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Warming effects on lizard gut microbiome depend on habitat connectivity

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Climate warming and landscape fragmentation are both factors well known to threaten biodiversity and to generate species responses and adaptation. However, the impact of warming and fragmentation interplay on organismal responses remains largely under-explored, especially when it comes to gut symbionts, which may play a key role in essential host functions and traits by extending its functional and genetic repertoire. Here, we experimentally examined the combined effects of climate warming and habitat connectivity on the gut bacterial communities of the common lizard (*Zootoca vivipara*) over three years. While the strength of effects varied over the years, we found that a 2°C warmer climate decreases lizard gut microbiome diversity in isolated habitats. However, enabling connectivity among habitats with warmer and cooler climates offset or even reversed warming effects. The warming effects and the association between host dispersal behaviour and microbiome diversity appear to be a potential driver of this interplay. This study suggests that preserving habitat connectivity will play a key role in mitigating climate change impacts, including the diversity of the gut microbiome, and calls for more studies combining multiple anthropogenic stressors when predicting the persistence of species and communities through global changes.

1. Introduction

Contemporary climate change is a major threat to biodiversity with an expected extinction rate of 15–37% of species by 2050 [1]. Species may respond to climate change through two compensatory processes. First, individuals can avoid extreme climatic conditions by dispersing towards more suitable thermal environments over small distances [2], a process that offsets climate impacts on populations and can lead to species range shifts. Second, species can adjust their phenotype to new environmental conditions through the selection of more adapted phenotypes or through intra- and inter-generational phenotypic plasticity [3,4]. Both processes strongly rely on the ability of individuals to disperse. Dispersal controls species movement distances and hence ability to track their shifting habitat [5]. It further influences the genetic composition of a population through individual/gene flows [6]. Dispersal is however hampered by the increasing destruction and fragmentation of habitats [7,8]. This reduces species abilities to track their

suitable thermal habitats [9] and influences species adaptation to local climate by reducing gene flows [10]. Assessing the effects of dispersal is much more challenging and requires better understanding of the complex interplay of climate and fragmentation for ecological and evolutionary processes [11].

A large body of literature already documented phenotypic changes with climate change and habitat fragmentation, including changes in reproduction phenology [12,13], physiology [14] or body size [15,16], as well as their interplay [16–18]. However, a still largely overlooked aspect is the role of host-associated microbiome responses to climate change. In animals, gut microbial symbionts play a key role in many essential host functions and traits related to, for exmaple, metabolism, nutrition, immunity, behaviour and morphology [19,20]. By harbouring its own genes and functions, the microbiota can thus extend both the functional and genetic repertoires of the host. The gut microbiome is therefore increasingly considered as the host's 'extended phenotype' [21] or even 'extended genotype' [22]. This, together with microbes' short generation time and rapid response to environmental changes [19], suggests that the gut microbiome could play a significant role in the host response to environmental changes [23]. For example, manipulating microbiome composition can have effects on host thermal tolerance, fitness and acclimation to heat stress [24,25]. However, because gut microbial community structures are complex and not necessarily adaptive for their host, the relationship between variations in gut communities and host fitness and their changes with environmental changes need to be clearly established to draw reliable conclusions on the evolutionary consequences of these changes [19,26,27].

As for any biological community, the gut microbiome is expected to be shaped by four fundamental assembly processes, namely selection by the host or its biotic/abiotic factors, dispersal, drift and speciation [28], of which exact nature and relative importance is context or scale dependent [29]. Climate change can positively or negatively affect certain taxa through environmental selection, direct climate effects, and/or climate-induced changes in host condition and physiology. Short-term responses of the gut microbiome diversity and composition to warmer temperatures have been reported in various animals (reviewed in [30]). As found in many vertebrates species [30], they usually translate into a reduction in diversity and/or a reduction of Firmicutes abundance, with potential subsequent negative consequences on host survival and health [31], for instance through a decrease of digestive efficiency or energy assimilation [32]. The gut microbiome composition can also exhibit greater variability among host individuals subjected to thermal stress. This may result from the decreased abundance of some bacteria taxa that usually fill the ecological niche space available in the gut habitat (e.g. in terms of food or adherence sites) and/or actively inhibit opportunistic colonization of the gut from the environmental microbial pool, including pathogens [30]. However, it remains unclear whether these effects remain through the host-generation time scale, since existing studies report only short-term responses (inferior to 1 year).

Likewise, the gut microbiome may be involved in the adaptation and phenotypic plasticity of the host through temporal changes in microbial diversity and composition. These microbial dynamics throughout an individual's lifespan (hereafter referred to as gut microbiome plasticity according to an extended phenotype viewpoint) can arise either from stochastic processes or from the host or environmental contexts [33] and may or may not have consequences on host phenotype and life history at different temporal scales [23,34]. Host dispersal can also influence the gut microbiome [35]. For example, high dispersal can increase the number of habitats, food resources, sexual and social partners experienced by hosts, hence exposing them to a greater diversity of environmental and/or gut bacterial species (reviewed in [36,37]). At the opposite, habitat fragmentation and host dispersal limitation might homogenize the gut microbiome across hosts, by increasing the density of individuals locally, and hence of contacts and bacterial transmission between hosts. Finally, dispersal limitation can also lead to a differentiation of the gut microbiome among populations at the regional scale.

Host dispersal may thus influence the way the gut microbiome responds to climate change. For example, a regional-scale study of the gut microbiome in isolated versus dispersing moose populations shows that only isolated populations are influenced by local temperatures, with potential implications in terms of metabolic adaptations [38]. However, such *in natura* studies do not allow to disentangle effects of potential confounding factors covarying with climate and habitat isolation. Experimental studies can circumvent this limitation, but have so far only singularly manipulated the effect of climate change [31,32,39] or connectivity [35,40], hence precluding potential interactive effects.

Here, we work on the gut microbiome data sampled during the experiment described in [16]. This study was built on a previous one year experiment examining the effect of climate change on the gut microbiome of the common lizard, *Zootoca vivipara* [31] to perform a new experiment where we investigated the dependency of climate effects on gut microbiome diversity to habitat connectivity for three years. The experiment is conducted in a semi-natural experimental set-up composed of connected or isolated mesocosms subjected to climate treatments, a present-day climate and approximately 2°C warmer climate, following IPCC's projections for southern Europe in 2080 [41]. This design allows us to study the impacts of warmer conditions on microbiome when lizards could move between thermal habitats and have access to a cooler microclimate or when they were facing warmer habitats only. The common lizard is a relevant model species to investigate these questions, because the body temperature, vital functions (e.g. nutrition), and a wide range of life history or extended traits (e.g. growth rate, survival, reproduction, dispersal propensity, gut microbiome) in ectotherms depend on external temperatures (e.g.[5,31,42–45]). We expect the gut bacterial diversity to be lower in warmer climate, in particular through a decrease of Firmicutes abundance, as well as changes in compositional similarity among host individuals. We further expect climate effects to be buffered in more connected habitats through the access to more diverse thermal habitats, food resources and microbial species pool, as observed for the impacts on life-history traits [16]. Finally, using an extended phenotype viewpoint, we studied whether changes in host microbial diversity resulted from host survival, microbiome plasticity and host dispersal.

2. Results

(a) Lizard gut diversity over years

We found an overall negative effect of warm climate on gut diversity varying with habitat connectivity (figure 1; electronic supplementary material, table S1). We also found that the interdependency between climate and connectivity became stronger over

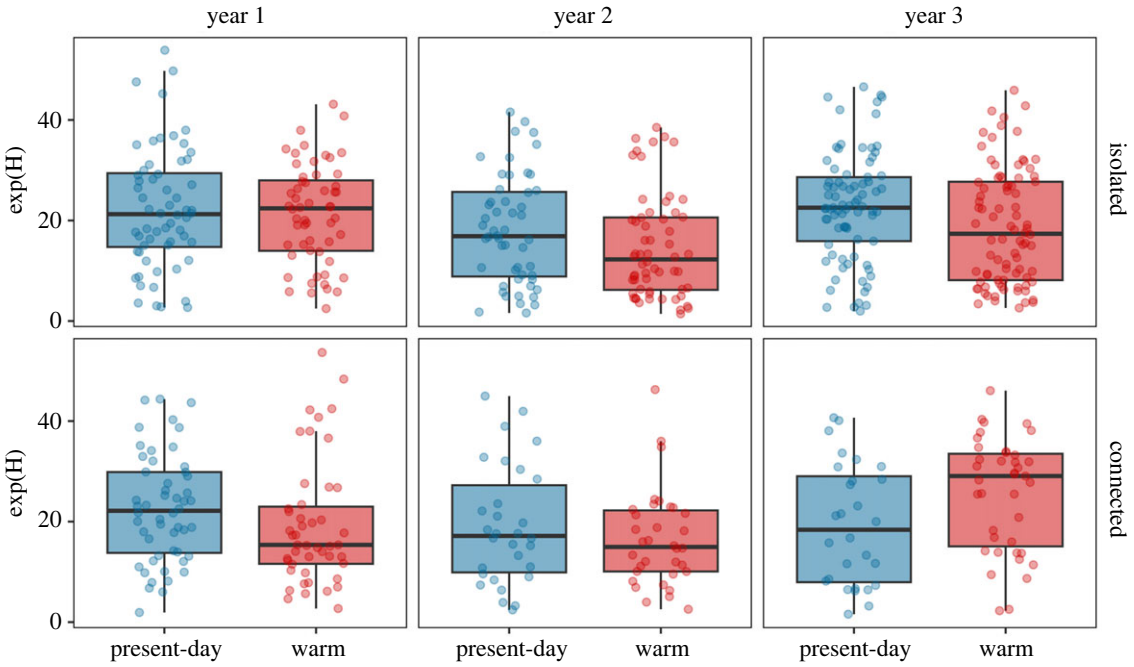

Figure 1. Gut diversity in each climate and habitat connectivity over time. Gut microbiota diversity, calculated as the exponential of Shannon index (exp(H)), in present-day and warm climates each year for isolated and connected mesocosms.

Table 1. Effects of centred years and warming interaction on the gut microbial diversity as expressed by the exponential Shannon index in isolated ($n_{lizards}$ = 395) and connected mesocosms ($n_{lizards}$ = 230). Age class, sex and snout–vent length were included as covariates. References levels of the factors are present-day climate, adults, and females. Interactions and parameters were excluded from models according to AIC procedure. Models explain 6.1% and 6.3% of the marginal variance and 6.1% and 7% of the conditional variance explained, respectively.

parameters	estimate	SE	*z*-value	RI	*p*-value
isolated mesocosms					
intercept	21.307	0.923	23.009	1	<0.001***
year 2	−4.079	2.082	1.953	1	0.051
year 3	0.598	1.870	0.319	1	0.750
warm climate	−2.924	1.111	2.623	1	0.009**
year 2 × warm climate	−2.453	2.915	0.839	1	0.402
year 3 × warm climate	−3.074	2.660	1.152	1	0.249
sex	−0.554	1.115	0.495	0.52	0.621
age class	0.407	1.134	0.358	0.51	0.720
connected mesocosms					
intercept	21.029	1.496	13.986	1	<0.001***
year 2	−3.340	2.656	1.351	1	0.211
year 3	−3.146	2.733	1.140	1	0.254
warm climate	−0.285	1.656	1.171	1	0.864
year 2 × warm climate	0.726	3.686	1.196	1	0.845
year 3 × warm climate	9.478	3.669	2.569	1	0.010*
age class	−1.152	1.551	0.739	0.63	0.460
sex	−0.293	1.515	0.193	0.56	0.847

time, as shown by the triple interaction between climate, connectivity between mesocosms and centred years effects, with a slightly stronger interaction between climate and connectivity for year 2 (RI = 1, *p*-value = 0.510; electronic supplementary material, table S1), and a much stronger interaction in year 3 (RI = 1, *p*-value = 0.005; electronic supplementary material, table S1).

In isolated mesocosms, the gut microbiome diversity was 14.4% lower in warm climate across years (table 1). This negative effect was slightly stronger through time. Indeed, the interactions between warm climate and years 2 and 3 were retained in the best model but not significant (RI = 1, *p*-values = 0.402 and 0.249; table 1). By contrast, there was no overall effect of

Table 2. Difference in diversity within major bacterial clades between present and warm climates each year within isolated ($n_{lizards} = 395$) and connected mesocosms ($n_{lizards} = 230$). Estimates show diversity in warm treatment minus diversity in present-day. Models explain 51% and 52% of the marginal variance and 53% and 54% of the conditional variance explained, respectively.

		isolated mesocosms				connected mesocosms			
	phyla	estimate	s.e.	*t*-ratio	*p*-value	estimate	s.e.	*t*-ratio	*p*-value
year 1	Actinobacteria	1.141	0.777	−1.469	0.142	0.158	0.851	0.186	0.853
	Alphaproteobacteria	0.024	0.777	0.030	0.976	−0.398	0.851	−0.469	0.640
	Bacteroidetes	0.054	0.777	0.069	0.945	−0.773	0.851	−0.908	0.365
	Deltaproteobacteria	−0.149	0.777	−0.192	0.848	−0.327	0.851	−0.384	0.701
	Firmicutes	−2.342	0.777	−3.016	0.003**	−3.129	0.851	−3.678	<0.001***
	Fusobacteria	−0.088	0.777	−0.113	0.910	−0.119	0.851	−0.140	0.889
	Gammaproteobacteria	−0.223	0.777	−0.287	0.774	−0.510	0.851	−0.599	0.549
year 2	Actinobacteria	−0.213	0.792	−0.269	0.788	0.993	1.051	0.945	0.345
	Alphaproteobacteria	0.231	0.792	0.292	0.770	0.503	1.051	0.479	0.632
	Bacteroidetes	−0.311	0.792	−0.393	0.694	−0.575	1.051	−0.547	0.584
	Deltaproteobacteria	−0.231	0.792	−0.292	0.771	0.380	1.051	0.361	0.718
	Firmicutes	−2.854	0.792	−3.605	< 0.001***	−3.058	1.051	−2.910	0.004**
	Fusobacteria	0.061	0.792	0.077	0.939	0.132	1.051	0.125	0.900
	Gammaproteobacteria	−0.661	0.792	−0.835	0.404	−0.015	1.051	−0.014	0.989
year 3	Actinobacteria	−0.316	0.646	−0.489	0.625	1.660	1.048	1.584	0.114
	Alphaproteobacteria	0.042	0.646	0.065	0.948	0.634	1.048	0.605	0.546
	Bacteroidetes	−0.226	0.646	−0.350	0.727	1.116	1.048	1.065	0.288
	Deltaproteobacteria	−0.002	0.646	−0.003	0.998	0.148	1.048	0.141	0.888
	Firmicutes	−2.211	0.646	−3.420	< 0.001***	3.955	1.048	3.775	<0.001***
	Fusobacteria	0.056	0.646	0.087	0.931	0.034	1.048	0.033	0.974
	Gammaproteobacteria	0.619	0.646	0.957	0.339	1.169	1.048	1.115	0.265

warm climate on gut microbiome diversity across years in connected mesocosms. Instead, we observed a strong positive effect in year 3 (RI = 1, *p*-value = 0.010; table 1). Moreover, we found no significant effects of age, sex and body length in models.

Most OTUs belonged to Firmicutes, Proteobacteria (mostly Gamma-, Delta- and Alphaproteobacteria), Bacteroidetes, Actinobacteria, Fusobacteria and Epsilonbacteraeota (electronic supplementary material, figure S1). Firmicutes was the only clade whose diversity was strongly affected by climate and connectivity treatments and was likely responsible for the diversity patterns observed for the whole community (table 2, electronic supplementary material, figure S2)

(b) Lizard gut composition over years

We found overall weak effects of the climatic and connectivity treatments on the bacterial community composition (PERMANOVA R^2 values < 1.5%; electronic supplementary material, table S2 and electronic supplementary material, method and results) suggesting either stronger effects of unmeasured biotic/abiotic parameters, or of stochastic assembly processes. Our null model analysis suggested that both explanations are possible, as 35–38% observed pairwise dissimilarities differed from those expected by chance (electronic supplementary material, figure S3).

We further analysed differences in OTUs abundance. Only a few OTUs were identified by analysis of compositions of microbiomes with bias correction (ANCOM-BC). These were mainly affiliated to Firmicutes (electronic supplementary material, table S3). Yet, analysing the distribution of log fold changes values from present-day to warm climates, on which the ANCOM-BC is based, suggests an accumulation of small non-significant differences in OTUs abundances between climate treatments across years. Indeed, log-fold changes distribution had lower kurtosis in year 1 regardless of the habitat connectivity (electronic supplementary material, figure S4) and exhibited values that were more negative in year 3 for isolated mesocosms and in year 2 and 3 for connected mesocosms.

(c) Host survival, dispersal and microbiome plasticity

Host survival between year t and $t + 1$ was not related to gut diversity at year t neither in present-day nor in warm climates (electronic supplementary material, table S4, figure S5). In accordance with previous study [16], climate-dependent survival differed according to habitat connectivity, with survival decreasing in warm climate in isolated mesocosms while increasing in connected ones and varying over years (electronic supplementary material, table S4).

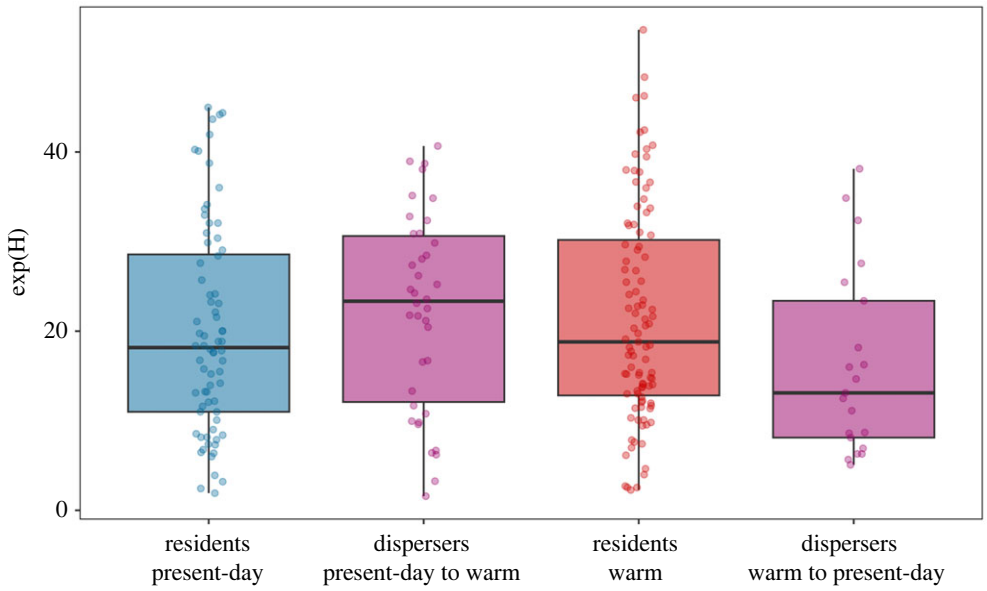

Figure 2. Gut microbiota diversity, calculated as the exponential of Shannon index [exp(H)] depending on dispersal status and climate over the three experimental years. Residents of present-day and warm climates are respectively in blue and red, and dispersers are in purple.

Table 3. Effects of dispersal status, climate treatment, year centred and their interaction on Shannon diversity. Snout–vent length, age and sex with the initial mesocosm were considered as covariates. Interactions and parameters not shown in the table were excluded from models according to AIC procedure. References levels of the factors are present-day climate, alive, adult and female. Interactions and parameters were excluded from models according to AIC procedure. Models explain 7.6% and 8.9% of the marginal and conditional variance explained, respectively. $n = 230$.

parameters	estimate	s.e.	*z*-value	RI	*p*-value
intercept	19.874	1.760	11.228	1	<0.001***
year 2	−3.722	2.921	1.267	1	0.205
year 3	−4.405	3.387	1.293	1	0.196
warm climate	1.443	2.034	0.706	1	0.480
dispersal status	2.719	2.337	1.157	1	0.247
dispersal status × warm climate	−6.906	3.697	1.858	1	0.063
year 2 × warm climate	1.728	3.754	0.458	1	0.647
year 3 × warm climate	10.508	3.952	2.644	1	0.008**
year 2 × dispersal status	0.693	4.308	0.160	1	0.873
year 3 × dispersal status	1.155	4.380	0.262	1	0.793
age class	−0.871	1.572	0.551	0.59	0.582
sex	−0.275	1.539	0.177	0.56	0.859

We also found that the gut microbiome plasticity, defined here as the intra-individual variation in microbiome diversity between two consecutive years, responded negatively to warm climate, warm climate being included in the best averaged model with a strong relative importance, despite a non-significant *p*-value (RI = 1, *p*-value = 0.476; electronic supplementary material, table S5 and figure S6). Gut microbiome plasticity in diversity varied across years in a similar fashion in both climate treatments (electronic supplementary material, table S5 and figure S6). However, in connected mesocosms, warm climate had a positive effect on gut microbiome plasticity at the end of the experiment, but with a small sample size in the present-day treatment (year 2 to year 3; electronic supplementary material, table S5 and figure S6).

Finally, we found lizards leaving warm climates display a less diverse microbiome than lizards staying in warm climate and conversely for present-day climates (figure 2, table 3). It is supported by the negative interaction between annual dispersal status and climate treatment maintain in the best averaged model with a relative importance of 1 and a marginally significant *p*-value (Table 3).

3. Discussion

The gut microbiome plays a crucial role on host phenotype, health and fitness [20] and is increasingly acknowledged as an essential component of species conservation [46]. However, its response to multiple anthropogenic stressors remains poorly understood assessed

mostly in the short term and in either captivity in artificial conditions, or *in natura* with confounding factors. Here, we investigated the response of the gut microbiome of lizards over 3 years in an experiment manipulating jointly climate and habitat connectivity.

We found that warmer climates reduced the diversity of the gut microbiome by 14% over the 3 experimental years in isolated habitats. However, the connectivity between climate treatments offset or even reversed the climate effects, with an increase in the gut microbiome diversity through time in warm climates. This suggests that connectivity between thermal habitats contributes to mitigating the effects of warming on gut microbiome diversity.

The reduction of gut diversity in warmer isolated conditions is consistent with previous short-term studies [30], including on the common lizard [31]. The present effects explained a slightly lower variance in diversity and varied over time compared to [31]. This difference may lie in the different diversity indices used in [31] and here. Bestion *et al.* [31] used bacterial OTU richness as a measure of diversity, while we used a Shannon index which is less subjected to under-sampling problems [47] and a more robust estimates of diversity from molecular data [48]. So the difference in conclusion could, at least partly lie in the weight given to taxa frequency. Differences in external climatic conditions may also explain the difference of results, the mesocosms being subjected here to inter-seasonal and inter-annual climatic fluctuations, which influence life-history traits and response of lizards to climate warming (environmental data and inter-annual variations are described in [16]). For example, in Pellerin *et al.* [16], the impact of warming on life-history traits varied across years and could be explained by inter-annual variation in climate treatments or/and by lizards adaptation/acclimation to warming [49]. Our results further suggest that the climate effects become stronger in the long term, consistent with another study that showed that the gut microbiome of the slender anole is resilient to warming in the short term but affected in the long term [50]. Both observations highlight that climate effects may progressively settle in time and emphasize the importance of long-term experiments when studying the response of the gut microbiome under climate change.

From an extended phenotype viewpoint, the observed loss of diversity in warmer conditions may either result from a lower survival of lizards harbouring a higher gut diversity, or by temporal changes in gut diversity during the lizard life (i.e. gut microbiome plasticity [3]). We show that changes in the gut microbiome diversity resulted more from plastic changes of microbial diversity than from differential survival.

A higher bacterial diversity index (i.e. Shannon's diversity index) is often associated with positive impacts on host fitness and performances [30–32], favour its own resilience [51] and prevent intestinal dysbiosis [52]. Thus, we could have expected a warming-induced reduction to impair host fitness and heat tolerance. But contrary to our expectations and short-term effects [31], our result suggests that the lower survival of adults [16] and the microbial changes in warmer treatments observed here over three years do not likely result from a relationship between gut microbiome diversity and host survival. This discrepancy can also be explained by differences in diversity metrics used or by temporal variation in climatic conditions, as discussed for diversity changes. The variation in external temperature across years may influence the strength of our climatic treatments and of the relationship between gut diversity and host survival. Moreover, given its correlative nature, this relationship may result from direct effects of diversity on survival or from effects on other traits (as thermal preference or optimal temperature) related to both microbiome and host survival responses to climate. Typically, the impact of warmer climates on the survival of the lizards, whose microbiome is under investigation in the present study has been shown to vary substantially across years [16]. Another possible explanation is that the diversity loss may be buffered by functionally redundant taxa preventing the loss of specific functions central to the host [53,54]. Our functional analysis shows no specific function affected by climate treatments, but many OTUs could not be functionally annotated (see electronic supplementary material, table S7–S8). In addition, we cannot exclude that the phylogenetic resolution of our DNA marker is insufficient to unveil eco-evo dynamics in microbes that would have functional consequences. This would require further functional analyses (e.g. with metagenomics).

Both stochastic and/or selection processes can generate variation in gut microbial composition in a non-exclusive manner [29,55]. Coupling multivariate analyses and null models, we found that our experimental treatments had weak effects on gut microbiome compositional dissimilarity patterns, which were already large between individuals from a same treatment. As such, about 65% of community changes did not differ from random expectation, the remaining dissimilarities out of the null distribution being potentially driven by drift with limited dispersal, or by selection by our treatments and/or unmeasured environmental parameters [28,56]. These results on community dissimilarity patterns contrast with that of diversity, which suggest an effect of our treatment on the community structure, regardless of the community taxonomic composition. For example, climate may affect the gut community carrying capacity (i.e. number of individuals, and hence potential number of species than can be present through a simple sampling process) without selecting specific bacterial clades [57].

Accordingly with the above we could not identify many bacterial OTUs whose abundance significantly changed in warmer climates. This might be due again to the intrinsic high compositional variability of the gut microbiome between lizards at the OTU level, together with the high conservatism of ANCOM-BC to detect subtle differences in abundance between climate treatments, in particular for low-abundance OTUs [58]. More OTUs exhibited small changes in abundance in the third year compared to the previous years, suggesting that small changes in abundance may accumulate over time without significance threshold in the ANCOM-BC. Here again it emphasizes the importance of longer studies when studying the response of the gut composition to climate changes.

Focusing back on more emergent properties of the community that are less likely heterogeneous, here the diversity of each phylum, we found that the decrease of gut diversity in warm climate was mainly driven by Firmicutes. This phylum is characteristic of vertebrate gut microbiome [59], and has been repeatedly found to decrease in abundance and/or diversity under warmer conditions in many species [30,60]. Firmicutes taxa are known to play a key role in the production of easily absorbable short fatty acids in human guts [61], which are involved in mass gain and metabolic efficiency [62,63]. As such, depletion of this clade has been associated with a decrease in the host digestive capacity in the red-backed salamander [32]. This may result from an investment of the host in the maintenance of particular beneficial members of Firmicutes against heat stress at the expense of others [64]. As certain members of Firmicutes, in particular Ruminococcaceae, have been found promoted by high-fat diet in mice [65], this observation might also suggest an increase of metabolic rate and energetic needs of lizards in warm climates [66].

Habitat connectivity buffered the effects of warming on gut diversity in the short term, and even reversed it at the end of the experiment, with higher diversity in warm conditions. Habitat connectivity may have influenced the climate impacts on gut microbiome, through effects on plasticity and/or selection, or through the spatial distribution of lizards according to their microbiome diversity. Corridors between present-day and warm mesocosms may allow lizards to access cooler climatic refugees to avoid at least temporary warming-induced physiological stress and potentially related impacts on gut microbiome. While habitat connectivity indeed reduced and even reversed the negative effects of warming on adult lizards' survival [16], it did not influence the climate-dependent selection or plasticity on microbiome diversity. However, for the final year in present-day climate, the sample size was too limited and prevented us to precisely estimate these mechanisms. Instead, lizards dispersed more from present-day climates to warm climates suggesting an effect of host dispersal on the buffering effect of connectivity on life-history traits and potentially on gut microbiome.

Individuals leaving warm climates for a present-day climate indeed tended to display a less diverse microbiome than individuals staying in warm climates, with a reversed pattern for individuals leaving present-day for warm climates. This dispersal-microbiome association may therefore counteract the negative effect of warming on the gut microbiome diversity and even reverse its effects, as observed in the third year, because immigrants dispersing to warmer climates had more diverse microbiome and emigrants leaving for present-day climates had less diverse ones. The question remains why dispersal behaviour is related to microbiome and why it varies with climatic conditions. Dispersing individuals often display a range of morphological, behavioural or physiological traits that differs from resident individuals (i.e. dispersal syndrome [67,68]) because phenotypic specialization reduces the costs of movements, increases the success of movements or is related to individuals' habitat preferences in heterogeneous landscapes [67,68]. The gut microbiome could be related to climate-dependent dispersal because gut microbiome influences the probability or the success of movements or the habitat choice among thermal habitats. For instance, gut microbiome has been shown to relate to hosts exploratory and cognitive behaviours [69,70] as well as locomotor behaviour [71]. Alternatively gut microbiome can be related to other host phenotypic traits [19] involved in or influencing this climate-dependent host dispersal. Gut microbiome diversity and composition can for example be related to host food preferences, metabolism or thermal performances, traits which can influence climate-dependent dispersal choices, as found for thermal preference in common lizards [43]. The dispersal-traits association could therefore carry along the gut microbiome without being directly related to individual performance in and preference for thermal habitats.

Regardless of the mechanisms, this climate-dependent relationship between dispersal and microbiome may further influence the spatial differentiation of microbiome composition among habitats and microclimates. Dispersal may favour the introduction of taxa and communities homogenization, modifying the strength of stochastic and selective processes [35,72]. Hence, the effects of local selection may be balanced. However, the mechanism at stake highly relies on the community structure and the dispersal rate [73,74]. Here, it appears that the connectivity among heterogeneous thermal habitats altered the effects of warming on gut diversity likely through a link between microbial diversity and climate-dependent dispersal decisions. However, other factors as changes on prey community or changes on lizard's prey preferences with climate [45] and connectivity may influence the lizards gut communities. A remaining objective will be to integrate the climate- and connectivity-dependent effects on all phenotypic traits and by considering jointly several factors internal or external to the host, including reproductive success, social interactions and diet [70,75,76] in a holistic understanding of global change impacts. [23,45]

4. Material and methods

(a) Experimental system and population monitoring

The common lizard (*Zootoca vivipara*) is a small ovoviviparous lacertid lizard widely distributed in Eurasia. In our study system (see below), it hibernates from November to March and mates right after emergence. Females lay approximately five soft-shelled eggs 2 months after mating, and juveniles emerge within one hour after laying. Life stages are juvenile (less than 1 year), yearling (1 to 2 years) and adults (greater than 2 years) for a lifespan of approximately 5 years.

Gut microbiome samples were collected during the experiment described in [16] in semi-natural mesocosms [77] (Metatron, Ariège, France; electronic supplementary material, figure S7). Each mesocosm (10 m × 10 m) is a small ecosystem composed of natural lizard habitat with rocks, logs, small water ponds and naturally occurring dense and diverse communities of plants (45.5 ± 5.2SD species per mesocosm in 2018) and invertebrates (36.2 ± 4.8SD families per mesocosm averaged between 2015 and 2018 [44]). The plant and invertebrate communities are naturally present on the Metatron site and are similar within and outside the mesocosms and between our different treatments [44]. To reduce predation and insure hermeticity, mesocosms are delimited by tarpaulins buried 50 cm into the soil and are covered with insect-proof nets, avoiding lizards to escape [77]. Within each mesocosm, temperature, illuminance and hygrometry are recorded every 30 min and can be manipulated using motor-driven shutters and a sprinkler system. Mesocosms can be connected through 19 m-long corridors, matching this species minimum dispersal distance [78]. The climate can be manipulated through shutters that close automatically when ambient temperature exceeds either 28°C to maintain conditions equivalent to the present-day climate, or 38°C to simulate warm climate [77]. The warm climate obtained is on average 1.4 and 2.6°C warmer (mean and maximal summer daily temperature) than the present-day climate, but these differences vary through time because the temperature within mesocosm depends on outdoor climatic conditions, hence allowing reproducing more realistic conditions with daily, seasonal or inter-annual climatic fluctuations [16]. Our experiment has been set up with 8 pairs of mesocosms (i.e. 16 mesocosms in total) composed of one 'present-day' and one 'warm' climate mesocosm, crossed with two levels of habitat connectivity (electronic supplementary material, figure S7). Four pairs of mesocosms had corridors opened to allow lizard movements between contrasted thermal habitats (connected mesocosms) while corridors remained closed for the four remaining mesocosms (isolated mesocosms).

Lizards used in this experiment were descendants of lizards previously captured in the Cevennes between 2010 and 2012. Populations were initiated in July 2015 (year 0) with 240 adults/yearling and 306 juveniles (10 females, 5 adults males and 19 ± 1 juveniles per mesocosm), matching the structure of natural populations. The genetic and phenotypic composition was homogenized among mesocosms and diversified within mesocosms by spreading juveniles of each family among different mesocosms. Populations were then maintained for three years with

the same procedures repeated each year. Each year in May before the laying period, all lizards were captured, identified, measured for body length (i.e. snout–vent length), weighted, sampled for their microbiome, and maintained in individual terraria in the laboratory. During captivity lizards are fed with two crickets daily and sprayed with water three times a day. Females laid eggs in the terraria and juveniles were immediately isolated from their mother, weighted and marked by toe-clipping to allow longitudinal monitoring. In early July, adults were released back into their mesocosms and juveniles into their mother's mesocosms where they spent the year until next May. We monitored annual survival status and phenotypic traits (e.g. body length, microbiome) by capturing all the lizards the following year in May through multiple capture sessions until no further lizard was found. Within connected mesocosms, after one year, lizards were recaptured either within the same or in another mesocosm than the previous year, and were classified as residents and dispersers respectively.

(b) Microbiome sampling

Lizard gut microbiome was sampled only on adults and yearling before egg laying and hatching. To sample hindgut bacterial communities, we used a cloacal flushing sampling method. This method allows to easily collect gut bacterial communities in a non-invasive way—a prerequisite for long-term monitoring. Moreover, cloacal sampling are also often considered as relatively good proxy of the hindgut microbiome due to their overall similarity with the lower intestines [79,80]. Prior to sampling, the edges of cloaca were cleaned with alcohol. Samples were then collected by flushing the inside of the hindgut twice with a sterile pipette filled with 50 ml of a sterile saline solution (Phosphate buffer saline, pH 7.4, Sigma) and gently introduced into the cloaca (0.5 mm). At least two flushes were performed on each lizard (range 2–5). Samples were stored at −20°C in sterile 1.5 ml vials. Two types of negative controls of cloacal sampling were also performed: using PBS buffer alone, to check for contaminants in this reagent, and using a saline solution collected with a pipette that remained around 10 s in the open air, to control for local contaminants.

(c) Molecular and bioinformatic analyses

The diversity, composition and structure of the microbiome were studied through amplification by PCR and high-throughput sequencing of the v5–6 region (approx. 250 bp length) of the bacterial 16S rRNA gene. DNA extraction, marker amplification and sequencing protocols were performed as in [31]. Briefly, after a total DNA extraction with the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands), PCR amplification was conducted using the BACTB-F (5′-GGATTAGATACCCTGGTAGT-3′) and BACTB-R (5′-CACGACACGAGCTGACG-3′) primers [81]. Both primers were labelled at their 5′ end with two different 8 nt tags to discriminate PCR reactions. PCR reactions were conducted for each sample in 30 µl containing 3 µl of 1/10 diluted DNA extract, 0.25 µM of each primer, 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 2.5 mM of MgCl2, 1x of Taq Buffer, 0.2 mM of each dNTP and 4 ng of bovine serum albumin (Promega Corporation, Madison, USA). The thermocycling conditions were as follows: 5 min of initial denaturation at 95°C, followed by 35 cycles of denaturation (95°C for 30 s), annealing (57°C for 30 s) and elongation (72°C for 30 s). PCR products were then pooled and purified with the QIAquick PCR purification Kit (Qiagen GmbH, Hilden, Germany). A library multiplexing all amplicons was prepared with Fasteris' MetaFast protocol, and included sampling, extraction and PCR negative controls. The library was sequenced on an Illumina MiSeq platform with the 2*250 paired-end chemistry at Fasteris SA (Plan-les-Ouates, Switzerland).

In total, we obtained 11 359 947 sequencing paired-end reads that we processed bioinformatically similarly to [31], using the `OBITools` package [82]. Briefly, paired-end reads were assembled accounting for sequences quality and assigned to their respective samples by authorizing no errors on the tag sequences, and no more than 2 mismatches on the primer sequences. After reads dereplication and exclusion of low-quality sequences (i.e. of length <70 bp, containing ambiguous bases, or being singletons), the remaining sequences were clustered into OTUs (Operational Taxonomic Units) using the sumaclust algorithm with a 97% similarity threshold [83]. We chose an OTU approach over an Amplicon Sequence Variance one (ASV) primarily because the biological relevance of ASVs has been questioned due to the intra-genomic variation of the 16S, a feature that has less impact when working with OTU-based approaches [84]. In addition, ASVs and OTUs tend to yield similar diversity trends, especially when down-weighting rare taxa as done here [85–87] (see below).

We used the SILVA database (release r132) and the taxonomic assignment tool from the SILVAngs pipeline [88] to assign each OTU a taxon, using default parameters. Taxonomic assignments with probability <80% were considered as unreliable. Finally, we used the `metabaR` R package [89] to curate the data from contaminants and potential tag-jumps based on all experimental blank controls, to exclude sequences assigned to chloroplasts or mitochondria, and to inspect the final dataset quality. At the end, the final data set included 10 017 573 reads, 7778 OTUs for 860 lizards sampled.

(d) Statistical analysis

(i) General statistical methodology

Statistical analyses were performed using R (v. 4.0.3, R Core Team 2020), and mainly consisted of linear mixed models and the following steps. First, using the `lme4` package [90], we built a full model including (i) climate treatment and habitat connectivity, the year and their interactions as fixed effects, (ii) age class, sex and snout–vent length as covariates, as these traits are influenced by climate treatment and habitat connectivity and are known drivers of survival and dispersal [16,31], and (iii) lizards and mesocosm identities as random intercepts. To interpret estimates of main climate and connectivity effects across years, years were treated as a categorical variable and then centred as described by [91]. Indeed, the inclusion of interactions in a model prevents from interpreting mean/simple effects of variables/factors involved in the interactions. For example, in a model with an interaction between years and climate treatment, the simple effect of climate treatment is estimated for a single year (i.e. the intercept year) and not across all years. To estimate the mean climate effects across all years, years should be centred as described by [91]. To this end, binomial variables (coded as 0 or 1) were created for each year (year 1, year 2 and year 3). For example, an individual sampled in year 2 was coded 0 for year 1 and year 3 variables and coded 1 for the year 2 variable. The variables for year 1, 2 and 3 were then centred by subtracting the mean value of each year variable. Models could then include the variables for each year and their interaction with treatments, allowing us to interpret simple effects of treatments on top of their year-specific effects. Note that only variables for year 2 and 3 were included, because the effect of year 1 variable is redundant with the additive effects of variables for year 2 and 3 together. The year 0 was before treatments and was hence not included in the analyses. All possible candidate models with the same random structure, from full to intercept only, were ranked by AIC and averaged for models with $\Delta AIC < 2$ [92]. Conditional estimates, standard errors, z-value, the relative importance of variables (RI) and p-values of variables kept in best averaged models were obtained using `MuMIn` package [93]. Normality and homoscedasticity were checked graphically

on residuals. When the interaction between climate treatment and connectivity was maintained in the best averaged model, we ran separate models for each connectivity treatment. We did so to assess more directly the effect of climate across years in each connectivity conditions, as the full model yielded dealing with triple interactions that are too challenging to interpret. Each computed model is summarized in electronic supplementary material, table S6.

(ii) Lizard gut diversity over years

We first quantified the diversity of the gut microbiome for each lizard at each sampling year with Hill numbers [94,95]. Rarefaction curves indicated a good coverage of sample diversity, in particular for $q = 1$, which corresponds to the exponential of Shannon index (exp(H); electronic supplementary material, figure S8). This index further down weights the impact of potential remaining rare molecular artefacts in the dataset [48], as well as of insufficient sampling [47]. We hence used this index to quantify OTUs diversity using the `vegan` R package [96]. We ensured that climate treatment and habitat connectivity had no effects on the gut microbiome diversity in year 0 at the beginning of the experiment (electronic supplementary material, figure S9), and then tested for these effects over the experimental years (electronic supplementary material, table S6). We also tested for same effects on the diversity within the top 7 most abundant bacterial clades (electronic supplementary material, table S6). We further ran contrast analyses between climate treatments and clades with a Bonferroni correction for multiple testing.

(iii) Lizard gut composition over year

We investigated what OTUs differed between climate treatments and habitat connectivity and years with an ANCOM-BC [97] (electronic supplementary material, table S6).

We complemented the above analyses with a null model approach [56] to assess whether changes in community composition resulted from stochastic processes rather than deterministic ones caused by unmeasured parameters [98]. For each sample, we resampled a fixed number of reads, as defined by the rarefaction analysis, from the whole experiment meta-community while maintaining the sample observed richness [56]. This procedure was repeated 999 times, hence producing a distribution of pairwise Bray–Curtis dissimilarities under null expectations. Overall deviation of the distribution of observed dissimilarities from that of null expectation was assessed using the overlap coefficient (shared area under both density curves).

(iv) Microbiome-dependent host survival, dispersal and microbiome plasticity

Considering gut microbiome as the host's extended phenotype, we first studied whether climate-induced changes of microbiome resulted from differential selection. More specifically, we studied the relationship between lizard survival and gut diversity (i.e. selection-like process), changes in gut diversity over a lizard lifetime (i.e. plasticity-like process), and the relationship between lizard dispersal and gut diversity. First, we analysed the effect of gut microbiome diversity at year t on lizards' survival until year $t + 1$ (i.e. annual survival). We considered three time periods for survival: year 0 to year 1, year 1 to year 2 and year 2 to year 3 (electronic supplementary material, table S6).

Second, we studied whether the gut microbiome plasticity could explain the observed effects of climate on the gut microbiome. To this end, we first defined as 'plasticity' the extent to which the gut microbiome in lizards differ between two consecutive years (i.e. survivors only) by calculating the difference of diversity values (i.e. exp(H)) between a given year and the preceding one. We then analysed how this parameter varied between warm and present-day treatments (electronic supplementary material, table S6).

Finally, we investigated how dispersal could explain the effect of climate on the gut microbial diversity. In common lizards, dispersal mostly occurs during the first year of life [99], but the small size of juveniles prevent their microbiome to be sampled. To consider all lizards, including juveniles, we studied the relationship between the dispersal status from year t to year $t + 1$ and gut microbiome diversity at year $t + 1$ (electronic supplementary material, table S6).

Ethics. The Station d'Ecologie Theorique et Experimentale has a national agreement for use of animals in the laboratory (number B09583). Experiments were made in accordance with French ethics regulations (ethics permit numbers APAFIS#15897-2018070615164391 v3 and APAFIS#19523-201902281559649 v3). Lizards were initially captured in the wild under licence number 2010-189-16 DREAL and 2013-274-0002.

Data accessibility. R1 and R2 fastq files of raw data are available on Dryad [100]. R script and data used in the present manuscript are also available on Zenodo [101].

Supplementary material is available online [102].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. E.F.: conceptualization, data curation, formal analysis, methodology, software, visualization, writing—original draft, writing—review and editing; L.Z.: conceptualization, data curation, formal analysis, methodology, software, supervision, validation, writing—original draft, writing—review and editing; F.P.: data curation, investigation, methodology; L.D.G.: data curation, investigation, methodology, project administration, resources; S.J.: data curation, methodology, writing—review and editing; L.W.: investigation, methodology, writing—review and editing; R.A.: methodology, writing—review and editing; N.P.: methodology, resources; A.I.: methodology, resources; J.W.: methodology, conceptualization, writing—review and editing; E.B.: data curation, methodology, writing—review and editing; J.C.: conceptualization, data curation, funding acquisition, investigation, methodology, project administration, supervision, validation, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

1. Barnosky AD *et al.* 2011 Has the Earth's sixth mass extinction already arrived? *Nature* **471**, 51–57. (doi:10.1038/nature09678)
2. Suggitt AJ *et al.* 2018 Extinction risk from climate change is reduced by microclimatic buffering. *Nat. Clim. Change* **8**, 713–717. (doi:10.1038/s41558-018-0231-9)
3. Merilä J, Hendry AP. 2014 Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl* **7**, 1–14. (doi:10.1111/eva.12137)
4. Kelly M. 2019 Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Phil. Trans. R. Soc. B* **374**, 20180176. (doi:10.1098/rstb.2018.0176)
5. Massot M, Clobert J, Ferrière R. 2008 Climate warming, dispersal inhibition and extinction risk: climate warming and dispersal inhibition. *Glob. Change Biol.* **14**, 461–469. (doi:10.1111/j.1365-2486.2007.01514.x)
6. Crispo E. 2008 Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *J. Evol. Biol.* **21**, 1460–1469. (doi:10.1111/j.1420-9101.2008.01592.x)
7. Cote J, Bestion E, Jacob S, Travis J, Legrand D, Baguette M. 2017 Evolution of dispersal strategies and dispersal syndromes in fragmented landscapes. *Ecography* **40**, 56–73. (doi:10.1111/ecog.02538)
8. Fahrig L. 2007 Non-optimal animal movement in human-altered landscapes. *Funct. Ecol.* **21**, 1003–1015. (doi:10.1111/j.1365-2435.2007.01326.x)
9. Heller NE, Zavaleta ES. 2009 Biodiversity management in the face of climate change: A review of 22 years of recommendations. *Biol. Conserv.* **142**, 14–32. (doi:10.1016/j.biocon.2008.10.006)
10. Lavergne S, Mouquet N, Thuiller W, Ronce O. 2010 Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. *Annu. Rev. Ecol. Evol. Syst.* **41**, 321–350. (doi:10.1146/annurev-ecolsys-102209-144628)
11. Legrand D, Cote J, Fronhofer EA, Holt RD, Ronce O, Schtickzelle N, Travis JM, Clobert J. 2017 Eco-evolutionary dynamics in fragmented landscapes. *Ecography* **40**, 9–25. (doi:10.1111/ecog.02537)
12. Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC. 2008 Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* **320**, 800–803. (doi:10.1126/science.1157174)
13. Massot M, Legendre S, Fédérici P, Clobert J. 2017 Climate warming: a loss of variation in populations can accompany reproductive shifts. *Ecol. Lett.* **20**, 1140–1147. (doi:10.1111/ele.12811)
14. Dillon ME, Wang G, Huey RB. 2010 Global metabolic impacts of recent climate warming. *Nature* **467**, 704–706. (doi:10.1038/nature09407)
15. Sheridan JA, Bickford D. 2011 Shrinking body size as an ecological response to climate change. *Nat. Clim. Change* **1**, 401–406. (doi:10.1038/nclimate1259)
16. Pellerin F, Bestion E, Winandy L, Di Gesu L, Richard M, Aguilée R, Cote J. 2022 Connectivity among thermal habitats buffers the effects of warm climate on life-history traits and population dynamics. *J. Anim. Ecol.* **91**, 1365–2656. (doi:10.1111/1365-2656.13814)
17. Tuff KT, Tuff T, Davies KF. 2016 A framework for integrating thermal biology into fragmentation research. *Ecol. Lett.* **19**, 361–374. (doi:10.1111/ele.12579)
18. Latimer CE, Cooper SJ, Karasov WH, Zuckerberg B. 2018 Does habitat fragmentation promote climate-resilient phenotypes? *Oikos* **127**, 1069–1080. (doi:10.1111/oik.05111)
19. Alberdi A, Aizpurua O, Bohmann K, Zepeda-Mendoza ML, Gilbert MTP. 2016 Do vertebrate gut metagenomes confer rapid ecological adaptation? *Trends Ecol. Evol.* **31**, 689–699. (doi:10.1016/j.tree.2016.06.008)
20. Macke E, Tasiemski A, Massol F, Callens M, Decaestecker E. 2017 Life history and eco-evolutionary dynamics in light of the gut microbiota. *Oikos* **126**, 508–531. (doi:10.1111/oik.03900)
21. Hunter P. 2018 The revival of the extended phenotype: after more than 30 years, Dawkins' extended phenotype hypothesis is enriching evolutionary biology and inspiring potential applications. *EMBO Rep.* **19**, e46477. (doi:10.15252/embr.201846477)
22. Henry LP, Bruijning M, Forsberg SKG, Ayroles JF. 2021 The microbiome extends host evolutionary potential. *Nat. Commun.* **12**, 5141. (doi:10.1038/s41467-021-25315-x)
23. Kolodny O, Schulenburg H. 2020 Microbiome-mediated plasticity directs host evolution along several distinct time scales. *Phil. Trans. R. Soc. B* **375**, 20190589. (doi:10.1098/rstb.2019.0589)
24. Baldassarre L, Ying H, Reitzel AM, Franzenburg S, 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)
25. Fontaine SS, Mineo PM, Kohl KD. 2022 Experimental manipulation of microbiota reduces host thermal tolerance and fitness under heat stress in a vertebrate ectotherm. *Nat. Ecol. Evol.* **6**, 405–417. (doi:10.1038/s41559-022-01686-2)
26. Fontaine SS, Kohl KD. 2023 Ectotherm heat tolerance and the microbiome: current understanding, future directions and potential applications. *J. Exp. Biol.* **226**, jeb245761. (doi:10.1242/jeb.245761)
27. Reese AT, Dunn RR. 2018 Drivers of microbiome biodiversity: a review of general rules, feces, and ignorance. *mBio* **9**, 10–128. (doi:10.1128/mBio.01294-18)
28. Vellend M. 2010 Conceptual synthesis in community ecology. *Q Rev. Biol.* **85**, 183–206. (doi:10.1086/652373)
29. Kohl KD. 2020 Ecological and evolutionary mechanisms underlying patterns of phylosymbiosis in host-associated microbial communities. *Phil. Trans. R. Soc. B* **375**, 20190251. (doi:10.1098/rstb.2019.0251)
30. Sepulveda J, Moeller AH. 2020 The effects of temperature on animal gut microbiomes. *Front. Microbiol.* **11**, 384. (doi:10.3389/fmicb.2020.00384)
31. Bestion E, Jacob S, Zinger L, Di Gesu L, Richard M, White J, Cote J. 2017 Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. *Nat. Ecol. Evol.* **1**, 0161. (doi:10.1038/s41559-017-0161)
32. Fontaine SS, Novarro AJ, Kohl KD. 2018 Environmental temperature alters the digestive performance and gut microbiota of a terrestrial amphibian. *J. Exp. Biol.* **221**, jeb187559. (doi:10.1242/jeb.187559)
33. Rosenberg E, Zilber-Rosenberg I. 2018 The hologenome concept of evolution after 10 years. *Microbiome* **6**, 78. (doi:10.1186/s40168-018-0457-9)
34. Moeller AH, Sanders JG. 2020 Roles of the gut microbiota in the adaptive evolution of mammalian species. *Phil. Trans. R. Soc. B* **375**, 20190597. (doi:10.1098/rstb.2019.0597)
35. Custer GF, Bresciani L, Dini-Andreote F. 2022 Ecological and evolutionary implications of microbial dispersal. *Front Microbiol* **13**, 855859. (doi:10.3389/fmicb.2022.855859)
36. Weinhold A. 2022 Bowel movement: integrating host mobility and microbial transmission across host taxa. *Front Microbiol* **13**, 826364. (doi:10.3389/fmicb.2022.826364)
37. Miller ET, Svanbäck R, Bohannan BJM. 2018 Microbiomes as metacommunities: understanding host-associated microbes through metacommunity ecology. *Trends Ecol. Evol.* **33**, 926–935. (doi:10.1016/j.tree.2018.09.002)
38. Chen S, Holyoak M, Liu H, Bao H, Ma Y, Dou H, Li G, Roberts NJ, Jiang G. 2022 Global warming responses of gut microbiota in moose (*Alces alces*) populations with different dispersal patterns. *Journal of Zoology* **318**, jzo.12998. (doi:10.1111/jzo.12998)
39. Kohl KD, Yahn J. 2016 Effects of environmental temperature on the gut microbial communities of tadpoles. *Environ. Microbiol.* **18**, 1561–1565. (doi:10.1111/1462-2920.13255)

40. Mony C, Uroy L, Khalfallah F, Haddad N, Vandenkoornhuyse P. 2022 Landscape connectivity for the invisibles. *Ecography* **2022**, e06041. (doi:10.1111/ecog.06041)
41. Intergovernmental Panel on Climate Change. 2021 *Climate change 2021: the physical science basis. Contribution of Working Gooup I to the sixth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.
42. Le Galliard JF, Marquis O, Massot M. 2010 Cohort variation, climate effects and population dynamics in a short-lived lizard: cohort effects in a short-lived lizard. *J. Anim. Ecol.* **79**, 1296–1307. (doi:10.1111/j.1365-2656.2010.01732.x)
43. Bestion E, Clobert J, Cote J. 2015 Dispersal response to climate change: scaling down to intraspecific variation. *Ecol. Lett.* **18**, 1226–1233. (doi:10.1111/ele.12502)
44. Bestion E, Teyssier A, Richard M, Clobert J, Cote J. 2015 Live fast, fie young: experimental evidence of population extinction risk due to climate change. *PLoS Biol.* **13**, e1002281. (doi:10.1371/journal.pbio.1002281)
45. Bestion E *et al.* 2019 Altered trophic interactions in warming climates: consequences for predator diet breadth and fitness. *Proc R Soc B* **286**, 20192227. (doi:10.1098/rspb.2019.2227)
46. Trevelline BK, Fontaine SS, Hartup BK, Kohl KD. 2019 Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proc. R. Soc. B* **286**, 20182448. (doi:10.1098/rspb.2018.2448)
47. Haegeman B, Hamelin J, Moriarty J, Neal P, Dushoff J, Weitz JS. 2013 Robust estimation of microbial diversity in theory and in practice. *ISME J.* **7**, 1092–1101. (doi:10.1038/ismej.2013.10)
48. Calderón-Sanou I, Münkemüller T, Boyer F, Zinger L, Thuiller W. 2020 From environmental DNA sequences to ecological conclusions: how strong is the influence of methodological choices? *J. Biogeogr.* **47**, 193–206. (doi:10.1111/jbi.13681)
49. Bestion E, San-Jose LM, Di Gesu L, Richard M, Sinervo B, Côte J, Calvez O, Guillaume O, Cote J. 2023 Plastic responses to warmer climates: a semi-natural experiment on lizard populations. *Evolution* **77**, 1634–1646. (doi:10.1093/evolut/qpad070)
50. Williams CE *et al.* 2022 Sustained drought, but not short-term warming, alters the gut microbiomes of wild *Anolis* lizards. *Appl. Environ. Microbiol.* **88**, e00530-22. (doi:10.1128/aem.00530-22)
51. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. 2012 Diversity, stability and resilience of the human gut microbiota. *Nature* **489**, 220–230. (doi:10.1038/nature11550)
52. Belkaid Y, Hand TW. 2014 Role of the microbiota in immunity and inflammation. *Cell* **157**, 121–141. (doi:10.1016/j.cell.2014.03.011)
53. Rosenfeld JS. 2002 Functional redundancy in ecology and conservation. *Oikos* **98**, 156–162. (doi:10.1034/j.1600-0706.2002.980116.x)
54. Zhang Z, Lu Y, Wei G, Jiao S. 2022 Rare species-driven diversity–ecosystem multifunctionality relationships are promoted by stochastic community assembly. *mBio* **13**, e00449-22. (doi:10.1128/mbio.00449-22)
55. Sieber M *et al.* 2019 Neutrality in the metaorganism. *PLoS Biol.* **17**, e3000298. (doi:10.1371/journal.pbio.3000298)
56. Stegen JC, Lin X, Konopka AE, Fredrickson JK. 2012 Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* **6**, 1653–1664. (doi:10.1038/ismej.2012.22)
57. Storch D, Okie JG. 2019 The carrying capacity for species richness. *Global Ecol. Biogeogr.* **28**, 1519–1532. (doi:10.1111/geb.12987)
58. Nearing JT *et al.* 2022 Microbiome differential abundance methods produce different results across 38 datasets. *Nat. Commun.* **13**, 342. (doi:10.1038/s41467-022-28034-z)
59. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. 2008 Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* **6**, 776–788. (doi:10.1038/nrmicro1978)
60. Huus KE, Ley RE. 2021 Blowing hot and cold: body temperature and the microbiome. *mSystems* **6**, e00707-21. (doi:10.1128/mSystems.00707-21)
61. Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. 2020 The controversial role of human gut Lachnospiraceae. *Microorganisms* **8**, 573. (doi:10.3390/microorganisms8040573)
62. Liao XD, Ma G, Cai J, Fu Y, Yan XY, Wei XB, Zhang RJ. 2015 Effects of *Clostridium butyricum* on growth performance, antioxidation, and immune function of broilers. *Poult. Sci.* **94**, 662–667. (doi:10.3382/ps/pev038)
63. Zheng A *et al.* 2016 Probiotic (Enterococcus faecium) induced responses of the hepatic proteome improves metabolic efficiency of broiler chickens (*Gallus gallus*). *BMC Genom.* **17**, 89. (doi:10.1186/s12864-016-2371-5)
64. Earle KA, Billings G, Sigal M, Lichtman JS, Hansson GC, Elias JE, Amieva MR, Huang KC, Sonnenburg JL. 2015 Quantitative imaging of gut microbiota spatial organization. *Cell Host Microbe* **18**, 478–488. (doi:10.1016/j.chom.2015.09.002)
65. Kim K-A, Gu W, Lee I-A, Joh E-H, Kim D-H. 2012 High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. *PLoS ONE* **7**, e47713. (doi:10.1371/journal.pone.0047713)
66. Liu W, Yang J, Meng Y, Wu D, Cui L, Li T. 2022 The divergent effects of moderate climate warming on the gut microbiota and energetic state of cold-climate lizards from open and semi-closed microhabitats. *Front. Microbiol.* **13**, 1050750. (doi:10.3389/fmicb.2022.1050750)
67. Clobert J, Le Galliard J-F, Cote J, Meylan S, Massot M. 2009 Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecol. Lett.* **12**, 197–209. (doi:10.1111/j.1461-0248.2008.01267.x)
68. Edelaar P, Siepielski AM, Clobert J. 2008 Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. *Evolution* **62**, 2462–2472. (doi:10.1111/j.1558-5646.2008.00459.x)
69. Bruce-Keller AJ, Salbaum JM, Luo M, Blanchard E, Taylor CM, Welsh DA, Berthoud HR. 2015 Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biol. Psychiatry* **77**, 607–615. (doi:10.1016/j.biopsych.2014.07.012)
70. Ezenwa VO, Gerardo NM, Inouye DW, Medina M, Xavier JB. 2012 Animal behavior and the microbiome. *Science* **338**, 198–199. (doi:10.1126/science.1227412)
71. Schretter CE. 2020 Links between the gut microbiota, metabolism, and host behavior. *Gut Microbes* **11**, 245–248. (doi:10.1080/19490976.2019.1643674)
72. Mouquet N, Loreau M. 2003 Community patterns in source-sink metacommunities. *Am. Nat.* **162**, 544–557. (doi:10.1086/378857)
73. Leibold MA *et al.* 2004 The metacommunity concept: a framework for multi-scale community ecology. *Ecol. Lett.* **7**, 601–613. (doi:10.1111/j.1461-0248.2004.00608.x)
74. Evans S, Martiny JBH, Allison SD. 2017 Effects of dispersal and selection on stochastic assembly in microbial communities. *ISME J.* **11**, 176–185. (doi:10.1038/ismej.2016.96)
75. Münger E, Montiel-Castro AJ, Langhans W, Pacheco-López G. 2018 Reciprocal interactions between gut microbiota and host social behavior. *Front. Integr. Neurosci.* **12**, 21. (doi:10.3389/fnint.2018.00021)
76. Soriano EL, Ramírez DT, Araujo DR, Gómez-Gil B, Castro LI, Sánchez CG. 2018 Effect of temperature and dietary lipid proportion on gut microbiota in yellowtail kingfish *Seriola lalandi* juveniles. *Aquaculture* **497**, 269–277. (doi:10.1016/j.aquaculture.2018.07.065)
77. Legrand D *et al.* 2012 The Metatron: an experimental system to study dispersal and metaecosystems for terrestrial organisms. *Nat. Methods* **9**, 828–833. (doi:10.1038/nmeth.2104)

78. Clobert J, Massot M, Lecomte J, Sorci G, de Fraipont M, Barbault R. 1994 Determinants of dispersal behavior: the common lizard as a case study. In *Lizard ecology* (eds LJ Vitt, ER Pianka), pp. 183–206. Princeton, NJ: Princeton University Press.
79. Colston TJ, Noonan BP, Jackson CR. 2015 Phylogenetic analysis of bacterial communities in different regions of the gastrointestinal tract of agkistrodon piscivorus, the Cottonmouth Snake. *PLoS ONE* **10**, e0128793. (doi:10.1371/journal.pone.0128793)
80. Bunker ME, Martin MO, Weiss SL. 2022 Recovered microbiome of an oviparous lizard differs across gut and reproductive tissues, cloacal swabs, and faeces. *Mol. Ecol. Resour.* **22**, 1693–1705. (doi:10.1111/1755-0998.13573)
81. Fliegerova K *et al.* 2014 Effect of DNA extraction and sample preservation method on rumen bacterial population. *Anaerobe* **29**, 80–84. (doi:10.1016/j.anaerobe.2013.09.015)
82. Boyer F, Mercier C, Bonin A, Le Bras Y, Taberlet P, Coissac E. 2016 obitools : a unix -inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* **16**, 176–182. (doi:10.1111/1755-0998.12428)
83. Mercier C, Boyer F, Bonin A, Coissac E. 2013 SUMATRA and SUMACLUST: fast and exact comparison and clustering of sequences. In *Programs and abstracts of the SeqBio 2013 workshop. Abstract*, pp. 27–29. Citesser.
84. Johnson JS *et al.* 2019 Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat. Commun.* **10**, 5029. (doi:10.1038/s41467-019-13036-1)
85. Glassman SI, Martiny JBH. 2018 Broadscale ecological patterns are robust to use of exact sequence variants versus operational taxonomic units. *mSphere* **3**, e00148-18. (doi:10.1128/mSphere.00148-18)
86. Nearing JT, Douglas GM, Comeau AM, Langille MGI. 2018 Denoising the Denoisers: an independent evaluation of microbiome sequence error-correction approaches. *PeerJ* **6**, e5364. (doi:10.7717/peerj.5364)
87. Caruso V, Song X, Asquith M, Karstens L. 2019 Performance of microbiome sequence inference methods in environments with varying biomass. *mSystems* **4**, e00163-18. (doi:10.1128/mSystems.00163-18)
88. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2012 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)
89. Zinger L, Lionnet C, Benoiston A, Donald J, Mercier C, Boyer F. 2021 metabaR: an r package for the evaluation and improvement of DNA metabarcoding data quality. *Methods Ecol. Evol.* **12**, 586–592. (doi:10.1111/2041-210X.13552)
90. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Soft* **67**, 1–48. (doi:10.18637/jss.v067.i01)
91. Schielzeth H. 2010 Simple means to improve the interpretability of regression coefficients: *Interpretation of regression coefficients*. *Methods Ecol. Evol.* **1**, 103–113. (doi:10.1111/j.2041-210X.2010.00012.x)
92. Burnham KP, Anderson DR, Huyvaert KP. 2011 AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* **65**, 23–35. (doi:10.1007/s00265-010-1029-6)
93. Bartoń K. 2022 MuMIn: multi-model inference. R package version 1.46.0. See https://CRAN.R-project.org/package=MuMI.
94. Hill MO. 1973 Diversity and evenness: a unifying notation and its consequences. *Ecology* **54**, 427–432. (doi:10.2307/1934352)
95. Chao A, Chiu C-H, Jost L. 2014 Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill numbers. *Annu. Rev. Ecol. Evol. Syst.* **45**, 297–324. (doi:10.1146/annurev-ecolsys-120213-091540)
96. Oksanen J. 2020 vegan: community ecology package. R package version 2.5-7.
97. Lin H, Peddada SD. 2020 Analysis of compositions of microbiomes with bias correction. *Nat. Commun.* **11**, 3514. (doi:10.1038/s41467-020-17041-7)
98. Vellend M, Srivastava DS, Anderson KM, Brown CD, Jankowski JE, Kleynhans EJ, Kraft NJ, Letaw AD. 2014 Assessing the relative importance of neutral stochasticity in ecological communities. *Oikos* **123**, 1420–1430. (doi:10.1111/oik.01493)
99. Galliard JL, Ferrière Clobert J. 2003 Mother–offspring interactions affect natal dispersal in a lizard. *Proc. R. Soc. Lond. B* **270**, 1163–1169. (doi:10.1098/rspb.2003.2360)
100. Fromm E *et al.* 2024 Data from: Warming effects on lizard gut microbiome depend on habitat connectivity. *Dryad Digital Repository*. (doi:10.5061/DRYAD.ZPC866TG1)
101. Fromm E *et al.* 2024 emmafromm/Warming-effects-on-lizard-gut-microbiome-depend-on-habitat-connectivity: Code for: Warming effects on lizard gut microbiome depend on habitat connectivity. *Zenodo*. (doi:10.5281/ZENODO.10829521)
102. Fromm E *et al.* 2024 Warming effects on lizard gut microbiome depend on habitat connectivity. Figshare. (doi:10.6084/m9.figshare.c.7165769)