© 2026. Published by The Company of Biologists | Journal of Experimental Biology (2026) 229, jeb251650. doi:10.1242/jeb.251650

RESEARCH ARTICLE

Composition and stability of the gut microbiome are associated with thermal tolerance and its plasticity in *Anolis* lizards

Claire E. Williams[1,*], Akhila C. Gopal[1], Wayne Wen-Yeu Wang[2], Jia-Syuan Chen[3], Hei Yuen Cheung[4,5], Anthony Strickler[2], Michael L. Logan[1] and Alex R. Gunderson[2]

ABSTRACT

Ectotherms are thought to be particularly vulnerable to climate change as they rely directly on environmental temperatures to regulate their physiology. One of the pathways by which ectotherms can alter their physiology in a warming environment is through phenotypic plasticity, which is usually treated as resulting from interactions between the organism's genetics and the environment. However, ectotherms also host communities of microbes which can change quickly within the host and affect host physiology. To date, little is known about the extent to which gut microbes affect thermal plasticity in the non-model host organisms that will be the most affected by climate change. We investigated relationships between gut microbiome composition and host heat tolerance plasticity in three species of *Anolis* lizards: *Anolis cristatellus*, *Anolis sagrei* and *Anolis carolinensis*. We brought wild-caught lizards into the lab and tested for (1) effects of experimental warming on the gut microbiomes and (2) associations between microbiome composition and compositional dynamics with heat tolerance and its plasticity across host individuals and species. We found that each anole species hosted a distinct gut microbial community, but that all host species had microbiomes that were largely resilient to temperature increases. However, several key aspects of microbiome composition were correlated with baseline host heat tolerance. Finally, microbiome composition and its stability were associated with the magnitude of plasticity in host heat tolerance. Our results indicate that gut microbes may play a role in the ability of ectotherms to mount plastic responses to rapidly changing thermal environments.

KEY WORDS: Ectotherm, Microbiota, Climate change, Thermal biology, Temperature

INTRODUCTION

Climate change is one of the greatest threats facing species on Earth. Increases in greenhouse gas emissions have led to rapid rises in the average global surface temperature and in temporal thermal variability, which already impact the survival and reproductive success of wild populations (IPCC, 2021; Kannan and James, 2009; Williams et al., 2008; Urban et al., 2012). Of the organisms that are being affected by climate change, ectotherms are considered particularly vulnerable as environmental temperatures directly dictate their physiology (Huey et al., 2012). One pathway by which ectotherms might respond to changing thermal environments is through phenotypic plasticity (Richter et al., 2012). Plastic responses can be a powerful avenue of resilience because they happen within the lifetime of individuals. In other words, they represent rapid responses that can generate changes in phenotype on time scales similar to the stresses that arise from rapid climate change (e.g. heat waves).

Phenotypic plasticity is typically defined as an interaction between host genotype and the environment to produce a particular phenotype (Whitman and Agrawal, 2009). With respect to thermal tolerance plasticity, environmental temperatures are thought to influence host gene expression through their effect on body temperature. However, ectotherms host extensive communities of microbes in and on their bodies – their microbiomes – which may also play a role in host plasticity by mediating complex interactions between the environment, microbial symbionts and host genotype (Kolodny and Schulenburg, 2020; Voolstra and Ziegler, 2020). Animal microbiomes serve as a rich source of metabolic potential for their hosts which can ultimately influence host physiology in important ways (Lynch and Hsiao, 2019). The gut, in particular, houses dense and diverse microbial communities, with trillions of bacteria that interact with each other and with their host (Ley et al., 2008). As a result, microbial proteins and metabolites produced in the gut can scale up to influence a wide array of host traits (Amenyogbe et al., 2017; Baniel et al., 2021; Cao et al., 2022; Cox et al., 2022).

Like any ecological community, microbiomes are not static (Gerber, 2014), and their composition can change over time in response to numerous variables, including climate, host diet consumption and host stress (Williams et al., 2023a; David et al., 2014; Hicks et al., 2018; Fernandes et al., 2023). These changes can occur through several mechanisms, including microbial evolution within individual hosts, shifts in microbial gene expression, and changes in community composition arising from shifting microbial population sizes and the acquisition or extirpation of taxa. Given that microbiomes influence host phenotypes, and that these communities can change rapidly within the lifetime of individual hosts, it has been suggested that the microbiome might contribute to plasticity in host traits (Kolodny and Schulenburg, 2020). Yet, studies on the extent to which microbiomes mediate the plasticity of climate-relevant traits in hosts are exceptionally rare.

Associations between gut microbes and static measures of host thermal tolerance have been demonstrated in numerous ectothermic animals, including aphids (Dunbar et al., 2007), frogs (Fontaine et al., 2022; Dallas et al., 2024) and lizards (Fontaine and Kohl, 2023a; Moeller et al., 2020; Sepulveda and Moeller, 2020). However, it remains almost entirely unknown whether microbiome composition is associated with plasticity in host thermal tolerance. To

[1]Department of Biology, University of Nevada, Reno, NV 89557, USA. [2]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118, USA. [3]Department of Life Science, National Taiwan University, Taipei 10617, Taiwan, Republic of China. [4]School of Medicine, Tulane University, New Orleans, LA 70112, USA. [5]Celia Scott Weatherhead School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, USA.

*Author for correspondence (williams.claire.e@gmail.com)

C.E.W., 0000-0002-8970-0032; A.C.G., 0000-0002-0925-4950; W.W.-Y.W., 0000-0001-7987-7697; J.-S.C., 0000-0002-6587-9197

Received 25 September 2025; Accepted 10 February 2026

address this knowledge gap, we tested the relationship between the composition of gut microbial communities and the plasticity of host thermal tolerance in three species of *Anolis* lizards: the crested anole (*Anolis cristatellus*), the brown anole (*Anolis sagrei*) and the green anole (*Anolis carolinensis*). *Anolis* lizards are a longstanding model in ecology and evolution (Losos, 2011), yet few studies have characterized their microbiome diversity or links with host physiology (Williams et al., 2022; Ren et al., 2016). Moreover, lizards are considered to be highly vulnerable to climate warming, hence the urgent need to investigate the factors shaping thermal physiology in this group (Huey et al., 2009; Mi et al., 2022; Sinervo et al., 2010). We brought wild-caught lizards into the lab and tested how the composition and diversity of gut microbiota responded to short-term warming. Afterwards, we measured heat tolerance and its plasticity across host individuals and species and related these to microbiome composition and its stability within individual lizards. We hypothesized that (1) species would have distinct gut microbiomes, (2) heat exposure would be associated with a change in gut microbiome composition and (3) gut microbiome composition would be associated with baseline thermal tolerance and thermal tolerance plasticity among individuals.

MATERIALS AND METHODS

Lizard collection and husbandry

All animal protocols were approved by the Tulane University Institutional Animal Care and Use Committee (504) with permits from the Louisiana Department of Wildlife and Fisheries (LNHP-18-100) and the Puerto Rican Departamento de Recursos Naturales y Ambientales (2019-IC-008). We collected 25 male crested anoles, *Anolis cristatellus* A.M.C. Duméril & Bibron 1837, in July 2019 by hand or hand-held loop in Bosque Estatal de Cambalache in Puerto Rico (18°26.998 N; 66°35.642 W), 24 male brown anoles, *Anolis sagrei* Duméril and Bibron 1837, and 22 male green anoles, *Anolis carolinensis* Voigt 1832, in August 2019 in New Orleans (29.9511°N, 90.0715°W). These three species occupy similar ecological niches and yet represent distant branches of the *Anolis* tree of life separated by tens of millions of years. Thus, we sought to test whether common relationships between temperature, thermal physiology and microbiome composition are maintained across evolutionary time. We brought all lizards to the laboratory, measured mass with a spring scale and snout–vent length with a ruler for each individual, and placed them in individual plastic terraria (Kritter Keepers; 18.03×11.18×13.97 cm) with mesh lining on the bottom and a wooden dowel for perching. All terraria were kept in climate-controlled incubators (Percival Model I30-NL) set to 28°C, 75% relative humidity, and a 12 h:12 h photoperiod for 2 weeks as a laboratory acclimation period. Lizards were fed crickets three times per week and misted with distilled water twice a day.

Temperature treatments

After the acclimation period, lizards were randomly assigned to one of two treatment groups: cool or warm (Fig. S1). The cool treatment group was maintained at a constant 25°C and 75–80% humidity and the warm treatment group was maintained at a constant 33°C and 75–80% humidity. These temperatures are ecologically relevant as they approximate the cool and warm extremes, respectively, of field active body temperatures for all three species (Gunderson and Leal, 2015; Ryan and Gunderson, 2021). For the 25°C treatment, sample sizes were as follows: crested anole n=11, brown anole n=11, green anole n=11. For the 33°C treatment, sample sizes were as follows: crested anole n=13, brown anole n=13, green anole n=11. Lizards were kept in the treatment for 2 weeks.

Fecal sample collection

For each lizard, we collected three fecal samples. Fecal samples are considered to be an accurate and non-invasive method for sampling the gut microbiome in lizards (Hernández et al., 2023; Kohl et al., 2017). The first was collected during the second week of acclimation, prior to the beginning of the temperature treatments. The second and third fecal samples were collected at the end of weeks 1 and 2, respectively, of the temperature treatments. All fecal samples were collected with sterile forceps, placed in 1 ml cryotubes with 750 µl RNAlater, and immediately stored in a −80°C freezer. All other fecal pellets were removed and discarded.

Measurement of critical thermal maxima

At the end of the temperature treatments, we measured the heat tolerance of each individual lizard as the critical thermal maximum (CT_{max}) following published protocols (Deery et al., 2021; Leal and Gunderson, 2012; Gunderson et al., 2018). In brief, a thermocouple probe was inserted into the cloaca of each lizard and secured with a small dot of super glue to monitor internal body temperature throughout the assay. Lizards were then placed individually inside an open cardboard box (31×31×16 cm) under a heat lamp such that the body temperature of each lizard would rise at a constant rate of 2°C min^{-1} (Gunderson, 2024). Starting at 36°C, we tested the righting response at 1°C intervals. The temperature at which the lizard could not right itself within 10 s was scored as its CT_{max} (estimated as the half degree between the second to last and the last testing temperature) (Gunderson et al., 2018). Following the first CT_{max} measurement, lizards were given a rest period of 3 h and CT_{max} was tested once more. The difference between these two measurements was considered to be the heat hardening response, a measure of heat tolerance plasticity (Deery et al., 2021).

DNA extraction and library preparation

Prior to DNA extraction, samples were defrosted at room temperature. DNA was extracted using the Qiagen DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with minor modifications. Briefly, each fecal sample was placed in a PowerBead Pro tube containing beads for mechanical lysis and combined with 800 µl of Solution C1 to initiate cell lysis. The tubes were vortexed at maximum speed for 8 min to ensure disruption of the cells. Following vortexing, samples were centrifuged at 15,000 ***g*** for 1 min, and the supernatant was collected. Next, 200 µl of Solution C2 was added to the supernatant, vortexed for 1 min, and centrifuged again at 15,000 ***g*** for 1 min. The supernatant was then combined with 600 µl of Solution C3, vortexed for 1 min and placed on ice for 10 min. The mixture was loaded onto an MB Spin Column and centrifuged at 15,000 ***g*** for 1 min; this step was repeated twice. The DNA pellet was then washed first with 500 µl of Solution EA, followed by 500 µl of Solution C5, with each wash step followed by centrifugation at 15,000 ***g*** for 1 min. The pellet was air-dried for 10 min, and 60 µl of Solution C6 was added to dissolve the DNA. DNA concentration and quality of each sample were measured using a NanoDrop 1000 (Thermo Scientific, Waltham, MA, USA). All samples were stored at −80°C until they were sent for amplification and sequencing.

The extracted DNA was then sent to the Integrated Microbiome Resource (IMR) at Dalhousie University (Halifax, NS, Canada) for 16S rRNA gene amplification and sequencing. IMR used primers targeting the 16S rRNA gene V4-V5 variable region, 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGY-CAATTYMTTTRAGTTT-3′), for amplification. PCR amplification

Journal of Experimental Biology (2026) 229, jeb251650. doi:10.1242/jeb.251650

followed a standardized protocol optimized for the Illumina MiSeq platform. After amplification, the PCR products were purified, quantified, normalized and pooled. Sequencing was performed using a 2×300 bp paired-end V3 kit on the Illumina MiSeq platform.

Bioinformatics and statistical analysis

We used a previously published and automated Snakemake pipeline to process raw sequencing files (available from GitHub: https://github.com/claireewilliams/SnakeMakeQIIMECW; Williams et al., 2023b). In brief, we imported fastq files into the Quantitative Insights into Microbial Ecology 2 package (QIIME2) (Bolyen et al., 2019). We then used the DADA2 plug-in for QIIME2 to quality filter and trim sequences to remove adapters and regions of low quality in order to generate amplicon sequence variants (ASVs) (Callahan et al., 2016). ASVs were taxonomically classified using the SILVA taxonomy database (version 138) via a naive Bayes classifier in QIIME2 (Pedregosa et al., 2011; Quast et al., 2013). We then removed all non-bacterial reads. A phylogenetic tree was generated and rooted using the Mafft and fasttree QIIME2 plug-ins (Katoh et al., 2002; Price et al., 2010). For all diversity metrics, we rarefied samples (subsampled without replacement) at 5000 reads after first verifying with a rarefaction curve (Fig. S2) that this was sufficient to capture the diversity of the community. We calculated the number of microbial ASVs, Jaccard dissimilarity (presence/absence of microbial taxa across samples; Jaccard, 1908) and weighted UniFrac metrics (similarity between samples based on the relative abundance of microbial taxa and their phylogenetic relationships) (Lozupone and Knight, 2005; Lozupone et al., 2007). Using the phyloseq (McMurdie and Holmes, 2013) and qiime2R (https://github.com/jbisanz/qiime2R) packages in R (version 4.2.3) (http://www.R-project.org/), we imported the QIIME2 feature tables, taxonomy assignments, diversity calculations and phylogenetic trees for further statistical analysis.

We first tested for baseline differences in microbial community composition between lizard species using pre-treatment samples (week 1). We visualized sample separation by using the distance matrices to conduct a principal coordinates analysis (PCoA). We then used PERMANOVA implemented via adonis2 in the vegan package in R to test for species differences in microbial community composition (https://CRAN.R-project.org/package=vegan).

To test whether individual lizards hosted stable and unique microbiomes over time, we compared the Jaccard and Weighted UniFrac distance between repeated samples from the same individual versus samples from different individuals of the same species. We used linear models with species as an interaction term, followed by Tukey *post hoc* tests to evaluate species-specific patterns in microbiome stability.

To determine whether the ASV richness and Shannon diversity of the microbiome were affected by time in captivity, we used linear mixed models (LMMs) with species and week as interacting effects, and individual lizard as a random effect. We then used *post hoc* pairwise comparisons to assess species-specific trends. To determine whether the microbial community composition was affected by time in captivity, we used PERMANOVA with Jaccard or weighted UniFrac distance as response variables, and week and species as interacting effects, with permutations stratified by individual lizard to account for repeated measures.

To determine whether CT_{max} was affected by temperature treatment, we used a linear model with treatment and species as interacting effects, followed by Tukey *post hoc* tests for species-specific patterns. To test whether the heat hardening response varied between treatment groups, we calculated the difference between the first and second CT_{max} measurements. We then followed a similar approach to that described above, using a linear model first to assess consistent differences across species and then *post hoc* comparisons by species to understand the relationship within each species.

To determine whether microbiome community composition and diversity were related to baseline CT_{max}, we used several complementary approaches. First, we conducted PERMANOVA relating CT_{max} to Jaccard or Weighted UniFrac distance by species using only week 1 or week 3 microbiome data so as not to introduce repeated measures. Next, to integrate the full dataset and account for repeated measures, we extracted principal coordinate 1 (PC1) scores and overall dispersion for each sample from global PCoAs (Figs S4 and S5) and regressed raw and absolute PC1 scores and sample dispersion against baseline CT_{max} using LMMs with species as an interaction term and individual as a random effect. PC1 was used because it captured the primary gradient in microbiome compositional variation across individuals (explaining 9.73% of variance for Jaccard and 36.1% for Weighted UniFrac). We identified the predominant ASVs correlated with PC1 by creating a biplot and extracting the top 20 features that were most highly correlated with PC1 (Table S8). We used similar LMMs to test for associations between ASV richness or Shannon diversity and CT_{max}. Treatment was initially included as a covariate but excluded from final models because of non-significance.

To determine whether there were differentially abundant ASVs or microbial families contributing to differences in individual heat tolerance, we used Maaslin2 in R (Mallick et al., 2021). However, after correcting for multiple comparisons, we were unable to identify any microbial ASVs or families for which relative abundance was significantly linearly related to thermal tolerance. As a complementary approach, we examined whether the relative abundance of dominant microbial phyla was non-linearly related to CT_{max}. We identified the six most abundant phyla based on average relative abundance and modeled each phylum's relative abundance as a function of baseline CT_{max}, including both linear and quadratic terms.

Finally, we examined how the microbiome is related to heat tolerance plasticity in two ways. We first tested whether characteristics of the static microbial community (richness, Shannon diversity and composition) were related to the degree of heat hardening. We used the same approach as that described for static CT_{max}, but used plasticity in CT_{max} (the difference between the first and second CT_{max} measurements) as the predictor instead of baseline CT_{max}. Second, we tested whether microbiome plasticity (turnover) over the course of the experiment was related to the magnitude of the heat hardening response in hosts. To do so, we compared the Jaccard distance between the first and final microbiome sample of each individual with the absolute value of the heat hardening response using a linear model with species as a covariate (https://CRAN.R-project.org/package=nlme).

RESULTS

Anole species and individual lizards hosted unique and consistent microbiome communities

In total, we sequenced 212 fecal DNA samples from 24 brown anoles, 22 green anoles and 25 crested anoles. Sequencing resulted in a total of 4,535,451 reads and 5823 ASVs after quality control, with a mean content of 21,448 sequences in each sample. All three lizard species were dominated by the same six phyla: Bacteroidota, Firmicutes, Proteobacteria, Verrucomicrobiota, Fusobacteriota and Desulfobacterota (Fig. S3). Similarly, the most common

genera were largely consistent across the three lizard species, with microbial communities dominated by *Bacteroides*, *Parabacteroides*, *Akkermansia*, *Odoribacter*, *Fusobacterium*, *Alistipes*, *Enterococcus*, *Clostridium sensu stricto*, *Faecalitalea* and *Bilophila* (Fig. S3).

'Host species' was the predominant factor shaping gut microbiome composition in our dataset, followed by 'individual' (Fig. 1). We evaluated gut microbiome composition using two complementary diversity metrics: Weighted UniFrac (phylogenetic, abundance weighted) and Jaccard distance (presence–absence). In week 1 of our experiment, anole microbiomes clustered distinctly by species, particularly when considering Jaccard distance (Fig. 1A; Jaccard PERMANOVA pseudo-F=4.84, P<0.001). When accounting for taxonomic distance of microbial taxa as well as their relative abundance, microbiomes were more similar but species still hosted significantly different microbial communities (Fig. 1B; Weighted UniFrac PERMANOVA pseudo-F=9.39, P<0.001).

Overall, lizard microbiomes changed only subtly in captivity, and individuals retained distinct microbiome compositions over the course of the experiment. There was no overall effect of time in captivity on richness (P=0.25; Table S1), though richness declined slightly but significantly over time in crested anoles only (B=−11.30, P=0.02; Table S1). Shannon diversity increased modestly over time (B=0.097, P=0.033; Table S1), and this change was not driven by an increase in evenness (P=0.096; Table S1). Microbiome community membership also shifted subtly with time in captivity (Jaccard, R^2=0.003, P=0.004; Table S1), but the overall phylogenetic structure of the microbiome did not change significantly (Weighted UniFrac, P=0.343; Table S1).

We compared within-individual temporal stability ('self') with between-individual dissimilarity ('other') to test for the presence of stable, individualized microbiomes. Across all three species, the Jaccard distance analysis revealed significantly lower dissimilarity within individuals than between conspecifics (B=−0.315, P<0.001; Fig. 1C; Table S2). Using Weighted UniFrac distances, we observed significant differences in green anoles (B=−0.03, P<0.001) and crested anoles (B=−0.2, P=0.002), but not brown anoles (B=0.007, P=0.139; Fig. 1D; Table S2).

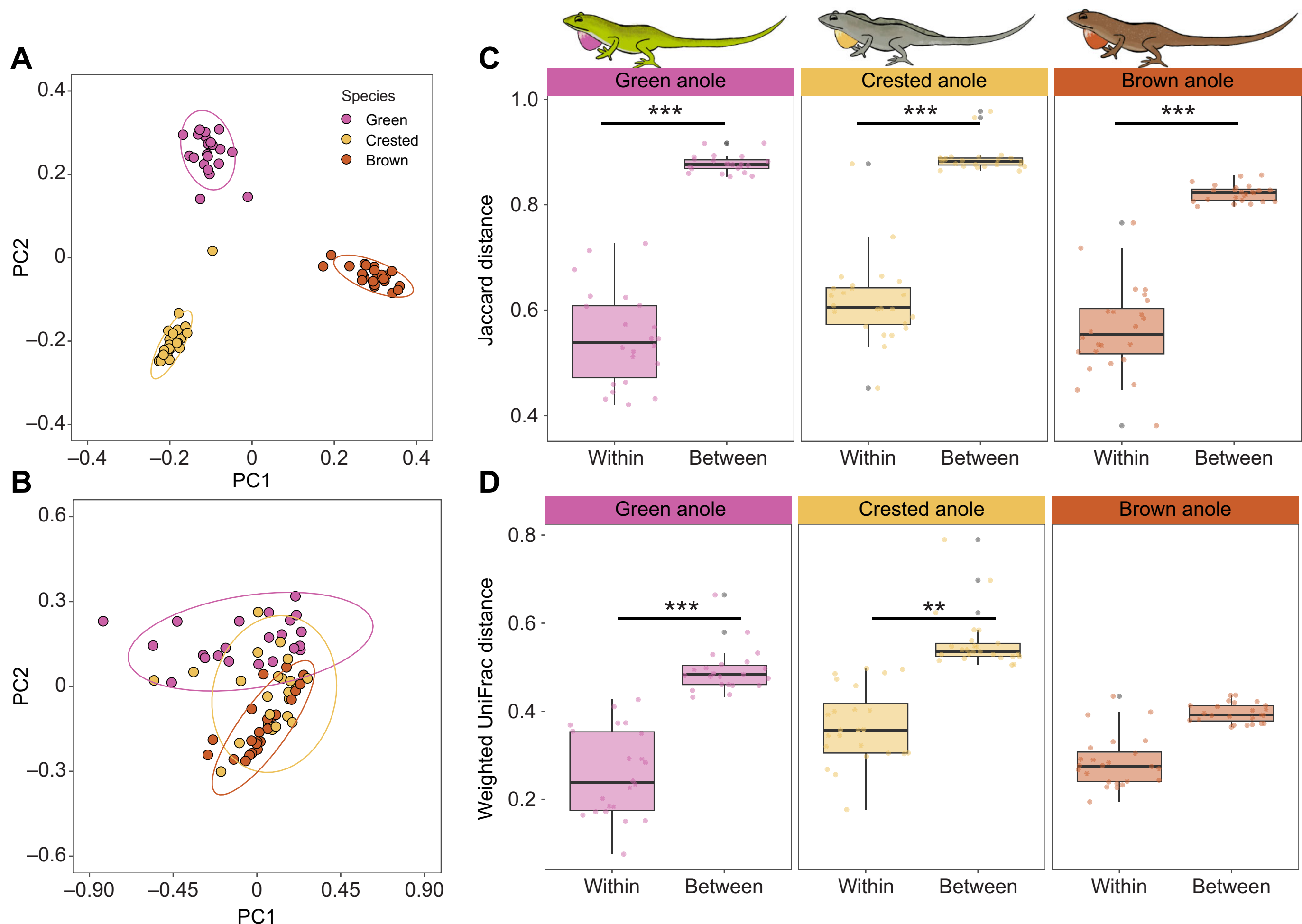

Fig. 1. 'Species' and 'individual' are strong factors shaping microbiome composition in *Anolis* lizards. Ordinations based on the presence/absence of microbial taxa (Jaccard; A) and incorporating microbial taxonomy and relative abundance (Weighted UniFrac; B) show that species cluster distinctly. Individual lizards host consistent microbiomes across sampling points. Differences in microbiome composition based on presence/absence (Jaccard; C) and incorporating microbial taxonomy and relative abundance (Weighted UniFrac; D) were higher between individuals of the same species than within repeat samples of the same individual. Box plots show median, upper and lower quartiles and 1.5× the interquartile range. Colored points indicate individual samples and grey points indicate the position of outliers within the dataset. Asterisks indicate significant comparisons: ***P*<0.01, ****P*<0.001.

Journal of Experimental Biology (2026) 229, jeb251650. doi:10.1242/jeb.251650

Host heat tolerance, but not microbiome composition, was strongly influenced by temperature treatment

CT_{max} was higher for lizards exposed to experimental warming in two of three anole species (Fig. 2A). Warming significantly increased CT_{max} (B=1.67, P=0.004); however, *post hoc* tests revealed that this effect was driven by plasticity in crested and green anoles, but not brown anoles. In green anoles and crested anoles, lizards in the warm treatment had a higher CT_{max} by between 1 and 2°C when compared with lizards in the cool treatment (green anoles: treatment P=0.004, B=1.67°C; crested anoles: treatment P=0.038, B=1.08°C). Conversely, the CT_{max} of brown anoles was not significantly influenced by the temperature treatment (treatment P=0.89). The heat hardening response was not significantly influenced by temperature treatment in any species (Fig. 2B; P>0.05 for all species).

Despite significant differences in CT_{max} based on temperature treatment in two of the three anole species, gut microbial communities were only modestly altered by temperature treatment (Fig. 3). Temperature treatment did not have a consistent effect on the richness of gut microbial communities across species. After 1 week of treatment, warming marginally increased ASV richness (B=36.60, P=0.051; Table S3), but at week 3, this warming effect was no longer significant (B=27.56, P=0.18). Similarly, Shannon diversity was significantly elevated by warming after 1 week in treatment in green anoles only (B=0.52, P=0.008; Table S3). This effect diminished by the following week (B=0.40, P=0.059).

Similarly, microbial community composition was largely unaffected by temperature treatment. Using the Jaccard index, the effect of treatment was significant at week 2 (pseudo-F=1.31, P=0.023) and marginally significant at week 3 (pseudo-F=1.22, P=0.054), suggesting a subtle influence of warming on community structure. However, treatment only accounted for 1.6–1.7% of the variance and pairwise PERMANOVA showed that only green anoles differed in community composition at week 2, while only brown anoles differed in community composition at week 3 (Table S3). Simultaneously, a PERMANOVA using weighted UniFrac distance revealed no significant effects of temperature treatment at week 2 or week 3, suggesting that while there were several non-overlapping microbial taxa in these species in the cool and warm treatment groups, warming did not shift the abundance or phylogenetic structure of the dominant community members (Table S3).

Gut microbiome composition was associated with host heat tolerance

We investigated the relationship between gut microbiome diversity and composition and host heat tolerance (CT_{max}) across the three anole species. We found no consistent effect of richness or Shannon

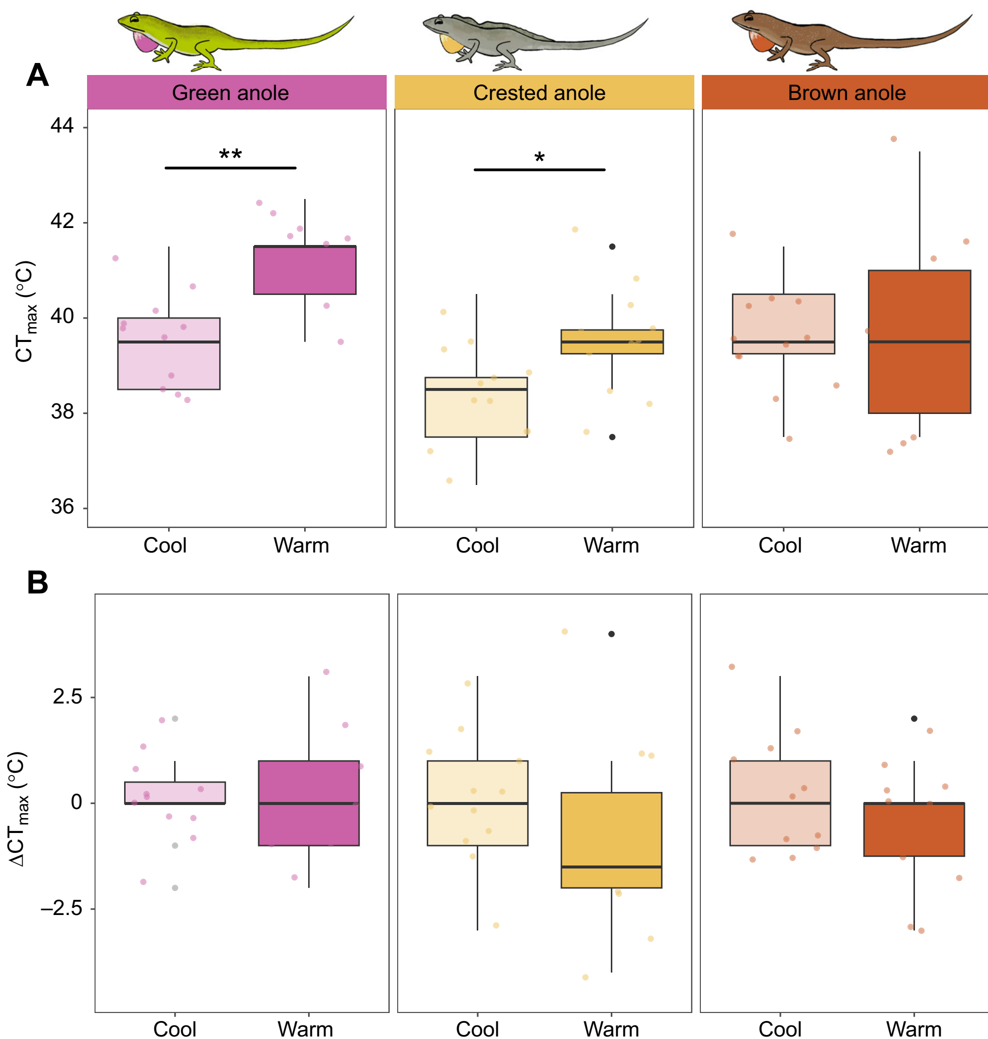

Fig. 2. CT_{max} of anoles exposed to experimental warming. Temperature treatment increased baseline CT_{max} in green and crested anoles (A). Temperature treatment did not significantly affect CT_{max} plasticity in any species (B). Box plots show median, upper and lower quartiles and 1.5× the interquartile range. Statistical differences were assessed by linear models. Asterisks indicate significant comparisons: *P<0.05, **P<0.01.

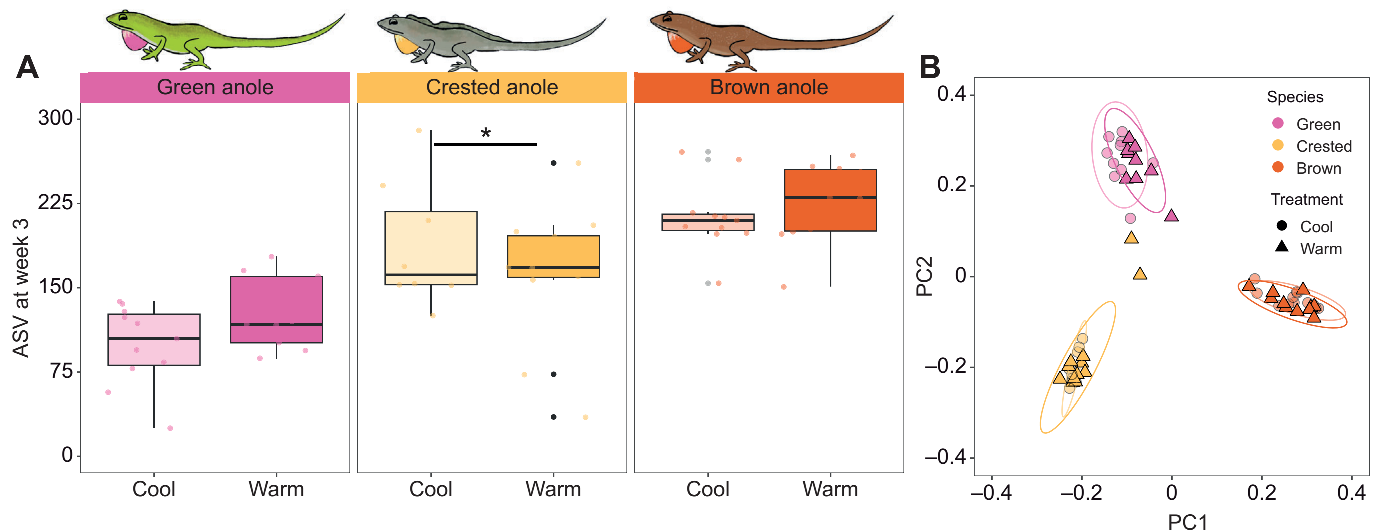

Fig. 3. Microbiome diversity and composition of anoles exposed to experimental warming. Temperature treatment did not result in consistent differences in microbiome amplicon sequence variant (ASV) richness in our study species (A) and only resulted in slight differences in composition in brown anoles (Jaccard distance; B). Box plots show median, upper and lower quartiles and 1.5× the interquartile range. Statistical differences were assessed by linear models in A and PERMANOVA in B. Asterisks indicate significant comparisons: *P<0.05.

diversity on CT_{max} across species (Table S4). However, in crested anoles, individuals with higher CT_{max} had significantly lower microbiome richness (B=−20.97, P=0.002) and Shannon diversity (B=−0.15, P=0.04).

Initial PERMANOVA analyses using only week 1 and week 3 microbiome data revealed no significant effect of CT_{max} on overall microbial community composition (all P>0.3). To integrate the entire dataset and account for repeated measures, we performed global ordinations of Jaccard and Weighted UniFrac distances across all samples and extracted PC1 scores for each sample. LMMs revealed a significant negative association between CT_{max} and Jaccard PC1 scores (Fig. 4; B=−0.014, P=0.014; Table S4), with species-specific interactions modifying this relationship, reflecting a different slope in brown anoles (interaction B=0.023, P=0.002). Individuals with PC1 values closer to zero, indicating more compositionally typical microbiomes, tended to exhibit higher CT_{max}, while those with more extreme PC1 scores had lower heat tolerance. This was reinforced by a significant negative relationship between the absolute value of PC1 and CT_{max} (B=−0.013, P=0.02; Table S4). Taxa contributing most strongly to

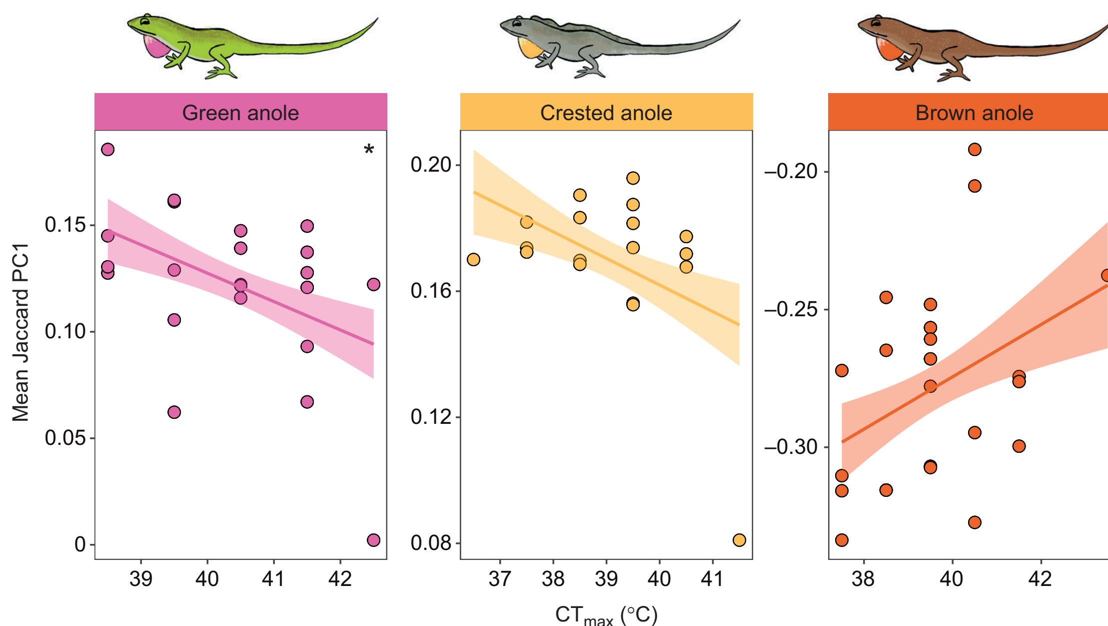

Fig. 4. Association between gut microbiome composition and anole heat tolerance. CT_{max} was significantly correlated with gut microbiome composition, though this relationship was different across species and primarily driven by a significant correlation in green anoles. In all species, higher CT_{max} values correspond to Jaccard PC1 scores that are closer to zero. Mean PC1 values are displayed such that each point represents an individual lizard's average microbiome composition along Jaccard PC1. Relationships were assessed via linear models. Asterisks indicate significant species-specific relationships: *P<0.05.

this axis included multiple ASVs from *Bacteroides*, Lachnospiraceae, Enterobacteriaceae and Verrucomicrobiota (Table S8). Neither weighted UniFrac PC1 scores (all *P*>0.2) nor overall sample dispersion (i.e. distance from global centroid; all *P*>0.05) for either beta diversity metric was significantly associated with CT_{max} across or within species.

Finally, at the taxonomic level, several of the dominant phyla in the anole gut microbiome had significant non-linear (quadratic) relationships with host heat tolerance (Fig. 5). These relationships were strongest when considering pretreatment (week 1) microbiome composition as compared with week 3 microbiome composition (Fig. S6, Tables S5 and S6). In green anoles, Bacteroidota (P=0.011, R^2=0.31) and Proteobacteria (P=0.006, R^2=0.36) abundance was significantly associated with lizard heat tolerance. In crested anoles, Fusobacteriota abundance was significantly associated with lizard heat tolerance (P=0.028, R^2=0.24). In brown anoles, there were no significant relationships between dominant bacterial phyla and host heat tolerance (Tables S5 and S6).

Microbiome composition and microbiome flexibility were associated with host heat hardening

Neither microbiome richness nor diversity was associated with the degree of heat hardening in a consistent way across species (Fig. 6A; richness *P*=0.27, Shannon *P*=0.67) or within species (all *P*>0.05). However, we found that individuals with closer-to-average (closer to 0) Jaccard PC1 scores in the global ordination had weaker heat hardening responses, particularly in green anoles (Fig. 6B; *B*=0.014, *P*=0.02; Table S7). Furthermore, across species, the degree of thermal tolerance plasticity within individual lizards was significantly correlated with microbiome turnover during the experiment: individuals with greater absolute changes in CT_{max} between the first and second trials also exhibited greater microbiome compositional shifts between weeks 1 and 3 (Fig. 7; Jaccard distance; *B*=0.03, *P*=0.017).

DISCUSSION

As climate change progresses, organisms may have to mount plastic responses to persist in changing thermal environments. Particularly for ectotherms, many of which are considered vulnerable to warming,

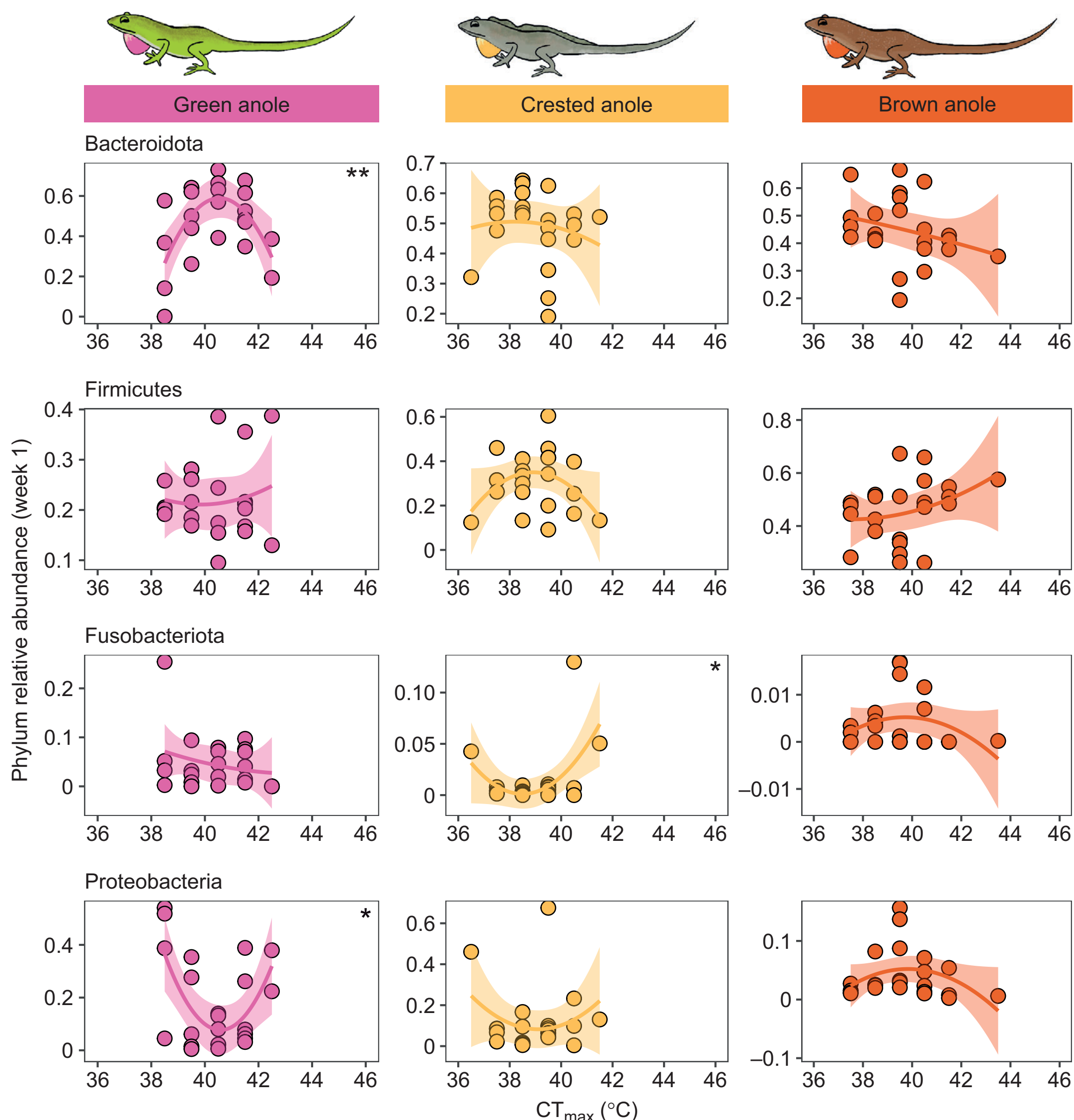

Fig. 5. Association between dominant bacterial phyla in the anole gut (measured in week 1) and host CT_{max} values. Relationships were assessed via linear models with a quadratic term. Asterisks indicate significant relationships: **P*<0.05, ***P*<0.01.

Journal of Experimental Biology

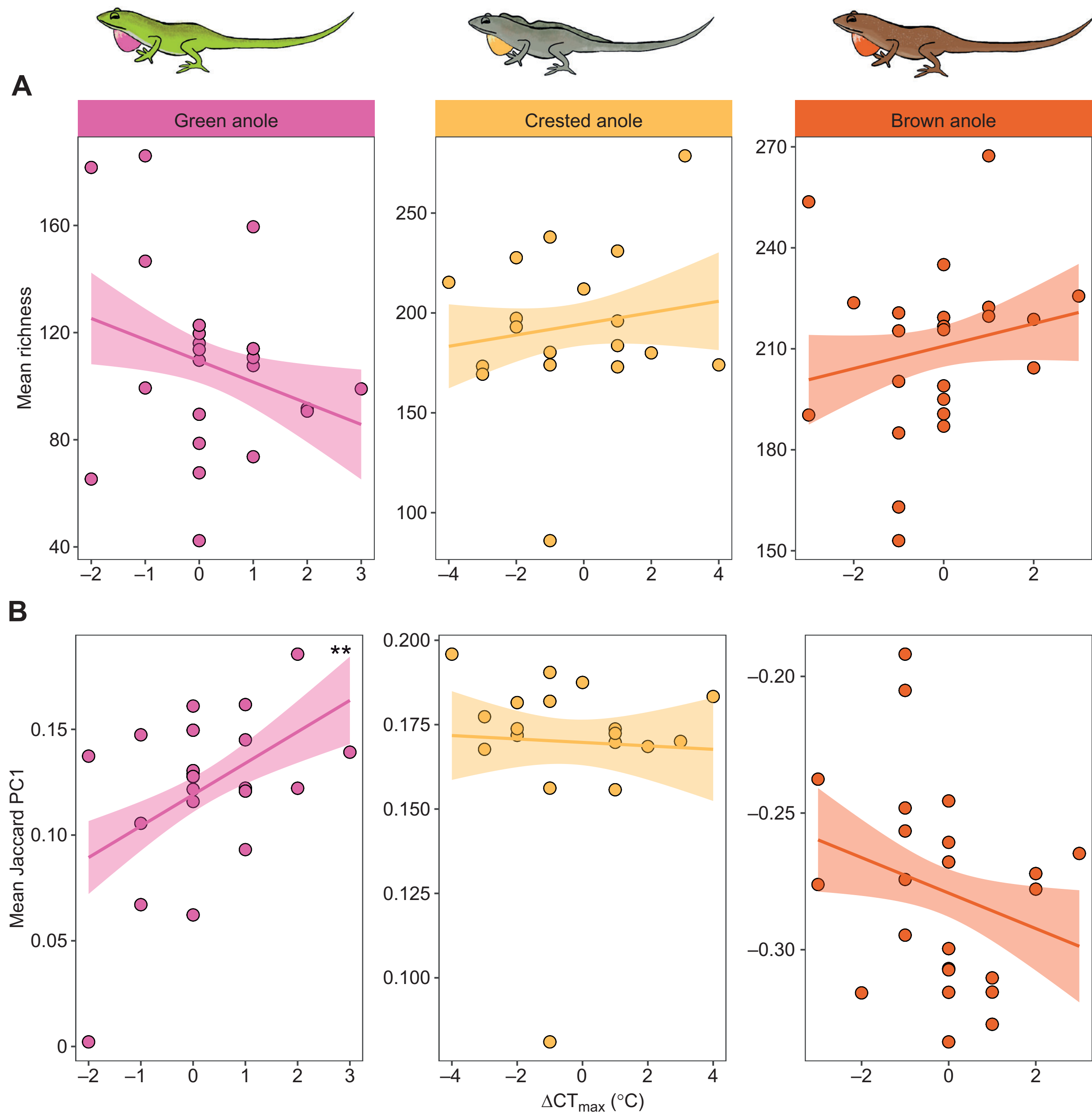

Fig. 6. Association of microbiome composition with heat hardening. Heat hardening (change in CT_{max}) was significantly correlated with gut microbiome composition, though this relationship was primarily driven by a strong and significant correlation in green anoles. Mean richness (A) and microbiome composition (PC1 values; B) are displayed such that each point represents an individual lizard's average microbiome richness or composition along Jaccard PC1. Relationships were assessed via linear models. Asterisks indicate significant comparisons: ***P*<0.01.

understanding the factors that facilitate or constrain the plastic response is crucial. One factor that can potentially shape host thermal physiology and its plasticity is the microbiome. Host-associated microbiota can affect baseline thermal tolerance and could mediate thermal tolerance plasticity via changes in microbial community composition, microbial gene expression or rapid microbial evolution. We sought to test how the microbiomes of three anole species responded to temperature changes and were associated with host physiology and thermal tolerance plasticity. Using three *Anolis* species with similar ecologies yet occupying distant branches of the *Anolis* evolutionary tree, we sought to identify common and divergent patterns in these relationships. Although anole gut microbiomes were largely insensitive to temperature change, we found that (1) microbiome composition was correlated with baseline host heat tolerance and (2) microbiome composition and the degree of gut microbiome plasticity were correlated with the magnitude of heat tolerance plasticity in individual lizards.

The gut microbiome was not strongly sensitive to temperature treatment in any of the species we studied. We saw only subtle effects of temperature treatment on alpha and beta diversity across our study species. While many ectothermic species do have microbiomes that respond to environmental temperature changes (Sepulveda and Moeller, 2020; Li et al., 2023), some studies have shown no responses of gut microbiota to warming (Vaziri et al.,

Journal of Experimental Biology (2026) 229, jeb251650. doi:10.1242/jeb.251650

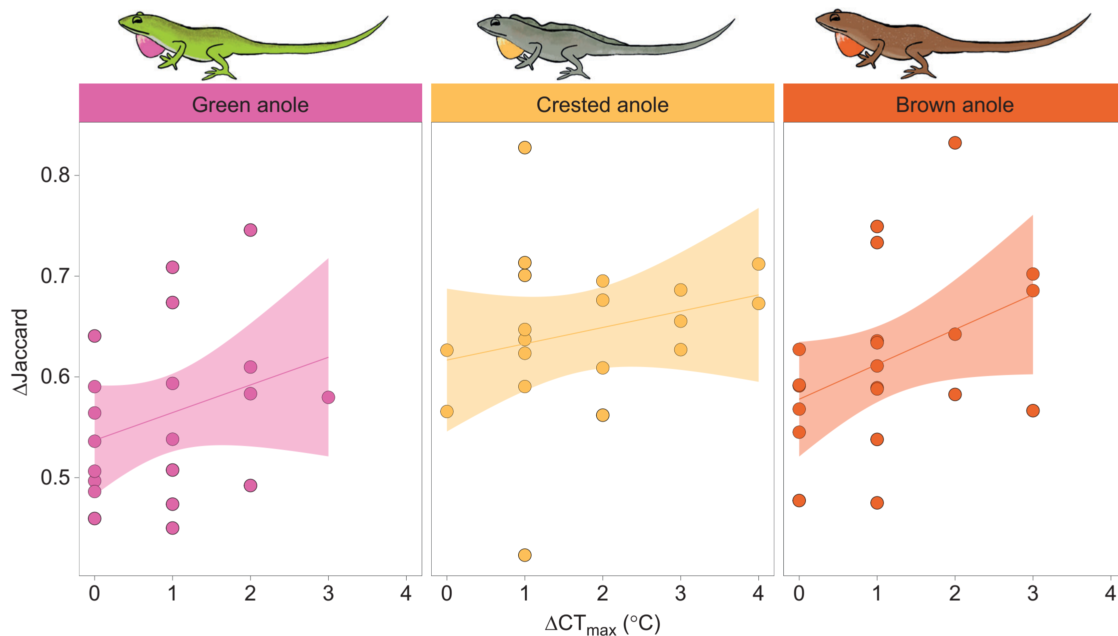

Fig. 7. Association between thermal tolerance plasticity and microbiome turnover of anoles. Larger changes in gut microbiome composition (difference in Jaccard index between week 1 and 3) were associated with greater heat hardening (larger absolute changes in CT_{max} after an initial CT_{max} measurement) across species. Relationships were assessed via linear models.

2025), including our previous research that tested the effect of controlled warming on gut microbiomes in anoles (Williams et al., 2022). Notably, we did not collect wild samples from the lizards in this study, and it is possible that the first week in captivity might have altered baseline microbiomes relative to the wild-type prior to commencement of the temperature treatment. However, our previous work also showed minimal differences between wild samples of a closely related *Anolis* species and those kept in captivity and fed a standardized diet for 6 weeks (Williams et al., 2022). Altogether, our findings and previous work support the idea that *Anolis* lizards harbor microbiomes which are largely heat insensitive and stable with regard to captivity. How sensitive a host's microbiota are to temperature changes likely depends on the composition of the gut microbiome as well as the ecology of the host. For example, terrestrial hosts typically have microbiomes that are less sensitive to temperature changes compared with aquatic hosts (Li et al., 2023). Additionally, both our previous study (Williams et al., 2022) and this one examined changes in microbiomes over just a few weeks of moderate warming, and it is possible that over longer periods or more severe warming, gut microbiomes would shift more substantially.

We found that variation in microbiomes along the dominant compositional axis (Jaccard PC1) was correlated with heat tolerance across species. Similar relationships between host thermal tolerance and microbiome composition have been reported in other vertebrate ectotherms, including lizards (Fontaine et al., 2022; Moeller et al., 2020). While not all species showed significant intraspecific associations, we found that lizards with microbiomes closer to the cross-species average microbiome (i.e. Jaccard PC1 values closer to 0) tended to have higher thermal tolerance, suggesting the existence of a conserved relationship between microbiome composition and thermal tolerance in anoles, despite species-level microbial variation. Importantly, this association was specific to the dominant compositional gradient captured by Jaccard PC1; overall community dispersion (i.e. distance from the global centroid) was not related to thermal tolerance. Thus, thermal tolerance does not appear to be associated with general microbiome variability, but rather with an individual's position along a specific, ecologically meaningful axis of community composition. One possible explanation is that microbial taxa shared across species form a functionally important core assemblage that supports host thermal physiology. Individuals whose microbiomes more closely resemble this shared community may harbor a stable set of microbial functions that enhance heat tolerance or buffer hosts against thermal stress (Zaneveld et al., 2017). Indeed, some of the taxa most correlated with PC1 were dominant members of the reptile gut microbiome, including many ASVs in the genus Bacteroides, which has been identified as a core member of the reptile gut (Hoffbeck et al., 2025). Alternatively, hosts with similar thermal tolerance, despite species identity, may select for a common set of microbial taxa (Mazel et al., 2018). Altogether, a relationship between microbiome composition and heat tolerance suggests that microbes may account for a portion of heat tolerance variation within and among species, raising the possibility that changes in microbiomes could contribute to variation in host heat tolerance.

Although microbiome characteristics have been linked to thermal traits in a range of species, an open question is whether the composition and flexibility of microbiome communities contributes to plastic responses in hosts. We tested this idea and found that microbiome composition and variability were related to the degree of plasticity in host heat tolerance as measured by our rapid heat-hardening experiment. Notably, we found that lizards with microbiomes closer to the cross-species average microbiome tended to have a reduced heat hardening response. In addition, the magnitude of change in individual lizard microbiomes corresponded to the magnitude of plasticity in host heat tolerance across all three species, highlighting a conserved relationship between microbiome plasticity and thermal tolerance plasticity. This finding suggests that hosts with certain microbiome compositions and more plastic microbiomes are more

Journal of Experimental Biology (2026) 229, jeb251650. doi:10.1242/jeb.251650

physiologically plastic. One possible explanation for this pattern is that microbiome composition can change readily in some hosts in a way that facilitates changes in host thermal tolerance (Voolstra and Ziegler, 2020). This idea is supported by the fact that green and crested anoles, which showed increases in baseline CT_{max} after warming, also exhibited greater shifts in microbiome diversity, whereas brown anoles showed neither long-term CT_{max} acclimation nor appreciable microbial responses to temperature treatment. Several studies have demonstrated that microbiome alterations can directly modify host thermal physiology (Santoro et al., 2021; Baldassarre et al., 2022) and broader syntheses have highlighted how microbial flexibility might underpin changes in host thermal tolerance traits (Li and King, 2025). These emerging data support a link between microbiome plasticity and host physiological plasticity. Alternatively, there may be other variables that we did not measure that link host phenotypic plasticity to microbiome flexibility. Regardless, taken together, our work and that of previous authors suggests that phenotypic plasticity in animals may co-vary with microbiome composition. Further research on the mechanisms by which changing microbial communities alter host trait expression is sorely needed.

Our study adds to a small but growing body of literature suggesting that microbiome variation might affect host phenotypes and their plasticity. Follow up work could seek to measure the relative effects of changes in host gene expression and microbiome composition, and their interactions, in the heat hardening response. Aagaard et al. (2024) followed this approach in spiders, but found no parallel changes in gene expression and microbiome changes, suggesting a limited role of the microbiome in the plastic response. In contrast, Fontaine and Kohl (2023a,b) experimentally manipulated the microbiomes of tadpoles and found evidence that host- and microbe-derived plasticity mechanisms might trade off. Plasticity of gene expression in hosts was greatly enhanced if the hosts had depleted microbiomes, while those with intact microbiomes exhibit stronger microbial gene expression responses (Fontaine and Kohl, 2023b). It is likely that the role of host-associated microbiota in host plastic responses varies dramatically among species, and characterizing these responses across the tree of life is a worthy avenue of future study.

As climate change progresses, many organisms will find themselves in rapidly changing thermal environments and may have to adjust their phenotypes to persist. In particular, ectotherms living at low latitudes are predicted to be highly vulnerable to climate warming (Huey et al., 2012, 2009), and for tropical species assemblages, even modest shifts in climate may have significant ecological consequences. Because the microbial mechanisms underlying thermal tolerance in lizards remain poorly understood, these three ecologically important and evolutionarily divergent species offer a valuable comparative framework for identifying how microbiome stability and flexibility may contribute to thermal responses in natural systems. Our results demonstrate that the gut microbiomes of *Anolis* lizards are associated with their baseline heat tolerance and the capacity of this tolerance to shift plasticly. This has important implications for our understanding of how lizards will respond to climate warming: if variation in microbiomes contributes to host phenotypes to an extent that is relevant to host survival and reproductive success, this could ultimately affect the persistence of these species and their evolutionary trajectories. Regardless, our study demonstrates that the link between microbiome flexibility and phenotypic plasticity deserves further attention to fully unravel how hosts and their microbes work together to shape emergent phenotypes and respond to changing environmental conditions.

Acknowledgements
We would like to acknowledge and thank John Anderson for his support in maintaining the University of Nevada Reno High Performance Computing System.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.R.G.; Data curation: C.E.W., W.W.-Y.W., A.R.G.; Formal analysis: C.E.W., J.-S.C.; Funding acquisition: A.R.G.; Investigation: A.C.G., W.W.-Y.W., H.Y.C., A.S.; Methodology: C.E.W., W.W.-Y.W., A.R.G.; Project administration: A.C.G., A.R.G.; Resources: A.R.G.; Software: C.E.W.; Supervision: M.L.L., A.R.G.; Visualization: C.E.W.; Writing – original draft: C.E.W.; Writing – review & editing: C.E.W., A.C.G., W.W.-Y.W., J.-S.C., H.Y.C., A.S., M.L.L., A.R.G.

Funding
This work was supported by Tulane University.

Data and resource availability
All raw sequences generated for this study have been deposited in the NCBI sequence read archive: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1270091. All processed microbiome data and physiological data used to conduct analyses are available from GitHub: https://github.com/claireewilliams/2025AnolisPlasticity. All other relevant data and details of resources can be found within the article and its supplementary information.

References

Aagaard, A., Bechsgaard, J., Sørensen, J. G., Sandfeld, T., Settepani, V., Bird, T. L., Lund, M. B., Malmos, K. G., Falck-Rasmussen, K., Darolti, I. et al. (2024). Molecular mechanisms of temperature tolerance plasticity in an arthropod. *Genome Biol. Evol.* **16**, evae165. doi:10.1093/gbe/evae165

Amenyogbe, N., Kollmann, T. R. and Ben-Othman, R. (2017). Early-life host–microbiome interphase: the key frontier for immune development. *Front. Pediatr.* **5**, 111. doi:10.3389/fped.2017.00111

Baldassarre, L., Ying, H., Reitzel, A. M., Franzenburg, S. and Fraune, S. (2022). Microbiota mediated plasticity promotes thermal adaptation in the sea anemone *Nematostella vectensis*. *Nat. Commun.* **13**, 3804. doi:10.1038/s41467-022-31350-z

Baniel, A., Amato, K. R., Beehner, J. C., Bergman, T. J., Mercer, A., Perlman, R. F., Petrullo, L., Reitsema, L., Sams, S., Lu, A. et al. (2021). Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas. *Microbiome* **9**, 26. doi:10.1186/s40168-020-00977-9

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F. et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852-857. doi:10.1038/s41587-019-0209-9

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A. and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581-583. doi:10.1038/nmeth.3869

Cao, Y., Liu, Y., Dong, Q., Wang, T. and Niu, C. (2022). Alterations in the gut microbiome and metabolic profile in rats acclimated to high environmental temperature. *Microb. Biotechnol.* **15**, 276-288. doi:10.1111/1751-7915.13772

Cox, T. O., Lundgren, P., Nath, K. and Thaiss, C. A. (2022). Metabolic control by the microbiome. *Genome Med.* **14**, 80. doi:10.1186/s13073-022-01092-0

Dallas, J. W., Kazarina, A., Lee, S. T. M. and Warne, R. W. (2024). Cross-species gut microbiota transplantation predictably affects host heat tolerance. *J. Exp. Biol.* **227**, jeb246735. doi:10.1242/jeb.246735

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A. et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559-563. doi:10.1038/nature12820

Deery, S. W., Rej, J. E., Haro, D. and Gunderson, A. R. (2021). Heat hardening in a pair of *Anolis* lizards: constraints, dynamics and ecological consequences. *J. Exp. Biol.* **224**, jeb240994. doi:10.1242/jeb.240994

Dunbar, H. E., Wilson, A. C. C., Ferguson, N. R. and Moran, N. A. (2007). Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biol.* **5**, 1006-1015. doi:10.1371/journal.pbio.0050096

Fernandes, K. E., Stanfield, B., Frost, E. A., Shanahan, E. R., Susantio, D., Dong, A. Z., Tran, T. D., Cokcetin, N. N. and Copeland, D. (2023). Low levels of hive stress are associated with decreased honey activity and changes to the gut microbiome of resident honey bees. *Microbiol. Spectr.* **11**, e00742-e00723. doi:10.1128/spectrum.00742-23

Fontaine, S. S. and Kohl, K. D. (2023a). Ectotherm heat tolerance and the microbiome: current understanding, future directions and potential applications. *J. Exp. Biol.* **226**, jeb245761. doi:10.1242/jeb.245761

Fontaine, S. S. and Kohl, K. D. (2023b). The microbiome buffers tadpole hosts from heat stress: a hologenomic approach to understand host–microbe interactions under warming. *J. Exp. Biol.* **226**, jeb245191. doi:10.1242/jeb.245191

Fontaine, S. S., Mineo, P. M. and Kohl, K. D. (2022). Experimental manipulation of microbiota reduces host thermal tolerance and fitness under heat stress in a vertebrate ectotherm. *Nat. Ecol. Evol.* **7**, 405-417. doi:10.1038/s41559-022-01686-2

Gerber, G. K. (2014). The dynamic microbiome. *FEBS Lett.* **588**, 4131-4139. doi:10.1016/j.febslet.2014.02.037

Gunderson, A. R. (2024). Disentangling physiological and physical explanations for body size-dependent thermal tolerance. *J. Exp. Biol.* **227**, jeb245645. doi:10.1242/jeb.245645

Gunderson, A. R. and Leal, M. (2015). Patterns of thermal constraint on ectotherm activity. *Am. Nat.* **185**, 653-664. doi:10.1086/680849

Gunderson, A. R., Mahler, D. L. and Leal, M. (2018). Thermal niche evolution across replicated *Anolis* lizard adaptive radiations. *Proc. R. Soc. B Biol. Sci.* **285**, 20172241. doi:10.1098/rspb.2017.2241

Hernández, M., Ancona, S., Hereira-Pacheco, S., Díaz De La Vega-Pérez, A. H. and Navarro-Noya, Y. E. (2023). Comparative analysis of two nonlethal methods for the study of the gut bacterial communities in wild lizards. *Integr. Zool.* **18**, 1056-1071. doi:10.1111/1749-4877.12711

Hicks, A. L., Lee, K. J., Couto-Rodriguez, M., Patel, J., Sinha, R., Guo, C., Olson, S. H., Seimon, A., Seimon, T. A., Ondzie, A. U. et al. (2018). Gut microbiomes of wild great apes fluctuate seasonally in response to diet. *Nat. Commun.* **9**, 1786. doi:10.1038/s41467-018-04204-w

Hoffbeck, C., Middleton, D. M. R. L., Wallbank, J. A., Boey, J. S. and Taylor, M. W. (2025). Culture-independent species-level taxonomic and functional characterisation of Bacteroides, the core bacterial genus within reptile guts. *Mol. Ecol.* **34**, e17685. doi:10.1111/mec.17685

Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Pérez, H. J. and Garland, T. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. B Biol. Sci.* **276**, 1939-1948. doi:10.1098/rspb.2008.1957

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M. and Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. B Biol. Sci.* **367**, 1665-1679. doi:10.1098/rstb.2012.0005

IPCC. (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. doi:10.1017/9781009157896

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. *Bull. Société Vaudoise Sci. Nat.* **44**, 223-270.

Kannan, R. and James, D. A. (2009). Effects of climate change on global biodiversity: a review of key literature. *Trop. Ecol.* **50**, 31-39.

Katoh, K., Misawa, K., Kuma, K. and Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**, 3059-3066. doi:10.1093/nar/gkf436

Kohl, K. D., Brun, A., Magallanes, M., Brinkerhoff, J., Laspiur, A., Acosta, J. C., Caviedes-Vidal, E. and Bordenstein, S. R. (2017). Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. *Mol. Ecol.* **26**, 1175-1189. doi:10.1111/mec.13921

Kolodny, O. and Schulenburg, H. (2020). Microbiome-mediated plasticity directs host evolution along several distinct time scales. *Philos. Trans. R. Soc. B Biol. Sci.* **375**, 20190589. doi:10.1098/rstb.2019.0589

Leal, M. and Gunderson, A. R. (2012). Rapid change in the thermal tolerance of a tropical lizard. *Am. Nat.* **180**, 815-822. doi:10.1086/668077

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R. et al. (2008). Evolution of mammals and their gut microbes. *Science* **320**, 1647-1651. doi:10.1126/science.1155725

Li, J. and King, K. (2025). Microbial Primer: microbiome and thermal tolerance – a new frontier in climate resilience? *Microbiology* **171**, 001523. doi:10.1099/mic.0.001523

Li, J., Bates, K. A., Hoang, K. L., Hector, T. E., Knowles, S. C. L. and King, K. C. (2023). Experimental temperatures shape host microbiome diversity and composition. *Glob. Change Biol.* **29**, 41-56. doi:10.1111/gcb.16429

Losos, J. B. (2011). *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. Univ of California Press.

Lozupone, C. and Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **71**, 8228-8235. doi:10.1128/AEM.71.12.8228-8235.2005

Lozupone, C. A., Hamady, M., Kelley, S. T. and Knight, R. (2007). Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl. Environ. Microbiol.* **73**, 1576-1585. doi:10.1128/AEM.01996-06

Lynch, J. B. and Hsiao, E. Y. (2019). Microbiomes as sources of emergent host phenotypes. *Science* **365**, 1405-1409. doi:10.1126/science.aay0240

Mallick, H., Rahnavard, A., McIver, L. J., Ma, S., Zhang, Y., Nguyen, L. H., Tickle, T. L., Weingart, G., Ren, B., Schwager, E. H. et al. (2021). Multivariable association discovery in population-scale meta-omics studies. *PLoS Comput. Biol.* **17**, e1009442. doi:10.1371/journal.pcbi.1009442

Mazel, F., Davis, K. M., Loudon, A., Kwong, W. K., Groussin, M. and Parfrey, L. W. (2018). Is host filtering the main driver of phylosymbiosis across the tree of life? *mSystems* **3**, e00097-e00018. doi:10.1128/mSystems.00097-18

McMurdie, P. J. and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**, e61217. doi:10.1371/journal.pone.0061217

Mi, C., Ma, L., Wang, Y., Wu, D., Du, W. and Sun, B. (2022). Temperate and tropical lizards are vulnerable to climate warming due to increased water loss and heat stress. *Proc. R. Soc. B Biol. Sci.* **289**, 20221074. doi:10.1098/rspb.2022.1074

Moeller, A. H., Ivey, K., Cornwall, M. B., Herr, K., Rede, J., Taylor, E. N. and Gunderson, A. R. (2020). The lizard gut microbiome changes with temperature and is associated with heat tolerance. *Appl. Environ. Microbiol.* **86**, e01181-e01120. doi:10.1128/AEM.01181-20

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Müller, A., Nothman, J., Louppe, G. et al. (2011). Scikit-learn: machine learning in python. *J. Mach. Learn. Res.* **12**, 2825-2830.

Price, M. N., Dehal, P. S. and Arkin, A. P. (2010). FastTree 2 – Approximately maximum-likelihood trees for large alignments. *PLoS ONE* **5**, e9490. doi:10.1371/journal.pone.0009490

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590-D596. doi:10.1093/nar/gks1219

Ren, T., Kahrl, A. F., Wu, M. and Cox, R. M. (2016). Does adaptive radiation of a host lineage promote ecological diversity of its bacterial communities? A test using gut microbiota of *Anolis* lizards. *Mol. Ecol.* **25**, 4793-4804. doi:10.1111/mec.13796

Richter, S., Kipfer, T., Wohlgemuth, T., Calderón Guerrero, C., Ghazoul, J. and Moser, B. (2012). Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *Oecologia* **169**, 269-279. doi:10.1007/s00442-011-2191-x

Ryan, L. M. and Gunderson, A. R. (2021). Competing native and invasive *Anolis* lizards exhibit thermal preference plasticity in opposite directions. *J. Exp. Zool. Part Ecol. Integr. Physiol.* **335**, 118-125. doi:10.1002/jez.2420

Santoro, E. P., Borges, R. M., Espinoza, J. L., Freire, M., Messias, C. S. M. A., Villela, H. D. M., Pereira, L. M., Vilela, C. L. S., Rosado, J. G., Cardoso, P. M. et al. (2021). Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. *Sci. Adv.* **7**, eabg3088. doi:10.1126/sciadv.abg3088

Sepulveda, J. and Moeller, A. H. (2020). The effects of temperature on animal gut microbiomes. *Front. Microbiol.* **11**, 384. doi:10.3389/fmicb.2020.00384

Sinervo, B., Méndez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., Lara-Resendiz, R., Martínez-Méndez, N., Calderón-Espinosa, M. L., Meza-Lázaro, R. N. et al. (2010). Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**, 894-899. doi:10.1126/science.1184695

Urban, M. C., Tewksbury, J. J. and Sheldon, K. S. (2012). On a collision course: competition and dispersal differences create no-analogue communities and cause extinctions during climate change. *Proc. R. Soc. B Biol. Sci.* **279**, 2072-2080. doi:10.1098/rspb.2011.2367

Vaziri, G. J., Caicedo, B., Dahrouge, N., Ryerson, W. G., Davenport, J. M., Stager, M., Jones, K. R., Frost, C., Seewagen, C. L., Rittenhouse, T. A. G. et al. (2025). Gut microbiomes are largely unchanged when exposed to their amphibian host's latitudinally variable upper thermal limit. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **302**, 111816. doi:10.1016/j.cbpa.2025.111816

Voolstra, C. R. and Ziegler, M. (2020). Adapting with microbial help: microbiome flexibility facilitates rapid responses to environmental change. *BioEssays* **42**, 2000004. doi:10.1002/bies.202000004

Whitman, D. and Agrawal, A. (2009). What is phenotypic plasticity and why is it important? In *Phenotypic Plasticity of Insects* (ed. D. Whitman and T. Ananthakrishnan). Science Publishers.

Williams, C. E., Kueneman, J. G., Nicholson, D. J., Rosso, A. A., Folfas, E., Casement, B., Gallegos-Koyner, M. A., Neel, L. K., Curlis, J. D., McMillan, W. O. et al. (2022). Sustained drought, but not short-term warming, alters the gut microbiomes of wild *Anolis* lizards. *Appl. Environ. Microbiol.* **88**, e0053022. doi:10.1128/aem.00530-22

Williams, C. E., Brown, A. E. and Williams, C. L. (2023a). The role of diet and host species in shaping the seasonal dynamics of the gut microbiome. *FEMS Microbiol. Ecol.* **99**, fiad156. doi:10.1093/femsec/fiad156

Williams, C. E., Williams, C. L. and Logan, M. L. (2023b). Climate change is not just global warming: multidimensional impacts on animal gut microbiota. *Microb. Biotechnol.* **16**, 1736-1744. doi:10.1111/1751-7915.14276

Williams, S. E., Shoo, L. P., Isaac, J. L., Hoffmann, A. A. and Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol.* **6**, e325. doi:10.1371/journal.pbio.0060325

Zaneveld, J. R., McMinds, R. and Vega Thurber, R. (2017). Stress and stability: applying the Anna Karenina principle to animal microbiomes. *Nat. Microbiol.* **2**, 1-8. doi:10.1038/nmicrobiol.2017.121