traits

Temperature can impact host-pathogen interactions to shape distinct disease outcomes, but the broad effect of warming on diverse host-pathogen systems are unclear. In Chapter 4, I investigated the effect of temperatures, with a focus on host-pathogen interaction and disease outcome. Using data from experimental studies, I broadly tested the effect of warming on the infection outcome, specifically pathogen-induced host mortality. I investigated whether host and pathogen biological traits (e.g. host-pathogen evolutionary history, pathogen thermal optimum), the scale of temperature increase and experimental variables (e.g. pathogen inoculation method, infection exposure time) could explain variations in the temperature-mortality relationship. Overall, I found that experimental warming drove higher mortality of infected hosts, with the magnitude of effects consistent across variable experimental protocols, and not impacted by host life-stage or immune complexity. Larger temperature increases were associated with more host deaths under infection. I further found that naturally established bacterial pathogens caused greater host mortality under warming, while novel infections were more lethal than established infections at low ambient temperatures. Fungal pathogens were more sensitive to temperature

changes, with the infection outcome worse when temperature shifted upwards their thermal optimum. Broadly, this study suggests that global warming could lead to excess deaths caused by infectious diseases in animal populations, with the magnitude of effects varying by pathogen type and evolutionary history. These factors should be considered when predicting the outcome of epidemics under climate change.

Chapter 5: Prolonged warming strengthens selection for host resistance to infection

Temperature broadly impacts infection outcomes across diverse host-pathogen systems, as shown by Chapter 4. Varying disease outcomes could be driven by host-pathogen interactions under temperature changes, with host resistance being an essential part in those interactions. Resistance is constantly evolving rather than be a fixed trait, the evolution of which greatly impact epidemics dynamics. In Chapter 5, I investigated the selective impacts of warming and infection on host resistance evolution. I conducted evolution experiments competing two *C. elegans* genotypes (susceptible wild-type vs. resistant mutant) across 10 host generations, varying the presence of infection by *L. musarum* and the time of warming. I found that persistent warming across host development and adulthood favoured genetic-based host resistance under infection, particularly as warming increased pathogen virulence. Conversely, periodic warming across the host's lifetime resulted in a loss of genetic-based resistance. Warming during host development conferred plastic pathogen resistance and thus weakened the selection for more costly genetic-based resistance under subsequent infection. Using mathematical modelling, I revealed that host evolutionary trajectories were likely driven by the combination of fitness constraints of genetic-based host resistance, temperature-mediated host plasticity,

pathogen virulence, as well as dilution effects on pathogen cells by resistant hosts. This study highlights the need to consider the multiple ways in which climate can shape host biology when forecasting patterns of evolution.

6.1 General themes and future work

6.1.1 Rapid microbial evolution can affect host performance

In Chapter 2, I showed that across a variety of host species, experimental warming and cooling could induce host microbiome diversity loss and compositional changes. I found that microbiome responses can be affected by the rate and scale of environmental change. A longer period of host-microbiome lab acclimation and ramped temperature treatments were associated with less microbiome diversity loss compared with sudden warming. Though the relationships between microbiome diversity and community emergent properties (eg. productivity, stability) are unresolved, understanding large-scale patterns in diversity changes driven by temperature is of great importance to predict and manage microbial communities (Shade 2017). The experimental studies I included in this chapter were conducted within a single host generation. Responses of microbiomes to perturbations, on extremely short timescales (minutes or hours) can involve metabolic or physiological changes (Sonnenburg et al. 2005; Turnbaugh et al. 2009; Townsend et al. 2019). Community alterations in host microbiomes can result in fundamental functional changes of the microbiome, may also be essential to buffer host against environmental challenges (reviewed in Kolodny & Schulenburg 2020). For example, coral microbiomes can mediate host protection against heat stress and CO_2 levels through functional alterations (Webster & Reusch 2017; Ziegler et al. 2017, 2019). Microbiome composition

can be manipulated using transplantation. This approach has been developed as an intervention method to improve host performance under stressful conditions (Moghadam et al. 2018; Gupta et al. 2016).

While much attention has been paid to host microbiome community ecological dynamics, less is known about microbial evolution within the community with consequences for host health (Lieberman 2022). Even on short timescales within a single host lifetime, microbiome evolution can occur (Zhao et al. 2019; Barreto & Gordo 2023; Frazão et al. 2022; King et al. 2020). Microbe evolution can occur within weeks or days (Rafaluk-Mohr et al. 2018; King et al. 2016; Ford et al. 2017; Robinson et al. 2018). Experiments on nematode *C. elegans* showed that the protective bacterium *Enterococcus faecalis* evolved within days of interaction with pathogens to provide greater host-defence (King et al. 2016). However, the genetic adaptation of microbes is less well characterized in complex communities stressed by environmental conditions. During a longer host acclimation, microbiomes might adapt via genetic changes *in vivo*, but it is unclear whether a gradual and longer time for host acclimation (Terblanche et al. 2011) could also facilitate adaptative evolution within the microbiomes. If rapid microbiome adaptation could ultimately overcome the slow or small environmental shifts (Voolstra & Ziegler 2020), host adaptation might not occur or be slow, as they will not provide a further fitness increase (Kolodny & Schulenburg 2020).

The above hypotheses can be tested by future evolution experiments. This approach provides a powerful tool to directly test the effect of microbiome evolution on host adaptation (Kolodny & Schulenburg 2020). Long-term evolutionary experiments can be a powerful tool

to reveal the impact of phenotypic plasticity mediated by microbial evolution on host genetic evolution. One challenge of conducting such co-evolutionary experiments is that it requires culturing and isolating individual member in the community to passage them across host generations. Recently, representative set of culturable microbes have been developed and characterized for two model animal hosts – mouse (GM15 composed of 15 bacterial strains, Darnaud et al. 2021) and *C. elegans* (CeMbio composed of 12 bacterial strains, Dirksen et al. 2020). These model systems provide opportunities for future research on how microbiome evolution shapes host health and adaptability.

6.1.2 Interspecific microbial interactions

In Chapter 3, I found that invading pathogens drove microbiota community composition, which decreased the abundance of dominant species and promoted abundance of rare species within hosts. These effects could be driven by inter-species interactions between pathogens and commensal microbes (Vonaesch et al. 2018). Microbial interactions can be direct, involving competition (e.g. colonization resistance) or cooperation (e.g. cross-feeding) (Fons 2000; Romero et al. 2011; Stevens et al. 2021). Such interactions among microbes can also occur indirectly from an altered host environment induced by pathogen infection (Kline et al. 2012). Microbial interactions can lead to a spectrum of outcomes, ranging from beneficial to harmful for the host (Armbruster et al. 2016; DeLeon et al. 2014; Pastar et al. 2013). Such interactions are often mediated by microbe-excreted metabolites, with their strength and direction altered by the modified physical environments over ecological timescales (Ratzke & Gore 2018). Thus, manipulation of environments might induce changes in the interactions among microbiomes and pathogens, eventually altering

host fitness outcomes under infection. Multiple theoretical studies have simulated the dynamics and complex interactions within microbiomes using community ecological modelling (Costello et al. 2012; Koskella et al. 2017; Coyte et al. 2015), which could be further developed to explore microbiome-pathogen interactions under different contexts.

Over a longer timescale, the changing environment could pose selective pressure on microbial interactions (Escalante et al. 2015), shaping community dynamics and host fitness consequences. Studies are needed to explore this field. One approach is to conduct *in vivo* experimental evolution, which is however normally limited to two-member microbiome communities. For example, Barroso-Batista et al. 2020 studied the adaptation of *Escherichia coli* upon colonization of germ-free mice, in the presence and absence of interspecies competition, and showed that the presence of competitor *Blautia coccoides* altered the evolutionary path of *E. coli* in the mouse gut. Another powerful approach is to use computational and mathematical models, which will allow for testing a wide range of environmental conditions and combination of species (Zaccaria et al. 2017; Gorter et al. 2020). Game theoretical models can address the benefits and costs associated with interspecies interactions for members within a community, which can be used to investigate the effect of variable abiotic and biotic factors on the evolution of microbe-microbe interactions (Frank 2010; Frey 2010; Zomorrodi & Segrè 2016).

In this chapter, I used a defined microbial community and conducted experiments in a restricted lab environment where dispersal or migration of microbes is lacking. Such environment can be different from *C. elegans*' natural habitats (eg. rotten fruits and stems,

compost), where the nematode hosts are constantly interacting with diverse microbes (Zhang et al. 2017). Thus, the representative CeMbio community might not fully capture the functional redundancies of worms' natural microbiomes (Zimmermann et al. 2020). It is unclear how the interactions among more diverse host microbiomes and the invading pathogen will shape microbiome structure and host fitness consequences in the wild, with the presence of environmental microbial dispersal. Future experiments under natural or semi-natural conditions will enhance the extrapolation of results from lab to the wild.

6.1.3 Temperature-disease relationship across time

In Chapter 4, I found a broad pattern that experimental warming exacerbated disease severity for ectothermic animals. These results revealed temperature's effect on infection outcomes on an ecological timescale. Less is known about the selective impacts of warming on host-pathogen interactions across multiple generations.

Disease outcomes might depend on the mismatch of host and pathogen's thermal tolerance curves, which are characterized by thermal optimum, maximum and minimum (Cohen et al. 2017). For both ectothermic hosts and pathogens, this curve can indicate the likelihood of population persistence under varying thermal conditions (Kearney et al. 2009). However, an organism's thermal tolerance curve is not fixed. Studies on multiple organisms, ranging from bacteria to insects, have shown that thermal optimum and maximum values can shift rapidly as the organism adapts to temperature changes (Listmann et al. 2016; Schulte et al. 2011; Geerts et al. 2015). Under rapid temperature changes, pathogens with shorter generation times and faster metabolisms might evolve more rapidly than their hosts

(Gillooly et al. 2001). However, hosts may adapt to temperature changes over longer timescales which might mitigate the negative impacts of infection and warming. Future investigations could track long-term host and pathogen adaptation to temperature changes using evolutionary experiments.

To date, much of our understanding of host or pathogen thermal adaptative responses are from experimental studies using constant rather than fluctuating warming regimes. The former does not resemble the temperature variability in nature (Schade et al. 2014). A recent study showed that fluctuations in temperature across 3-4 generations, compared to constant warming, can rapidly select for elevated thermal tolerance in phytoplankton, *Thalassiosira pseudonana*, while long-term fluctuations for 30-40 generations generated two genetically distinct populations varying in their thermal tolerance (Schaum et al. 2022). These findings indicate that the degree of host evolved thermal tolerance can vary by frequencies and duration of temperature changes. Future lab studies should consider effects of these experimental factors, for generating more ecologically relevant predictions on host performance under infection and thermal stress.

6.1.4 Loss of resistance

Natural populations are under constant selection from pathogens, which reduce the fitness of infected individuals. Pathogens have been broadly found to select for host resistance in lab (Buckling & Rainey 2002; Bérénos et al. 2009) and wild populations (Duffy & Sivars 2007; Thrall et al. 2012). However, in Chapter 5 and in studies of natural populations (Allen et al. 2004; Laine 2004), loss of resistance has also been detected. In Chapter 5, I observed

loss in population resistance facing pathogen infection, under constant and periodic warming regimes. In natural populations, resistance rarely fixes. Why is not always clear (Koskella 2018). Several hypotheses have been proposed, such as pathogen counteradaptation (Allen et al. 2004; Bondy-Denomy et al. 2013), fitness cost carried by resistance (Sheldon & Verhulst 1996), and host reversion to susceptibility (Weissman et al. 2018; Chaudhry et al. 2018). In Chapter 5, I found that such an outcome could be shaped by the combined effects of fitness constraints of genetic-based resistance, temperature-mediated host plasticity, and protection conferred by dilution effects.

The magnitude of resistance costs shaped by temperature is unclear. Such costs are hard to measure due to the context-dependent nature of host fitness, which is highly impacted by environments (Meaden et al. 2015). It is also unclear to what extent the cost of resistance and host plasticity contribute to loss of resistance under infection. As climate change generates more variable temperatures, future studies on resistance evolution should investigate these questions under varying temperature contexts.

6.2 Concluding remarks

Climate change is leading to shifts in average and extreme temperatures (Allen et al. 2018), as well as modifying biotic interactions between species (Parmesan et al. 1999; Hance et al. 2007; Cahill et al. 2013; Longdon et al. 2015). Work in this thesis was inspired by the disease pyramid (Bernardo-Cravo et al. 2020) to investigate host microbiome dynamics, infection outcomes and host resistance evolution, under the driving force of temperature. The meta-analytic and experimental work spanning both ecological and evolutionary

timescales are timely for understanding the importance of microbial symbiosis in animal responses to climate change. Future work could expand on work in this thesis, to investigate the fitness consequences from these interactions, as well as the molecular and metabolic mechanisms underlying them.

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