

RESEARCH ARTICLE

Microbial Responses to Temperature Change Mediated by Nutrient Enrichment

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ABSTRACT

Aim: Understanding of the mechanisms in community reorganisation and predicting species distribution are challenging because species responses to warming vary notably. We assessed thermal responses of aquatic bacterial communities in 167 stream biofilms and 480 field aquatic microcosms on subtropical and temperate mountainsides with contrasting climates, and examined the joint effects of temperature and nutrients on thermal responses.

Location: Galong and Qilian mountains of the Tibetan Plateau, China.

Time Period: July to September in 2018.

Major Taxa Studied: Bacteria.

Methods: We examined bacterial communities using high-throughput sequencing. We quantified aggregated thermal responses of bacterial communities for each sample based on changes in species abundance along temperature gradients. Finally, we studied the effects of temperature change and nutrient enrichment on thermal responses using structural equation models.

Results: Bacterial species showed consistent responses to temperature change within each climate zone in streams or microcosms. The magnitude of positive and negative thermal responses increased and decreased with lower rRNA operon copy numbers, respectively. In the two contrasting climate zones, the community-level thermal responses consistently increased with rising temperatures. Bacterial phyla and classes with diverse species thermal responses showed greater sensitivity of thermal responses to warming. Unexpectedly, thermal responses were more sensitive to warming at higher and lower nutrients in the subtropical wet and the temperate arid climate zones, respectively. The divergence is explained by the fact that nutrients showed stronger effects on thermal responses in the temperate arid than in the subtropical wet climate zones, while temperature was dominant in both climate zones. The result was consistent in streams and microcosms.

Wenqian Zhao and Ang Hu contributed equally to this work.

Main Conclusions: Our synthesis across two contrasting habitats and climates clearly shows consistent patterns in microbial thermal responses along temperature gradients. Sensitivity of thermal responses to warming is mediated by nutrient enrichment. Our findings provide a novel understanding of aquatic microbial biodiversity responses to global change.

1 | Introduction

Contemporary climate change is affecting species distribution ranges and causing rapid community reorganisations (Blowes et al. 2019; Poloczanska et al. 2013; Schuster et al. 2022), which could have far-reaching consequences for ecosystem functioning (García Molinos et al. 2016; Pecl et al. 2017). In both aquatic and terrestrial ecosystems, global warming favours species adapted to warmer environments, while it diminishes those adapted to cold conditions (Antao et al. 2020; De Frenne et al. 2013). Local species gains and losses, as well as shifts in the abundance of species with varying temperature tolerances, drive changes in community structure (Burrows et al. 2019; Smith et al. 2022). Microorganisms, as key drivers of ecosystem processes, are crucial to global biogeochemical cycles. Shifts in microbial community structure and their ability to respond to climate warming will severely impact global scale geochemistry and consequently ecosystem functioning, such as production, decomposition, and nutrient cycling (Seidel et al. 2023). Therefore, elucidating the response of microbial communities to climate warming is crucial for understanding the extents of community reorganisation and ecosystem responses under climate change (Wang et al. 2022).

Microbial responses to climate change may be shaped by their life history traits, which influence species growth rates and resilience to disturbances (Stearns 1998; Wang et al. 2020). These traits can be used to track complex ecosystem responses to environmental change (Green et al. 2008; Wieczynski et al. 2021). For instance, temperature fluctuations may alter the life history strategies of enteric bacterial communities in media, influencing their growth and survival strategies (Basan et al. 2020). Moreover, ecological communities with diverse traits tend to be more resilient to environmental change, as these diverse traits enhance adaptive potential (Burrows et al. 2019; Elmqvist et al. 2003). Microbial communities may acclimate to warming by altering their structure or through physiological adjustments (Alster et al. 2023; Cohen et al. 2023; Walker et al. 2018). Specifically, the taxonomic composition with different trophic strategies may strongly influence their responses to warming for aquatic bacterial communities (Arandia-Gorostidi et al. 2017). Quantifying microbial traits enables us to identify r-strategists and K-strategists and to assess whether these strategies are differentially favoured in varying thermal environments (Andrews and Harris 1986; Smith et al. 2022). Overall, exploring microbial life history traits can enhance our understanding of their responses to increasing temperatures.

The response of microbial communities to environmental factors varies with changing temperatures. Rising temperatures are expected to accelerate microbial growth and metabolism rates based on thermodynamic and metabolic theory (Brown et al. 2004; Walker et al. 2018). The intrinsic temperature sensitivity of microbial growth and respiration rates increases at higher temperatures (Nottingham et al. 2019; Ren et al. 2024). The temperature sensitivity of microbial processes reflects a combination

of the community characteristics and environmental constraints (Anderson et al. 2021; Louthan et al. 2021; Pacifici et al. 2017). For instance, the difference in the taxonomic composition and temperature sensitivities of taxa determines the temperature sensitivities of bacterial growth (Wang et al. 2021). Human impacts, such as nutrient enrichment that alters resource composition and supply in ecosystems, can either strengthen or dampen the temperature sensitivity to warming (Comte et al. 2021; López-Urrutia and Morán 2007). For instance, the temperature effects on heterotrophic bacterial communities are stronger during high-nutrient periods in marine ecosystems (Huete-Stauffer et al. 2015). However, the roles of community characteristics and human impacts on temperature sensitivity of microbial communities to climate warming remain poorly understood.

Here, we quantified the thermal response of bacterial communities along elevational gradients in streams and field microcosms, and examined how their responses were jointly influenced by temperature, nutrients and their life history traits. We analysed aquatic bacterial communities from 167 composite biofilms in natural streams and 480 sediments collected from field microcosms on the two mountainsides of the Tibetan Plateau, that is subtropical Galong Mountain and temperate Qilian Mountain. The two mountains exhibit contrasting climatic conditions, with Galong Mountain being wetter and warmer, while Qilian Mountain is drier and colder. We selected the two contrasting habitats (streams vs. microcosms) and two contrasting climates to maximise the generality of our main findings. We addressed the following four main questions: (1) At a species level, how do bacterial thermal responses vary with their life history traits, such as rRNA operon (*rrn*) copy number? (2) At a community level, how do bacterial thermal responses vary along temperature gradients? (3) How is the sensitivity of thermal responses to warming mediated by community characteristics and nutrient enrichment? (4) How are bacterial thermal responses jointly affected by temperature change and nutrient enrichment?

2 | Materials and Methods

2.1 | Study Area and Field Sampling

We sampled stream water and biofilms from 86 small streams along elevational gradients on Galongla Mountain (29.3930–30.0341 N; 92.3243–95.6863 E) in July 2018, and from 81 sites along three larger stony streams on Qilian Mountain (38.1755–39.7458 N; 97.95582–100.3375 E) in September 2018, both located in the Eastern Tibetan Plateau, China. The sampling sites ranged in elevation from 753 to 4750 m on Galongla Mountain and from 1494 to 4030 m on Qilian Mountain. Concurrently, field microcosm experiments were conducted for 1 month along elevational gradients on the two mountains, and overlying water and sediments were collected at the end of the experimental period. The two regions differ markedly

in climate, representing subtropical wet and temperate arid climates for Galongla Mountain and Qilian Mountain, respectively. Galongla Mountain experiences a wetter and warmer climate, with mean annual precipitation (MAP) ranging from 289 to 2412 mm and mean annual temperatures (MAT) from -0.7°C to 20.1°C . In contrast, Qilian Mountain is drier and colder, with MAP and MAT ranging from 100 to 364 mm and -6.1°C – 7.4°C , respectively. We had a similar number of streams sampled in the two climate zones and would expect the sample number would not affect our findings and conclusions between the two regions.

We followed the same protocols for collecting stream biofilms as shown in the previous study (Wang et al. 2011). Briefly, each sampling site was divided into five or 10 cross-sections based on the stream width. Along these transects, 10 stones from riffle or run habitats were randomly selected, and biofilm was scraped from a predefined 9 cm^2 area for subsamples using a sterilised sponge. At each site, water samples and the composite biofilm samples pooled from subsamples were immediately preserved at -18°C . More details on the sampling scheme were described in our previous reports (Zhao et al. 2024).

The field microcosm experiments were characterised by sterile aquatic ecosystems with consistent initial artificial lake water and lake sediments but different locally colonised microorganisms. The approach minimises the complexity of natural ecosystems by controlling the initial conditions, enabling direct investigation into the effects of elevated temperature and anthropogenic nutrient enrichment on microbial thermal responses. Briefly, 10 and six different elevations were selected on Galongla and Qilian Mountains, respectively. These elevations were 743, 947, 1240, 1578, 1850, 2072, 2328, 2658, 3116 and 3565 m a.s.l. on Galongla Mountain (29.3507–29.7414 N; 95.3412–95.6863 E), and 1750, 2214, 2670, 3050, 3477 and 3905 m a.s.l. on Qilian Mountain (39.3268–39.5974 N; 94.2733–94.3384 E).

We followed the same protocols for field microcosm experiments as in previous literature (Wang et al. 2016). At each elevation, 30 aquatic microcosms (1.5 L bottle) were established, including 10 nutrient levels and three replicates of each level. Each aquatic microcosm was composed of 1.2 L sterilised artificial freshwater and 15 g sterilised sediments. The 10 nutrient levels in overlying waters were 0, 0.45, 1.80, 4.05, 7.65, 11.25, 15.75, 21.60, 28.80, and 36.00 mg N L^{-1} of KNO_3 (Hu et al. 2024; Wang et al. 2016), which were selected based on eutrophication of Taihu Lake. To maintain stoichiometric balance, KH_2PO_4 was added to the bottles to ensure an N/P ratio of 14.93, closely aligning with the annual average ratio (14.49) in Taihu Lake during 2007. Therefore, we use ‘nutrient enrichment’ to refer to a set of nutrient levels of both nitrate and phosphate in our study, and the nitrate levels were used to represent nutrient enrichment in the statistical analyses. Lake sediments were collected from the centre of Taihu Lake, China, in October 2017, and autoclaved eight times at 121°C for 30 min. Sterility was confirmed by negative PCR results after DNA extraction using bacterial primers, with no detectable amplification observed. The common sterilised sediment can be regarded as a standardised ‘culture medium’ for microbial colonisation. In theory, we can collect standardised sediment from any lakes. This method provides greater confidence that changes in microbial species can be mainly attributed to variations in colonised

microorganisms across microcosms (i.e., different samples) and the two key environmental gradients of temperature and nutrients, rather than initial conditions.

The microcosms were left in the field for 1 month, allowing airborne microbes to freely colonise the water and sediments (Hu et al. 2024). The experimental setups were left uncovered, with the top 20% left empty to prevent overflow during heavy rain. The bottom 10% was buried in local soil to buffer against large air temperature fluctuations and reduce UV exposure to the sediments. During each experimental period, we inspected these sites twice and added sterilised water to maintain a final volume of approximately 1.2 L. At the end of the experimental period, we collected the water and sediments from each bottle, which were immediately frozen at -20°C .

We assessed microbial communities from two contrasting habitats, i.e., stream biofilms and microcosm sediments, which provide valuable insights into their responses to temperature change. Microbial communities in these two habitats differ fundamentally in habitat structure and microbial colonisation dynamics. In natural streams, microbial communities are influenced by a variety of environmental variables, such as surrounding vegetation and local physicochemical conditions (Giling et al. 2013; Wang et al. 2017). Stream biofilms typically form on exposed substrates (e.g., stones) under oxic and environmentally variable conditions, where microbes are subject to greater fluctuations in light, oxygen availability, and hydrodynamic forces (Battin et al. 2003). These conditions favour microbes adapted to fast-flowing water and the continuous export of nutrients and organic matter (Battin et al. 2016). In contrast, microcosms offer a simplified environment where ecosystem complexity is reduced by using consistent artificial lake water and lake sediments. Microcosm sediments provide a more stable, organic-rich habitat, with microbes being less exposed to external environmental fluctuations such as UV radiation (Wang et al. 2016).

2.2 | Physicochemical Variables

Several environmental variables were collected at each sample in natural streams and field microcosms. We measured in situ the longitude, latitude, elevation, water temperature, conductivity, and pH. In field microcosms, water temperature, pH, and conductivity at all elevations were measured within 2 h before noon on the day before the final sample collection to avoid the impact of daily temperature fluctuations. We also measured dissolved nutrients in the water using a continuous flow analyser (Skalar SA 1000, Breda, The Netherlands), that is NO_x^- , NO_2^- , NH_4^+ and PO_4^{3-} . In streams, shading (% canopy cover) was estimated, and 10 randomly selected stones were measured to calculate stone size. Stream width, depth, and current velocity were measured at 10 random locations along the cross-channel transects. Total phosphorus and total nitrogen in the stream water were analysed in the laboratory.

2.3 | Bacterial Communities

The bacteria communities in biofilms and sediments were examined using high-throughput sequencing of 16S rRNA genes,

following previous literature (Wang et al. 2017). Bacterial genomic DNA was extracted using the MoBio PowerSoil DNA Isolation Kit (MoBio, Carlsbad, USA). The V4 hypervariable region of the 16S rRNA gene was amplified in triplicate with universal primers 515F and 806R (Peiffer et al. 2013). PCR products from triplicate reactions were pooled at equal molarity and sequenced through the Miseq PE300/NovaSeq PE250 (Illumina, USA) (Caporaso et al. 2011; Caporaso et al. 2012). The sequence processing was achieved in QIIME v1.9.1 (Caporaso, Kuczynski, et al. 2010) and operational taxonomic units (OTUs) were clustered at 97% sequence similarity via the seed-based UCLUST algorithm (Edgar 2010). Representative sequences from each OTU were aligned to the Greengenes imputed core reference alignment (DeSantis et al. 2006) using PyNAST (Caporaso, Bittinger, et al. 2010), and were further identified taxonomically using the RDP classifier (Wang et al. 2007). Chloroplast and archaeal sequences were subsequently removed. Biofilm bacterial sequences were rarefied to 61,000 per sample, while sediment sequences were rarefied to 63,000 per sample. It should be noted that our results are based on amplicons, and the relative abundance may be biased due to PCR efficiencies. We encourage future studies to employ more advanced techniques without PCR to more precisely quantify the species relative abundance along environmental gradients. To simplify, we referred to OTUs as ‘species’ throughout this study, given the widespread use of the 97% sequence identity cut-off in community analysis.

2.4 | Estimating Community-Level Environmental Responses

The thermal responses of microbial communities could be quantified using community-level environmental response (iCER) of temperature for each sample based on changes in the species relative abundance along temperature gradients (Hu et al. 2024). The indicator iCER was originally developed for dissolved organic matter to quantify the degree to which DOM assemblages respond to temperature change for each sample, but could be well extended to microorganisms. The indicator was calculated by two primary procedures (Figure 1).

First, the species-specific environmental response of temperature (SERs) for individual species in each climate zone was calculated by the Spearman’s correlation coefficient ρ between the relative abundance of each species and water temperature. All corresponding p -values of SERs were adjusted using the Benjamini–Hochberg (BH) procedure to control the false discovery rate (FDR) at 0.05 (Benjamini and Hochberg 1995). The SER represents the effect size for the change in relative abundance of each species as a function of temperature across time, space, or treatments. Species observed in less than a third of the whole samples were removed to minimise errors in correlation analyses caused by low-occurrence species. For field microcosm experiments, we used the water temperature on the last day of the incubation, as it more accurately reflects the conditions experienced during the experiment than the mean annual temperature. It should be noted that all these temperatures should be continuous in both streams and microcosms. The continuous values are clear for stream samples. However, for microcosms, the temperature values were clustered for each

elevation. The experiments were designed to reflect the ambient climate conditions at 10 or 6 different elevations on each mountain. Moreover, since iCER depends on SER, it is necessary to use statistically independent data sets for their calculation—one for computing SERs and the other for computing iCERs. These two independent data sets ensured that the relative abundance of the same species was not used repeatedly in SER and iCER calculations. Practically, we randomly divided the whole data set into two independent subsets based on the 80:20 split ratio: 80% for calculating SER (the ‘SER dataset’) and 20% for calculating iCER (the ‘iCER dataset’). The common split ratio of 80:20 was also used in species distribution modelling or machine learning for the data splitting (Naimi and Araújo 2016). This unitless coefficient SER facilitates easy comparison among studies. Positive and negative SER values reflect whether species are accumulating and depleting with increasing temperatures, respectively.

Second, for each climate zone in streams and field microcosms, we calculated iCER of each sample. The iCER is calculated from the weighted average of all SERs in a given bacterial community. Thus, iCER reflects the response of a bacterial community to temperature changes in terms of the difference in magnitude of positive and negative SERs. Specifically, the iCERs were calculated as the sum of the product of the SER value for each species (SER_i) and relative abundance I_i divided by the sum of all intensities using the equation:

$$iCER = \frac{\sum (SER_i \times I_i)}{\sum (I_i)}$$

We calculated the iCER values for each sample using iCER dataset within each climate zone in streams and microcosms. Furthermore, we recalculated the iCERs using species with only statistically significant (adjusted $p \leq 0.05$) SERs. The species with only statistically significant (adjusted $p \leq 0.05$) SERs were included in the iCER calculation because the results were strongly positively correlated with those calculated using all SERs ($R^2 \geq 0.95$, $p < 0.001$) (Hu et al. 2024). The above data partitioning and the calculation of SER and iCER were randomly repeated 999 times, and their averaged values across randomisation values were used for the subsequent statistical analyses. The larger positive and negative iCER values indicate that communities are dominated by warm-accumulating and warm-depleting species, respectively, while an iCER of zero suggests the two groups of species equally dominate. That is, the larger absolute values of iCER reflect that communities will have greater shifts in their thermal responses with increasing temperatures. The SER and iCER values were computed using iDOM (Meng et al. 2024) and FD package in R (Dormann et al. 2009).

However, it is important to note that it is still challenging to quantify the abundances of microbial species in an absolute sense largely because 16S rRNA copy numbers in bacterial genomes vary by an order of magnitude. We used the change of relative abundance to reflect the effects of environmental gradients on microbial species as the relative abundance is also jointly controlled by the biotic factors such as species interactions. Thus, the iCER of a microbial taxon characterises its responses

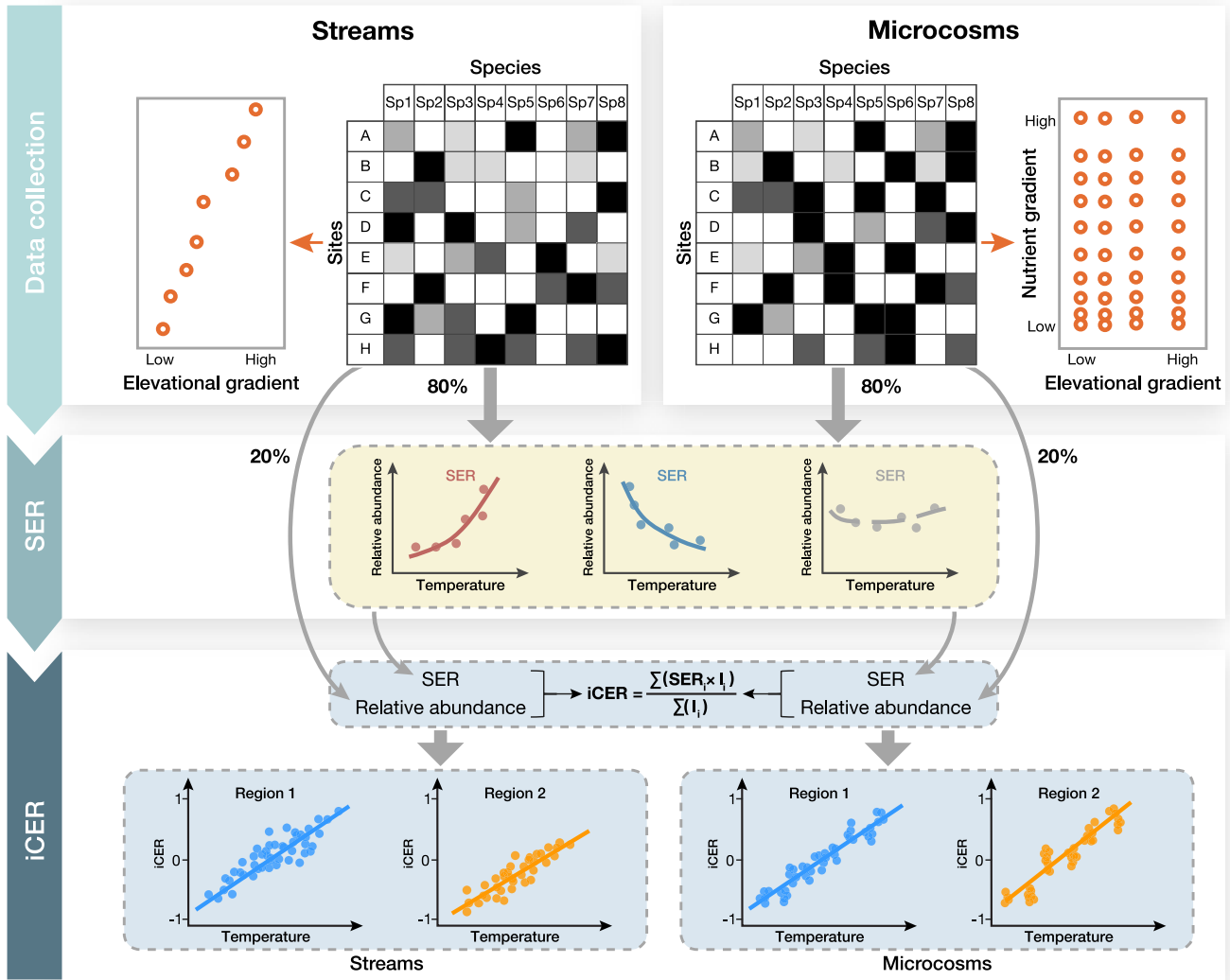


FIGURE 1 | Flowchart of community-level environmental response (iCER) of temperature. Specifically, the indicator development involved two primary procedures: (1) calculating the direction and magnitude of a species-specific environmental response (SER) of temperature and (2) producing the indicator of compositional-level thermal responses (iCER). Since iCER depends on the SER, we used two independent data sets to avoid the repeated use of species relative abundance data for SER and iCER calculations. The whole data was randomly divided into two subsets based on an 80:20 split ratio: 80% for calculating SER (the ‘SER dataset’) and 20% for calculating iCER (the ‘iCER dataset’). The data partitioning and the calculation of SER and iCER were randomly repeated 999 times, and their averaged values across randomisation values were used for the subsequent statistical analyses. First, the species-specific environmental response of temperature (SERs) for individual species in each climate zone was calculated by the Spearman’s correlation coefficient ρ between the relative abundance of each species and water temperature. Positive and negative SER values reflect whether species are accumulating and depleting with increasing temperatures, respectively. Second, the iCER is calculated from the weighted average of all SERs in a given bacterial community. Specifically, the iCERs were calculated as the sum of the product of the SER value for each species (SER_i) and relative abundance I_i divided by the sum of all intensities. The larger positive and negative iCER values indicate that communities are dominated by warm-accumulating and warm-depleting species, respectively, while an iCER of zero suggests the two groups of species equally dominate.

to temperature change in terms of relative dominance within a microbial assemblage, rather than the response of its strains in a pure culture.

We further confirmed the feasibility of using relative abundance for environmental responses via reviewing previous studies (Table S1). Changes in species relative abundance in response to environmental factors such as drought treatment, salt stress, and warming are commonly analysed using statistical models

(Dong et al. 2024; Lavallee et al. 2024; Saez-Sandino et al. 2025). Such changes in species relative abundance could be evaluated via the experimental setups with control and treatment groups (Figure S1) (Delgado-Baquerizo et al. 2020; Knight et al. 2024), and the field observations or experimental setups with continuous environmental gradients (Figure S1) (Dove et al. 2021; Mackelprang et al. 2011). Further details supporting the feasibility of using relative abundance to quantify thermal responses are provided in the [Supporting Information](#).

2.5 | Estimation of Bacterial *Rrn* Copy Number

The bacterial rRNA operon (*rrn*) copy numbers were estimated through the rrnDB database based on their closest relatives in the database (Stoddard et al. 2015) (version 5.9). Specifically, each OTU was matched with the database from the lowest confident (> 60%) taxonomic rank. Each OTU was matched with the database starting from the lowest rank. If the taxon within the same species/strain was available in the database, the *rrn* copy number of this strain was used. If the closest taxon was unavailable, the mean *rrn* copy number of the higher taxa (i.e., genus, family, class, etc.) for that OTU was assigned. The abundance-weighted average *rrn* copy number was then computed for each sample using the following equation:

$$\text{community - level } rrn \text{ copy number} = \frac{\sum_{i=1}^N R_i}{\sum_{i=1}^N \frac{R_i}{n_i}}$$

where N is the number of OTUs, R_i is the relative abundance of species i in the community and n_i is the estimated *rrn* copy number of OTU $_i$. This analysis was performed using FD V1.0.1260 package in R (Dormann et al. 2009).

2.6 | Statistical Analyses

We assessed whether a given species showed the same SER at the intra-regional and inter-regional scales, that is, spatial transferability. The degree of spatial transferability was quantified using the Pearson correlation coefficient r or linear regression slope between the SERs of two sample groups within each climate zone (intra-regional scale) or across climate zones (inter-regional scale). At the intra-regional scale, we divided whole samples of each climate zone into two groups based on randomly splitting water temperature (for streams) or nutrient levels (for microcosms) in half. To maximise environmental heterogeneity in microcosms, we randomly partitioned the data by nutrient levels rather than by samples. The Pearson correlation coefficient r or linear regression slope between the SERs of the two groups were calculated for all shared species at each climate zone. At the inter-regional scale, we examined the statistical relationships between the SERs of the two climate zones for all shared species. For instance, we correlated the SER of species X in the subtropical wet climate zone with the SER of species X in the temperate arid climate zone. All shared species with SER values were included to quantify the degree of spatial transferability for statistical analyses. At the intra-regional scale, in streams, there were 3850 and 3410 SERs included for the analyses of Pearson correlation or linear regression in the subtropical wet and the temperate arid climate zones, respectively (Figure 2b). In microcosms, there were 4638 and 3378 SERs used in the two climate zones, respectively.

We investigated the relationship between species thermal responses and their *rrn* copy numbers by gradually removing species with high and low *rrn* copy numbers, respectively. This is because slow-growing oligotrophs and fast-growing copiotrophs species may respond differently to temperature changes. We divided the species into two groups with positive and negative SERs and classified the species into 40 equal-sized bins for each

group according to their *rrn* copy numbers at each climate zone in streams and microcosms. For fast-growing copiotrophs, for instance, we removed the species starting from the bins of the highest *rrn* copy numbers and then calculated the mean SER values for the remaining species. The mean SER values were visualised by plotting against the mean *rrn* copy number of remaining species. To ensure a continuum of *rrn* copy numbers and reveal continuous transitions in species thermal responses, we included all species with SER values in the analyses, not just those with statistically significant SERs. We confirmed the robustness of the results by repeating the analyses using 20 equal-sized bins of all species (Figure S9).

At the community level, we applied linear models to explore the relationships between iCER and temperature along elevational gradients at both climate zones for streams and microcosms. Additionally, the relationships between iCER and temperature across different nutrient enrichment levels were visualised in microcosms.

Furthermore, the linear slopes between iCER and temperature were used to explore the sensitivity of thermal responses to increasing temperatures. At the phylum or class level of microbes, we used linear models to examine the relationships between the sensitivity of thermal responses to warming and community characteristics, which refer to the variability of species thermal responses. The variability of species thermal responses was calculated as the standard deviation of species thermal responses within each phylum or class.

The sensitivity of thermal responses to warming was related to nutrient enrichment, and these relationships were explored using linear and quadratic models. The model with the lower value of Akaike Information Criterion was chosen as the better option (Yamaoka et al. 1978). In addition, we used linear models to explore the relationships between the variability of species thermal responses and nutrient enrichment. We calculated the variability of species thermal responses of each sample, and their averaged values of each nutrient level were used.

We finally explored the effects of temperature and nutrient enrichment on thermal responses of bacterial communities using variation partitioning analysis (VPA) (Borcard et al. 1992), and structural equation modelling (SEM) (Grace et al. 2012). VPA was used to quantify the relative contributions of temperature and nutrients towards thermal responses. In streams, we partitioned the variation in thermal responses into temperature, nutrients, and other environmental variables (Table S2). In microcosms, the explanatory variables were categorised into temperature and nutrients (i.e., nutrient enrichment and measured nutrients) considering their initial conditions were consistent (Table S2). For instance, the nutrient variables in streams included total phosphorus and total nitrogen, NH_4^+ , NO_2^- , NO_x^- and PO_4^{3+} . In microcosms, nutrient variables consisted of the initial levels of added NO_3^- and the measured NH_4^+ , NO_2^- , NO_x^- and PO_4^{3+} . The final significant explanatory variables were selected by forward selection with AIC (Miller and Farr 1971). VPA was conducted using the R package *vegan* V2.5-5 (Oksanen 2010).

SEM was used to assess how temperature and human impacts (i.e., nutrient enrichment and measured local nutrients)

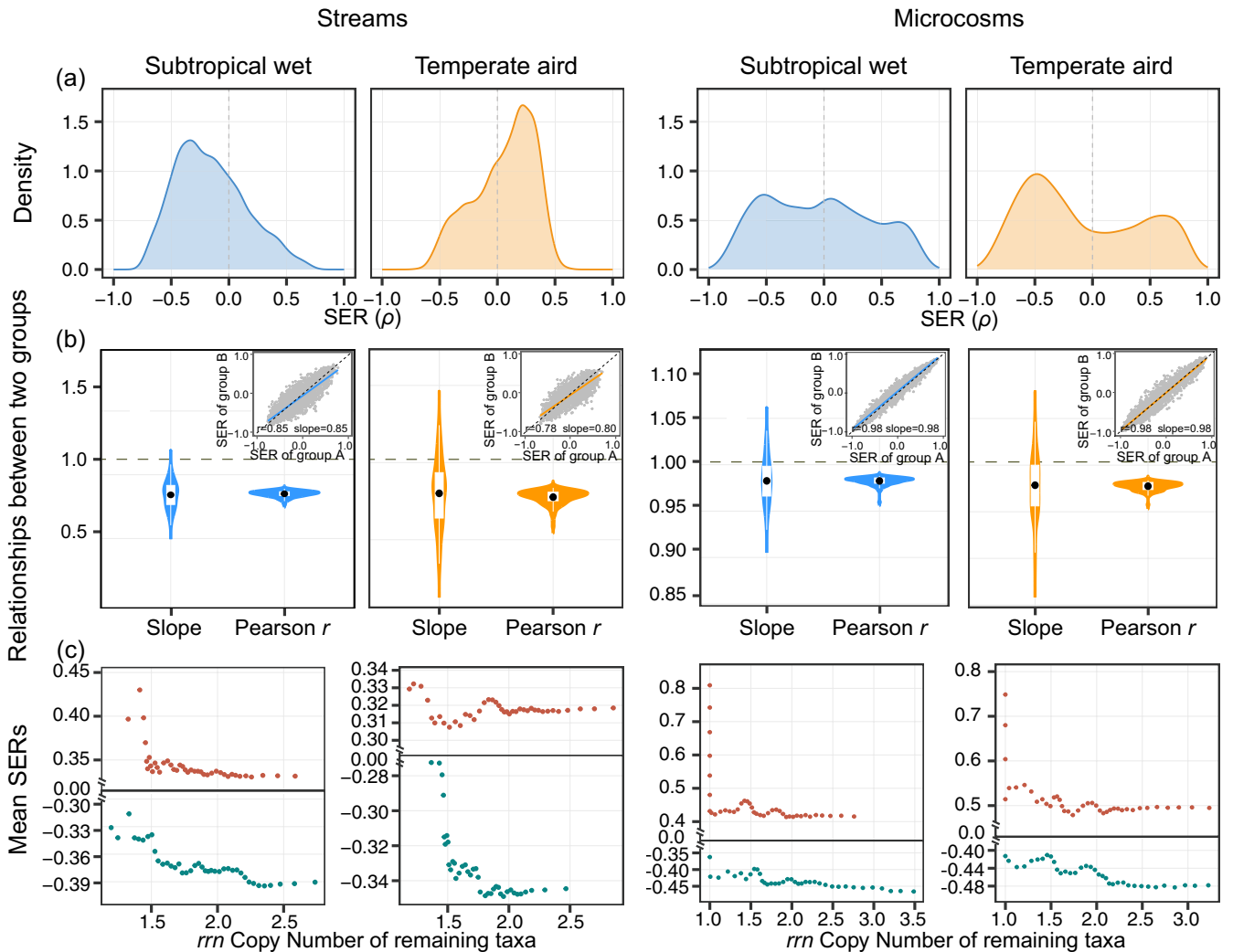


FIGURE 2 | Species thermal responses (SER) of bacteria and their correlation with *rrn* copy number. (a) The distribution of SERs for bacterial species in the subtropical wet (Galongla Mountain) and the temperate arid (Qilian Mountain) climate zones in streams and microcosms. To simplify, we referred to bacterial OTUs as ‘species’ in the results. We further assessed the spatial transferability of SERs at the intra-regional scale (b). In each climate zone, we randomly divided the whole samples (for streams) or 10 nutrient levels (for microcosms) into two groups with 100 randomisation times. The two-sided Pearson correlation coefficient r and linear slope of SERs between two random sample groups (A and B) were visualised using violin plots at each climate zone (b). For the violin plots, the width represents the density of the Pearson correlation coefficient r or linear slope, minima and maxima are the top and bottom of the violin. The lower and upper hinges of the boxplots correspond to the first and third quartiles (the 25th and 75th percentiles), and the black dots in the boxplot show the means ($n = 100$ randomisation times). The insert figures show an example of one of the randomisations, with a slope equivalent to the mean value of slopes in 100 randomisation times at each climate zone. The relationships of SERs between the two groups were visualised with linear regression models for all species (blue and yellow lines) in insert figures. In streams, 3850 and 3410 points were included in the analyses of Pearson correlation or linear regression in the subtropical wet and the temperate arid climate zones, respectively. In microcosms, 4638 and 3378 points were used in the two climate zones, respectively. Statistical significance of the model fits with F-statistics is indicated by solid ($p \leq 0.05$) or dotted ($p > 0.05$) lines. Dashed black line marks 1:1 relationship. We further showed the gradual changes in positive (red) and negative (blue) SERs along the continuum of species *rrn* copy numbers (c). We categorised all species into two groups with positive and negative SERs, and then divided all species of each group into 40 equal-sized bins according to their magnitude of *rrn* copy number to create the continuum of *rrn* copy numbers. For each group, the species from the bins with higher *rrn* copy number were sequentially removed and generated 40 removal scenarios. The mean SERs were plotted against the mean *rrn* copy number of the remaining species for each removal scenario.

directly and indirectly affected the thermal responses of bacterial communities. The hypothesised structure of causal links was established as shown in Figure S20. Five latent variables were used in streams and microcosms: water temperature, environmental variables or nutrient enrichment, nutrients (measured local nutrients), life history traits (community-level *rrn* copy number), and community-level thermal responses. All latent variables were transformed using z -scores before

modelling, and we used composite variables to integrate the effects of nutrients and environmental variables through multiple regressions (Table S3). The hypothesised links in the model were tested using AIC and overall model fit statistics (Grace et al. 2010). The final model was determined by identifying the lowest AIC value among those models with a non-significant χ^2 test ($p > 0.05$), high comparative fit index (CFI > 0.95) and low standardised root mean squared residual

(SRMR < 0.05) (Table S4). The analysis was performed with R package lavaan V.0.5.23 (Rosseel 2012). All the data and code supporting the analyses are deposited in FigShare repository (<https://doi.org/10.6084/m9.figshare.28514021.v1>).

3 | Results

Thermal responses of species (SERs) varied substantially among bacteria, while maintaining consistent magnitude within regions, indicating their spatial transferability (Figure 2). SER, quantified with Spearman's correlation coefficient, varied from -0.82 to 0.82 in streams and from -0.96 to 0.94 in field microcosms (Figure 2a). The negative median values were -0.30 and -0.46 in streams and microcosms, respectively, and positive median values were 0.23 and 0.42 . When we randomly divided samples into two groups for each climate zone with 100 randomisation times, we found that each species showed consistent responses to water temperature between the two groups (Figure 2b). This was indicated by higher mean Pearson r values of 0.85 and 0.78 , and linear regression slopes of 0.85 and 0.80 in the subtropical wet and the temperate arid climate zones for streams, respectively (Figure 2b). In microcosms, the mean Pearson r values and linear regression slopes were all 0.98 in the two climate zones. This result suggests spatial consistency was stronger in microcosms than in streams. The above findings were further supported at bacterial phylum and class levels (Figures S2–S7). For instance, among bacterial classes, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Planctomycetia showed the highly consistent SERs between the two groups, with mean linear regression slopes ranging from 0.78 to 0.96 in streams and microcosms (Figures S3 and S5). At

the inter-regional scale, each species in microcosms showed consistent responses to temperature between the two climate zones with the high Pearson correlation coefficient and linear regression slope of 0.55 and 0.57 (Figure S8). However, the associations between SERs were weak in streams at the inter-regional scale (Figure S8).

The divergent thermal responses of individual species could be related to their intrinsic traits such as life history traits and taxonomic compositions. When species with higher *rrn* copy numbers were removed gradually, the magnitude of mean positive SERs for warm-accumulating species increased in streams and microcosms, except for the subtropical wet climate zone in microcosms (Figure 2c and Figure S9). Conversely, the magnitude of mean negative SERs for warm-depleting species decreased with lower *rrn* copy numbers in both streams and microcosms. Moreover, the relative abundance of the major bacterial phyla or classes substantially varied among different SER values (Figures S10 and S11). For instance, the relative abundance of Alpha-proteobacteria gradually increased with increasing SERs in the two climate zones of microcosms, while the relative abundance of Bacteroidetes decreased (Figure S10). This indicates that warm-accumulating species with more positive SERs were characterised by increased Alpha-proteobacteria and declined Bacteroidetes.

At a community level, we found consistent responses to increasing temperatures for bacterial communities across the two contrasting climate zones in streams and microcosms. At each climate zone, iCER generally increased with water temperature (Figure 3). The stronger thermal responses with higher temperatures were also observed for the main bacterial phyla and classes (Figures S12 and S13). For example, bacterial classes, such as

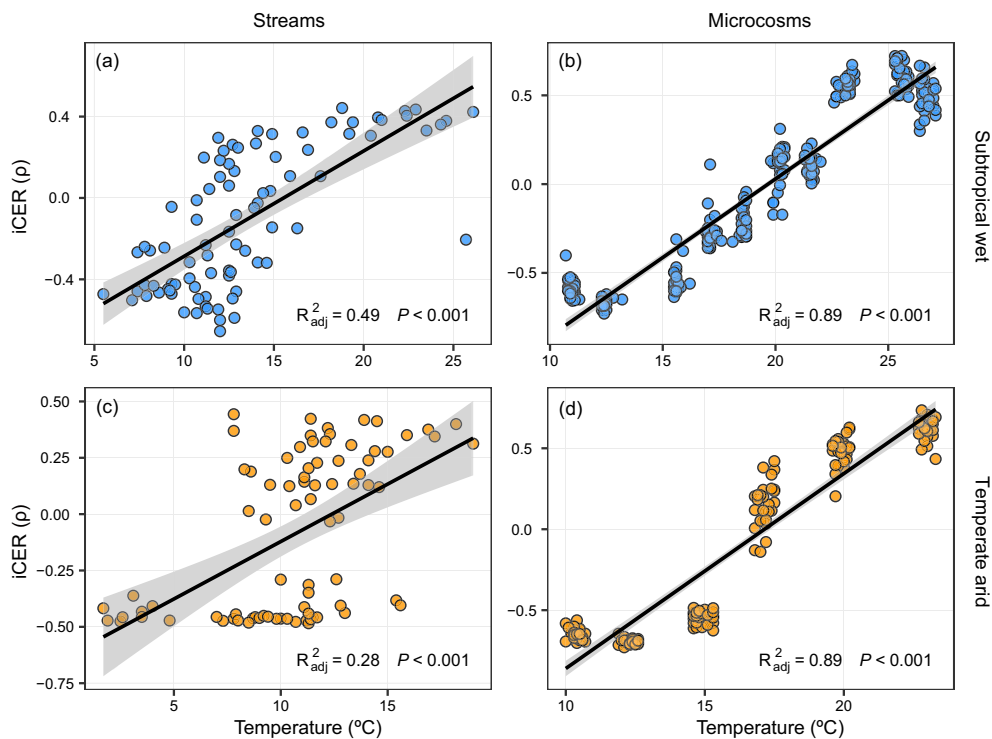


FIGURE 3 | The community-level thermal responses (iCER) of bacteria along increasing temperatures in streams and microcosms. The relationships between iCER and temperature were visualised with linear regression models in the subtropical wet (a, b) and the temperate arid (c, d) climate zones. Statistical significance of linear model fits with F -statistics is indicated by solid ($p \leq 0.05$) or dotted ($p > 0.05$) lines.

Agaricomycetes, Alphaproteobacteria, Betaproteobacteria, and Deltaproteobacteria, consistently showed the higher iCER values in warmer temperatures (Figure S13). The thermal responses of each bacterial community quantify the aggregated response of all species that positively and negatively associate with warming based on changes in the species relative abundance along temperature gradients.

We further examined the sensitivity of thermal responses to warming by the linear slope between iCER and temperature. More positive slopes indicate stronger sensitivity of thermal responses to warming. We found the slopes between iCER and temperature of main bacterial classes were significantly positively correlated with their variability of species thermal responses, which is true for each climate zone in both streams and microcosms ($p < 0.05$; Figures 4 and S15). This suggests a stronger sensitivity of thermal responses to warming in bacterial classes with higher variability of thermal responses. The positive correlation was generally consistent at bacterial phylum level (Figure S16).

The relationships between iCER and temperature were significantly positive across all nutrient levels at each climate zone in

microcosms (Figure 5 and Figure S17). The slopes between iCER and temperature were 1.32 times higher in the temperate arid than in the subtropical wet climate zones ($p < 0.01$; Figure 5a,c). Moreover, the sensitivity of thermal responses to warming was mediated by nutrient enrichment, though the effect of nutrients differed between the two contrasting climate zones. The slopes between iCER and temperature for bacterial communities significantly increased and decreased along nutrient enrichment in the subtropical wet and the temperate arid climate zones, respectively ($p < 0.01$; Figure 5a,c). Interestingly, the variability of species responses at the community level also showed increased and decreased trends along nutrient enrichment gradients in the two climate zones (Figure 5b,d).

We then explored the relative importance of temperature and nutrients on bacterial thermal responses. We found that much of the variation in thermal responses was explained by temperature in streams and microcosms. Specifically, the pure effects of temperature accounted for 6.2%–26.2% and 55.9%–83.8% of variation in thermal responses in streams and microcosms, respectively (Figure 6a,c,e,g). However, the pure effects of nutrients or environmental variables were low (0%–0.3%), except

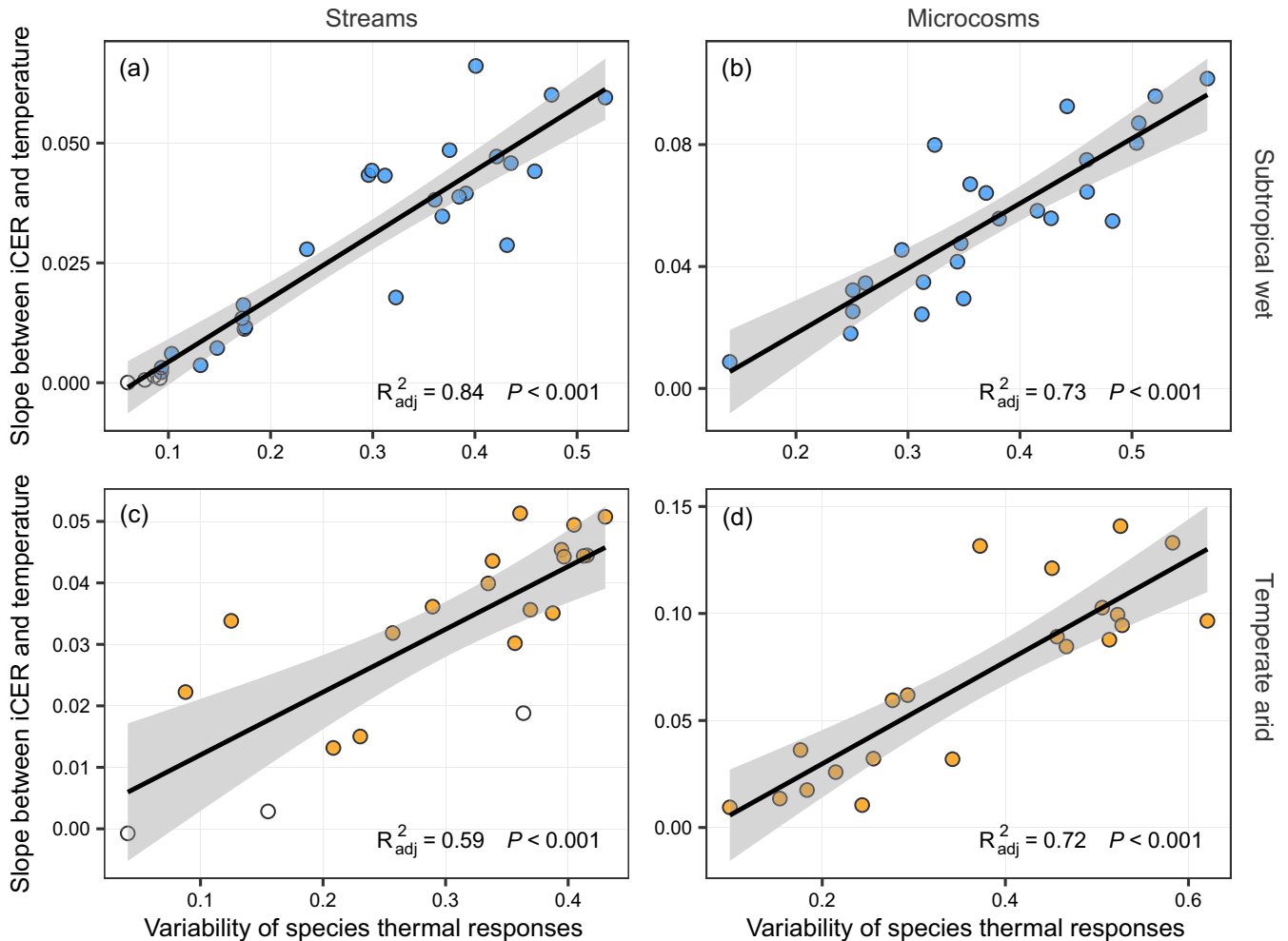


FIGURE 4 | The associations between sensitivity of thermal responses to warming and the variability of species thermal responses for bacterial classes in streams and microcosms. The relationships were visualised with linear regression models in the subtropical wet (a, b) and the temperate arid (c, d) climate zones. The iCER sensitivity was quantified by the slope of the relationship between iCER and temperature, and solid dots indicate the significant ($p \leq 0.05$) relationships. The variability of species thermal responses was calculated as the standard deviation of species thermal responses for each class. Statistical significance of linear model fits with F-statistics is indicated by solid ($p \leq 0.05$) or dotted ($p > 0.05$) lines.

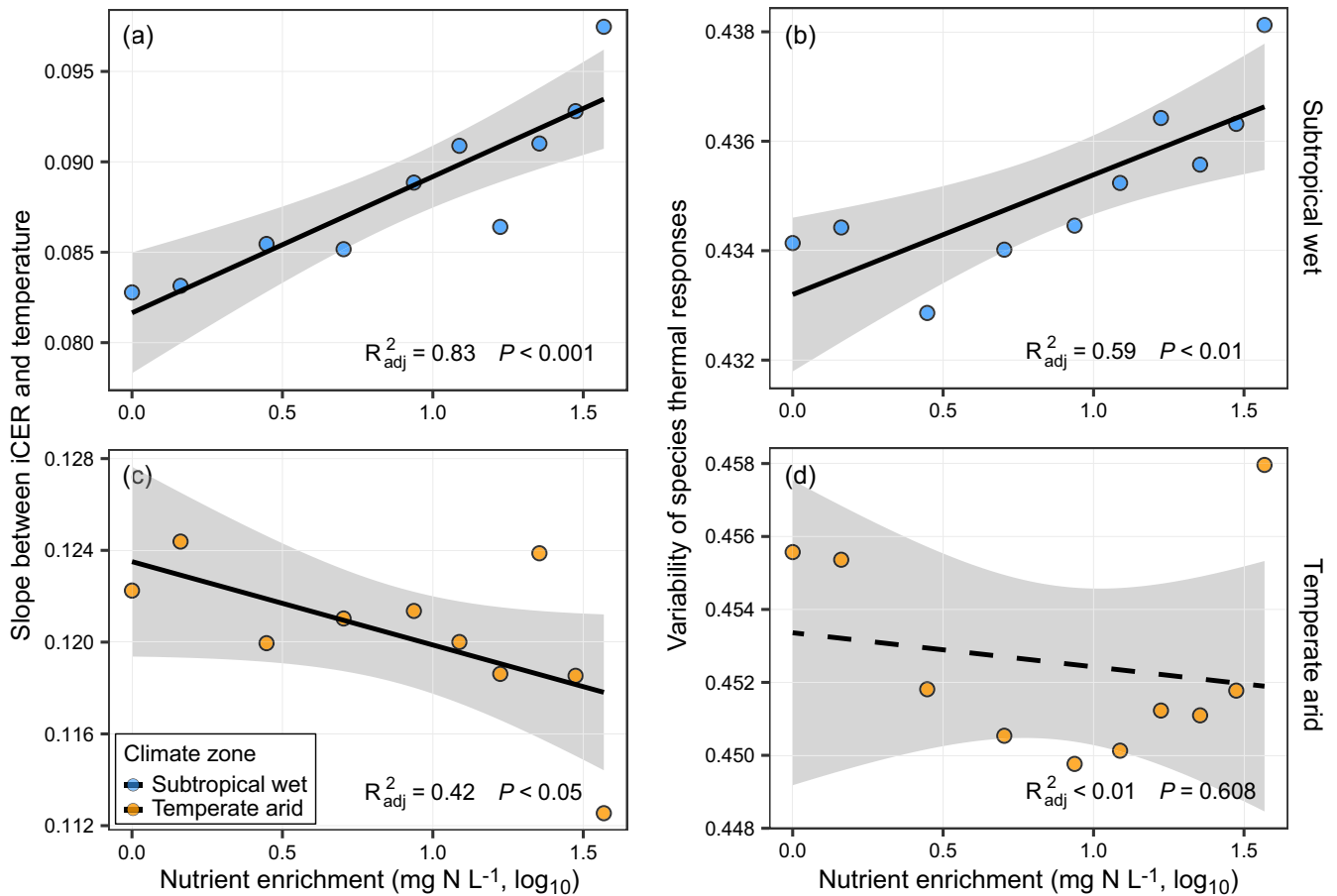


FIGURE 5 | The community-level thermal responses (iCER) and the variability of species thermal responses along nutrient enrichment levels in field microcosms. (a, c) The relationships between iCER sensitivity to increasing temperature and nutrient enrichment in the subtropical wet (a) and the temperate arid (c) climate zones. Solid dots indicate the significant ($p \leq 0.05$) relationships between iCER and temperature at different nutrient levels. (b, d) The relationships between the variability of species thermal responses and nutrient enrichment in the subtropical wet (b) and the temperate arid (d) climate zones. The variability of species thermal responses for each sample was calculated, and their averaged values at each nutrient level were plotted against nutrient enrichment within each climate zone. Statistical significance of linear model fits with F-statistics is indicated by solid ($p \leq 0.05$) or dotted ($p > 0.05$) lines.

for the temperate arid climate zone in streams (6.0%–10.5%). In contrast to the subtropical wet climate zone, nutrients and the joint effects between nutrients and temperature accounted for more variation in thermal responses in the temperate arid climate zone (Figure 6e,g).

Finally, according to SEM, temperature was the dominant factor affecting thermal responses in both streams and microcosms, except for the temperate arid climate zone in streams (Figure 6). Temperature directly and indirectly affected bacterial thermal responses through *rrn* copy number. In streams, compared to the subtropical wet climate zone, nutrients had larger total mean effects of 0.29 on the thermal responses in the temperate arid climate zone. This was also true in microcosms, where nutrient enrichment and local nutrients exerted direct and indirect effects on thermal responses through *rrn* copy number.

4 | Discussion

Climate regulates the distribution, reorganisation, and function of microbial communities (Antao et al. 2020; Lenoir et al. 2020;

Ren et al. 2024), yet predicting the responses of microbial communities to climate change remains a major challenge in ecology (Smith et al. 2019; Stuart-Smith et al. 2015). Here we used iCER to quantify thermal responses of aquatic bacterial communities on mountainsides of two contrasting climate zones in natural streams and field microcosms. We found consistently higher bacterial thermal responses towards high temperatures across the two contrasting climate zones in streams and microcosms, highlighting the dominant role of temperature in shaping their responses. Surprisingly, nutrient enrichment showed contrasting effects on the sensitivity of thermal responses to warming in the two climate zones. The difference may be attributed to the varying influence of nutrients on the variability of species thermal responses and bacterial thermal responses in the two climate regions, with stronger effects of nutrients observed in the temperate arid climate zone. Disentangling the effects of temperature and nutrient enrichment on thermal responses would enable us to track how climate change and human impacts drive microbial community reorganisation and assess the ecological consequences of these changes.

The magnitude of each species-specific thermal response was consistent within both climate zones in streams and field

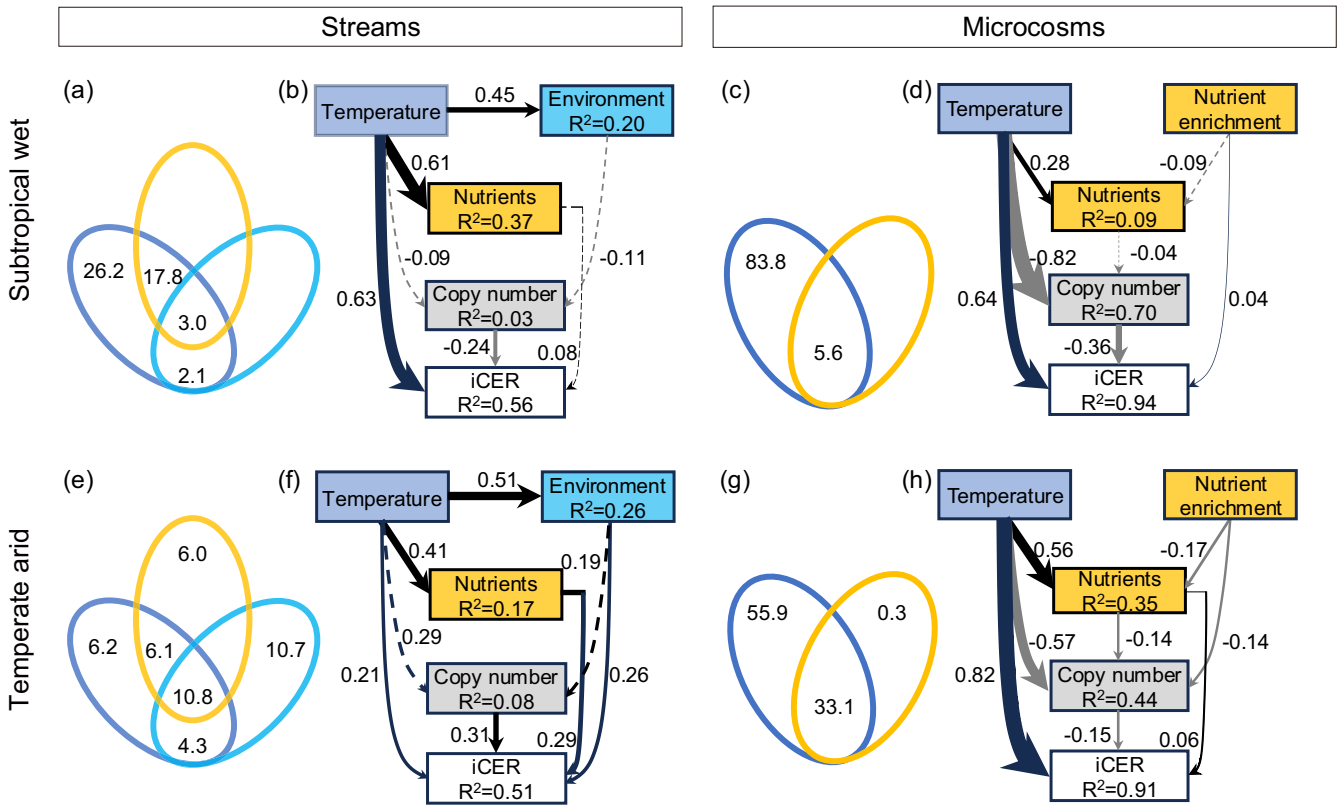


FIGURE 6 | The effects of temperature and nutrients on thermal responses in streams and microcosms. The variances in thermal responses associated with the temperature (purple circles), nutrients (yellow circles), and other environmental variables (blue circles) were obtained using variation partitioning in the subtropical wet (a, c) and the temperate arid (e, g) climate zones. For nutrients, total phosphorus and total nitrogen, NH_4^+ , NO_2^- and PO_4^{3+} were used in streams, while the initial levels of added NO_3^- and the measured NH_4^+ , NO_2^- and PO_4^{3+} were included in microcosms. Environmental variables were not used in microcosms due to the consistent initial conditions. These explanatory variables were described in detail in Table S2. The values are the adjusted coefficient of determination (adjusted R^2), and negative variance components are not shown. The statistical significance was according to the Monte Carlo permutation test (9999 permutations, $p \leq 0.01$). We further used structural equation models (SEMs) to examine the direct and indirect effects of temperature and nutrients on thermal responses in the subtropical wet (b, d) and the temperate arid (f, h) climate zones. In microcosms, the initial levels of added NO_3^- were used as nutrient enrichment, and measured NH_4^+ , NO_2^- and PO_4^{3+} of overlying water were used as nutrients. The distal drivers were described in detail in Table S3. Values along numbered paths are standardised partial regression coefficients. The width of each arrow is scaled to the absolute value of the standardised path coefficient. Positive paths in black, negative in grey, significant as solid, nonsignificant as dashed. R^2 denotes the proportion of variance explained for endogenous variables. All hypothesised pathways including in the SEMs before model simplification are shown in Figure S20.

experiments. The species thermal responses were used to quantify changes in their relative abundance along temperature gradients. Generally, the species thermal responses ranged from -0.82 to 0.82 in streams, and from -0.96 to 0.94 in microcosms. This consistency indicates that species responses to temperature are transferable and generalisable at least within each climate zone. Species thermal responses are an inherent property for each species, which may be transferred across spatial and temporal scales to a certain degree. We expect that species thermal responses could be amenable to the inclusion in models to predict restructuring of communities under global change.

Interestingly, we found that species thermal responses were related to their life history traits linked to growth rates, that is, rRNA operon (*rrn*) copy number. In streams and microcosms, the magnitude of species positive thermal responses increased with lower *rrn* copy numbers in both climate zones, while the magnitude of negative thermal responses showed decreased trends. The *rrn* copy number is positively correlated with the maximum growth rates, and species with low *rrn* copy number

are considered to be slow-growing oligotrophs (Lauro et al. 2009; Roller et al. 2016). Therefore, warm-accumulating species with strong positive thermal responses tend to have low growth rates, whereas those warm-depleting species with strong negative thermal responses are associated with high growth rates. This finding is consistent with recent studies showing that the growth rates of cold-adapted organisms are higher than those from warm sites (Ren et al. 2024), aligning with the latitudinal compensation hypothesis in macroecology (Conover and Present 1990; Levinton 1983). The hypothesis posits that organisms in cold environments increase their basal metabolic rates and growth rates to offset short growing seasons. Additionally, the relationships between species thermal responses and *rrn* copy number may be caused by potential trade-offs between energy efficiency and thermal tolerance (Dahlke et al. 2020). For warm-accumulating species, slow-growing oligotrophs with low *rrn* copy number can invest more energy in thermal adaptation due to the low intrinsic growth costs, resulting in more pronounced positive responses to higher temperatures. In contrast, for warm-depleting species, oligotrophs may allocate more resources to maintaining activity

and defending against climate stress (Chen et al. 2021; Gray et al. 2019), ultimately leading to weak negative responses to warming. Our results reflect that slow-growing oligotrophs with low *rrn* copy number could prioritise resources for stress resistance or climate adaptation, at the cost of reduced growth rates (Zhu and Dai 2024). Thus, species life history traits related to growth rate might provide mechanistic insights into the changes in species-level thermal responses.

At the community level, the thermal responses of bacteria increased towards warmer conditions such as lower elevations, which is true in the two contrasting climate zones regarding streams or microcosms. Such consistent thermal responses to temperature changes indicate that the increasing dominance of warm-accumulating species in warmer sites is a widespread phenomenon across a range of geographic contexts. Our results showed that temperature, strongly correlated with elevation, was a key driver for bacterial thermal responses (Figures 2 and 6 and Figures S18 and S19). Increasing temperature may alter *rrn* copy numbers of microbial communities, thereby causing changes in thermal responses. The relative abundance of species with positive SERs increased towards high water temperatures (Figure S14), indicating a shift towards a lower *rrn* copy number for microbial community (lower growth rate) with increasing temperatures. This aligns with the prevalence of oligotrophic microbial community in warmer sites (Jansson and Hofmockel 2020; Li et al. 2021; Wang et al. 2020). The changes of life history traits within microbial communities could influence their responses to increasing temperatures, given species' position in the fast-slow life-history continuum (Wang et al. 2020).

We further found that bacterial sensitivity of thermal responses to increasing temperatures could be influenced by community characteristics, such as the variability of species thermal response set by the standard deviation of species thermal responses, and human-related variables like nutrient enrichment. The sensitivity of thermal responses to warming was quantified by the slopes of above linear regressions between iCER and temperature, reflecting how fast the thermal responses change with increasing temperatures. The higher sensitivity of thermal responses to warming suggests a faster change in thermal responses with rising temperature.

The sensitivity of thermal responses to warming, varying among bacterial phyla or classes, was largely controlled by the variability of species thermal responses. For instance, bacterial phyla and classes with high variability in species thermal responses tended to exhibit greater sensitivity of thermal responses to warming compared to those with low variability. These phyla and classes contain more species with different thermal responses, and thus have greater potential for species gains, losses, or changes in relative abundance due to temperature rises. The observed effects of variability in species responses on their sensitivity of thermal responses to warming could be related to their life history strategies of microbial phyla and classes. Copiotrophs tend to respond more rapidly to environmental changes, such as temperature (Lauro et al. 2009). For example, Bacteroidota and Proteobacteria phyla classified as copiotrophs were found to have high variability in species thermal responses and strong sensitivity of thermal responses to warming (Figure 3 and Figure S16). Our results indicate a stronger capacity to respond

to environmental temperature changes for bacterial phyla and classes with high variability of species thermal responses.

Unexpected, we further found that the sensitivity of thermal responses to warming was mediated by nutrient enrichment, but the nutrient effects were divergent between the two contrasting climate zones. Specifically, the sensitivity of thermal responses to warming was strengthened and suppressed with the gradients of nutrient enrichment in the subtropic wet and the temperate arid climate zones, respectively. The contrasting effect of nutrient enrichment may reflect the different community assembly mechanisms constrained by temperature and nutrient gradients in the two contrasting climate zones.

How to explain this contrasting phenomenon? The contrasting effect of nutrient enrichment may primarily result from the different effects of nutrients on community characteristics and the thermal responses in the two contrasting climate zones. Firstly, the effects of nutrient enrichment on variability of species responses were divergent in the two contrasting climate zones. Specifically, the variability of species responses increased and decreased towards high nutrient levels in the subtropical wet and the temperate arid climate zones, respectively (Figure 5). The contrasting trend aligns with the higher or lower sensitivity of thermal responses to warming with nutrient enrichment gradients in the two climate zones. Our results further support the positive relationships between variability of species responses and the sensitivity of thermal responses to warming (Figure 4). The correlations observed in this study support previous findings showing that the variation in temperature sensitivity of microbial processes may stem from the influence of community composition (Smith et al. 2019). Thus, nutrient enrichment may influence the sensitivity of thermal responses to warming by mediating community characteristics.

The second explanation could be the different role of nutrients on thermal responses in the two contrasting climate zones. In microcosms, nutrients had a higher effect on bacterial thermal responses in the temperate arid than the subtropical wet climate zones, while temperature was dominant in the two climate zones. This is also true across the two contrasting climate zones in streams. Such divergent effects of nutrients in the two contrasting climate zones were statistically supported by random forest analyses (Breiman 2001) (Figure S19), variation partitioning analyses (Borcard et al. 1992) and structural equation modelling (Grace et al. 2012) (Figure 6). For instance, in the temperate arid climate zone, nutrients had larger total mean effects of 0.29 and 0.10 on bacterial thermal responses in streams and microcosms, respectively (Figure 6). In the subtropical wet region, nutrient eutrophication may stimulate the primary productivity of algae (Hu et al. 2024; Wang et al. 2016), which influence the bacterial community composition and strengthen bacterial responses to increasing temperature. For example, marine bacterial communities show stronger temperature responses in periods with high nutrients and phytoplankton biomass (Arandia-Gorostidi et al. 2017). Moreover, nutrient enrichment and warming may favour species with similar traits and accelerate community reorganisations (Monchamp et al. 2018), leading to stronger sensitivity of thermal responses to warming. However, in temperate arid climates, lower water levels and higher nutrient concentrations can cause excess nutrient buildup and algal blooms (Palmer et al. 2009), stimulating microbial metabolism while

excluding sensitive species and favouring more adapted species (Grzyb and Skłodowska 2022). This may lead to stronger roles of nutrients on microbial thermal responses in the temperate arid climate zone, and decreased sensitivity of thermal responses to warming along the nutrient enrichment gradient.

Furthermore, nutrients can indirectly influence thermal responses through interactions with *rrn* copy numbers in the temperate arid climate zone. High nutrient levels in environments may suppress bacterial growth, as bacteria require substantial resources to accumulate solutes and reduce the internal water potential (Harris 1981; Schimel et al. 2007). In addition, the continuous water level decline can cause the loss of aquatic microbial biogeochemical cycling functionality, thereby weakening ecosystem functionality (Zhang et al. 2021). Consequently, nutrient enrichment may act as an important environmental stressor for microbes in the temperate arid climate zone, diminishing microbial sensitivity of thermal responses to warming along the nutrient enrichment gradient. Taken together, the different effects of nutrient enrichment on sensitivity of thermal responses to warming in subtropical wet versus temperate arid climate zones suggest that climate and anthropogenic impacts can have interactive ecological impacts. Such contrasting effects could potentially be a result of the different changes in community characteristics along the nutrient enrichment gradient and the divergent role of nutrients on thermal responses in the two contrasting climate zones.

Nevertheless, there may be caveats to consider regarding the thermal responses of microbial communities. First, the species thermal responses were based on the OTUs with 16S rRNA, which may deviate from the true microbial species due to the high phylogenetic conservatism of the marker gene. We encourage future studies to examine the species thermal responses using other techniques, such as metagenomics, via which the species could be defined with finer phylogenetic information. Second, the water temperature measured on the last day of incubations may not fully reflect the overall temperature patterns throughout the entire study period. However, it would be challenging to directly measure the water temperature variations experienced by the microbial communities as microcosms were incubated in remote mountain regions. The water temperature recorded on the last day in the microcosms is a feasible way to represent the field conditions. This could be supported by the fact that the measured temperature significantly decreased along elevational gradients (Spearman $r=0.92$ and 0.62 for streams, and Spearman $r=0.99$ and 0.99 for microcosms), and could better capture the local conditions during our incubations than the extrapolated mean annual temperature.

5 | Conclusion

In conclusion, our study found consistently increasing community-level thermal responses towards high temperatures across two contrasting climate zones in both natural streams and field microcosms, showing the dominant role of temperature. Such sensitivity of thermal responses to warming could be affected by community characteristics and human-related factors, such as the variability of species thermal responses and nutrient enrichment. Surprisingly, nutrient enrichment exhibited different effects on the sensitivity of thermal responses to

warming in the two contrasting climate zones, with increasing effects along nutrient gradients in the subtropical wet climate zone and decreasing effects in the temperate arid climate zone. This discrepancy could be explained by the different impacts of nutrients on community characteristics and thermal responses, with nutrients showing stronger effects in the temperate arid climate zones as excess nutrient buildup acts as an important environmental stressor for microbes. Overall, microbial species with positive thermal responses are replacing those with negative thermal responses under climate warming, and anthropogenic activities, such as nutrient enrichment, may amplify or dampen the effects of warming depending on local climates and environmental conditions. Intriguingly, the thermal responses of microbes showed similar patterns to dissolved organic matter (Hu et al. 2024), indicating shared mechanisms underlying both microbes and organic molecules along environmental gradients. Our study strengthens the understanding of climate-induced spatial variation in microbial thermal responses and provides thoughtful insights into how nutrient enrichment alters the sensitivity of thermal responses under future climate change. Given that different regions of the world may display distinct climates and the strength of human activities, further research is urgently required to foster broader conclusions regarding the effects of climate change and human impacts on thermal responses.

Author Contributions

J.W. conceived the idea. J.W. and W.Z. performed the field experiments and sampling, generated the biological and environmental data. W.Z. performed the data analyses, with contributions from J.W. and A.H. W.Z. wrote the first draft of the manuscript. W.Z., J.W. and A.H. finished the manuscript with contributions from other co-authors. All authors approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw sequences that support the findings of this study were submitted to NODE (<https://www.biosino.org/node>) with accession numbers: OEP004862, OEP00005650.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** geb70111-sup-0001-supinfo.docx.