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Abstract

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 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 to global changes.

Keywords

Climate change – Habitat connectivity – Host-microbiome interactions – Gut microbiome – Dispersal – Dispersal syndrome

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 e.g., 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'

74 short generation time and rapid response to environmental changes [19], suggests that the gut
75 microbiome could play a significant role in the host response to environmental changes [23].
76 For example, manipulating microbiome composition can have effects on host thermal
77 tolerance, fitness, and acclimation to heat stress [24,25]. However, because gut microbial
78 community structures are complex and not necessarily adaptive for their host, the relationship
79 between variations in gut communities and host fitness and their changes with environmental
80 changes need to be clearly established to draw reliable conclusions on the evolutionary
81 consequences of these changes [19,26,27].

82 As for any biological community, the gut microbiome is expected to be shaped by four
83 fundamental assembly processes, namely selection by the host or its biotic/abiotic factors,
84 dispersal, drift, and speciation [28], of which exact nature and relative importance is context
85 or scale dependent [29]. Climate change can positively or negatively affect certain taxa either
86 through environmental selection, due to direct climate effects, and/or climate-induced changes
87 in host condition and physiology. Short-term responses of the gut microbiome diversity and
88 composition to warmer temperatures have been reported in various animals (reviewed in
89 [30]). As found in many vertebrates species [30], they usually translate into a reduction in
90 diversity and/or a reduction of Firmicutes abundance, with potential subsequent negative
91 consequences on host survival and health [31], for instance through a decrease of digestive
92 efficiency or energy assimilation [32]. The gut microbiome composition can also exhibit
93 greater variability among host individuals subjected to thermal stress. This may result from
94 the decreased abundance of some bacteria taxa that usually fill the ecological niche space
95 available in the gut habitat (e.g. in terms of food or adherence sites) and/or actively inhibit
96 opportunistic colonization of the gut from the environmental microbial pool, including
97 pathogens [30]. However, it remains unclear whether these effects remain through the host-

98 generation time scale, since existing studies report only short-term responses (inferior to 1
99 year).

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

114 Host dispersal may thus influence the way the gut microbiome responds to climate change.
115 For example, a regional-scale study of the gut microbiome in isolated vs. dispersing moose
116 populations shows that only isolated populations are influenced by local temperatures, with
117 potential implications in terms of metabolic adaptations [38]. However, such *in natura* studies
118 do not allow to disentangle effects of potential confounding factors covarying with climate
119 and habitat isolation. Experimental studies can circumvent this limitation, but have so far only
120 singularly manipulated the effect of climate change [31,39,40] or connectivity [35,41], hence
121 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 a ~2°C warmer climate, following IPCC's projections for southern Europe in 2080 [42]. 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.,[5,31,43–46]). 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.

Results

Lizard gut diversity over years

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

connectivity became stronger over time, as shown by the triple interaction between climate, connectivity between mesocosms and centered years effects, with a slightly stronger interaction between climate and connectivity for year 2 (RI = 1, p-value = 0.510 in table S1), and a much stronger interaction in year 3 (RI = 1, p-value = 0.005, 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 = 0.249 & 0.402, table 1). By contrast, there was no overall effect of 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 (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, figure S2)

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%, table S2 and supplementary 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 (figure S3).

We further analyzed differences in OTUs abundance. Only a few OTUs were identified by ANCOM-BC. These were mainly affiliated to Firmicutes (table S3). Yet, analyzing the distribution of log fold changes values from present-day to warm climates, on which is based the ANCOM-BC, 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 (figure S4) and exhibited values that were more negative in year 3 for isolated mesocosms and in year 2 and 3 for connected mesocosms.

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 (table S4). 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 (table S4).

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, table S5, figure S6). Gut microbiome plasticity in diversity varied across years in a similar fashion in both climate treatments (table S5, 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, table S5, 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).

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 [47]. 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 condition 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 [48] and a more robust estimates of diversity from molecular data [49]. 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.* 2022 [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 [50]. 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 [51]. 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 [52] and prevent intestinal dysbiosis [53]. 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 [54,55]. Our functional analysis shows no specific function affected by climate treatments, but many OTUs could not be functionally annotated (see 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 with e.g. metagenomics.

Both stochastic and/or selection processes can generate variation in gut microbial composition in a non-exclusive manner [29,56]. 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 [28,57]. 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 [58].

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 [59]. 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 [60], and has been repeatedly found to decrease in abundance and/or diversity under warmer condition in many species [30,61]. Firmicutes taxa are known to play a key role in the production of easily absorbable short fatty acids in human guts [62], which are involved in mass gain and metabolic efficiency [63,64]. 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 [65]. As certain members of Firmicutes, in particular Ruminococcaceae, have been found promoted by high-fat diet in mice [66], this observation might also suggest an increase of metabolic rate and energetic needs of lizards in warm climates [67].

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, [68,69]) because phenotypic specialization reduces the costs of movements, increases the success of movements or is related to individuals' habitat

preferences in heterogeneous landscapes [68,69]. 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 [70,71] as well as locomotor behaviour [72]. 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 [44]. 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 [35,73]. Hence, the effects of local selection may be balanced. However, the mechanism at stake highly relies on the community structure and the dispersal rate [74,75]. 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 [46] 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 [71,76,77] in a holistic understanding of global change impacts. [23,46]

Material and Methods

Experimental system and population monitoring

The common lizard (*Zootoca vivipara*) is a small ovoviviparous lacertid lizard widely distributed in Eurasia (IUCN, 2009). In our study system (see below), it hibernates from November to March and mates right after emergence. Females lay ~ 5 soft-shelled eggs 2 months after mating, and juveniles emerge within one hour after laying. Life stages are juvenile (<1 year), yearling (1 to 2 years) and adults (>2 years) for a lifespan of ~5 years.

Gut microbiome samples were collected during the experiment described in [16] in semi-natural mesocosms, (Metatron, [78], Ariège, France, figure S7). Each mesocosm (10m x 10m) 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, [45]). 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 [45]. 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 [78]. Within each mesocosm, temperature, illuminance and hygrometry are recorded every 30 minutes and can be manipulated using motor-driven shutters and a sprinkler system. Mesocosms can be connected through 19 meter-long corridors, matching this species minimum dispersal distance [79]. 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 [78]. 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

362 temperature within mesocosm depends on outdoor climatic conditions, hence allowing
363 reproducing more realistic conditions with daily, seasonal or inter-annual climatic fluctuations
364 [16]. Our experiment has been set up with 8 pairs of mesocosms (i.e. 16 mesocosms in total)
365 composed of one "present-day" and one "warm" climate mesocosm, crossed with two levels
366 of habitat connectivity (figure S7). Four pairs of mesocosms had corridors opened to allow
367 lizard movements between contrasted thermal habitats ("connected mesocosms") while
368 corridors remained closed for the four remaining mesocosms ("isolated mesocosms").

369 Lizards used in this experiment were descendants of lizards previously captured in the
370 Cevennes between 2010 and 2012 (Licence for capture no. 2010-189-16 DREAL and no.
371 2013-274-0002, Ethical permits for the experimental procedures below: APAFIS#19523-
372 201902281559649 v3). Populations were initiated in July 2015 (Year 0) with 240
373 adults/yearling and 306 juveniles (10 females, 5 adults males and 19 ± 1 juveniles per
374 mesocosm), matching the structure of natural populations. The genetic and phenotypic
375 composition was homogenized among mesocosms and diversified within mesocosms by
376 spreading juveniles of each family among different mesocosms. Populations were then
377 maintained for three years with the same procedures repeated each year. Each year in May
378 before the laying period, all lizards were captured, identified, measured for body length (i.e.,
379 snout-vent length), weighted, sampled for their microbiome, and maintained in individual
380 terraria in the laboratory. During captivity lizards are fed with two crickets daily and sprayed
381 with water three times a day. Females laid eggs in the terraria and juveniles were immediately
382 isolated from their mother, weighted and marked by toe-clipping (Ethical permits:
383 APAFIS#15897-2018070615164391 v3) to allow longitudinal monitoring. Early July, adults
384 were released back into their mesocosms and juveniles into their mother's mesocosms where
385 they spent the year until next May. We monitored annual survival status and phenotypic traits
386 (e.g., body length, microbiome) by capturing all the lizards the following year in May through

multiple captures 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.

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 [80,81]. 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 seconds in the open air, to control for local contaminants.

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 (ca 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'-

411 CACGACACGAGCTGACG-3') primers [82]. Both primers were labelled at their 5' end with
412 two different 8 nt tags to discriminate PCR reactions. PCR reactions were conducted for each
413 sample in 30 µL containing 3 µL of 1/10 diluted DNA extract, 0.25 µM of each primer, 1U of
414 AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 2.5 mM of
415 MgCl2, 1x of Taq Buffer, 0.2mM of each dNTP and 4 ng of bovine serum albumin (Promega
416 Corporation, Madison, USA). The thermocycling conditions were as follows: 5 min of initial
417 denaturation at 95°C, followed by 35 cycles of denaturation (95°C for 30 s), annealing (57°C
418 for 30 s) and elongation (72°C for 30 s). PCR products were then pooled and purified with the
419 QIAquick PCR purification Kit (Qiagen GmbH, Hilden, Germany). A library multiplexing all
420 amplicons was prepared with Fasteris' MetaFast protocol, and included sampling, extraction
421 and PCR negative controls. The library was sequenced on an Illumina MiSeq platform with
422 the 2*250 paired-end chemistry at Fasteris SA (Plan-les-Ouates, Switzerland).

423 In total, we obtained 11,359,947 sequencing paired-end reads that we processed
424 bioinformatically similarly to [31], using the `OBITools` package [83]. Briefly, paired-end
425 reads were assembled accounting for sequences quality and assigned to their respective
426 samples by authorizing no errors on the tag sequences, and no more than 2 mismatches on the
427 primer sequences. After reads dereplication and exclusion of low-quality sequences (i.e., of
428 length < 70 bp, containing ambiguous bases, or being singletons), the remaining sequences
429 were clustered into OTUs (Operational Taxonomic Units) using the sumaclust algorithm with
430 a 97% similarity threshold [84]. We chose an OTU approach over an Amplicon Sequence
431 Variance one (ASV) primarily because the biological relevance of ASVs has been questioned
432 due to the intra-genomic variation of the 16S, a feature that has less impact when working
433 with OTU-based approaches [85]. In addition, ASVs and OTUs tend to yield similar diversity
434 trends, especially when down-weighting rare taxa as done here ([86–88], see below).

We used the SILVA database (release r132) and the taxonomic assignment tool from the SILVAngs pipeline [89] 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 [90] 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.

Statistical analysis

General statistical methodology

Statistical analyses were performed using R (version 4.0.3, R Core Team 2020), and mainly consisted of linear mixed models and the following steps. First, using the `lme4` package [91], 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 year, years were treated as a categorical variable and then centered as described by [92]. 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 centered as described by [92]. 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 centered 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 $\Delta AIC < 2$ [93]. 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 [94]. 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 table S6.

Lizard gut diversity over years

We first quantified the diversity of the gut microbiome for each lizard at each sampling year with Hill numbers [95,96]. Rarefaction curves indicated a good coverage of sample diversity, in particular for q=1, which corresponds to the exponential of Shannon index [exp(H)] (figure S8). This index further down weights the impact of potential remaining rare molecular artifacts in the dataset [49], as well as of insufficient sampling [48]. We hence used this index to quantify OTUs diversity using the `vegan` R package [97]. 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 (figure S9), and then tested for these effects over the

484 experimental years (table S6). We also tested for same effects on the diversity within the top 7
485 most abundant bacterial clades (table S6). We further ran contrast analyses between climate
486 treatments and clades with a Bonferroni correction for multiple testing.

487 Lizard gut composition over year

488 We investigated what OTUs differed between climate treatments and habitat connectivity and
489 years with an Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC,
490 [98], table S6).
491 We complemented the above analyses with a null model approach [57] to assess whether
492 changes in community composition resulted from stochastic processes rather than determinis-
493 tic ones caused by unmeasured parameters [99]. For each sample, we resampled a fixed num-
494 ber of reads, as defined by the rarefaction analysis, from the whole experiment meta-
495 community while maintaining the sample observed richness [57]. This procedure was repeat-
496 ed 999 times, hence producing a distribution of pairwise Bray-Curtis dissimilarities under null
497 expectations. Overall deviation of the distribution of observed dissimilarities from that of null
498 expectation was assessed using the overlap coefficient (shared area under both density
499 curves).

500 Microbiome-dependent host survival, dispersal and microbiome plasticity

501 Considering gut microbiome as the host's extended phenotype, we first studied whether
502 climate-induced changes of microbiome resulted from differential selection. More
503 specifically, we studied the relationship between lizard survival and gut diversity (i.e.,
504 selection-like process), changes in gut diversity over a lizard lifetime (i.e., plasticity-like
505 process), and the relationship between lizard dispersal and gut diversity. First, we analyzed
506 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 (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 analyzed how this parameter varied between warm and present-day treatments (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 [100], 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 $t+1$ (table S6).

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Table 1. Effects of centered 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 condition 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

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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	SE	t-ratio	p-value	Estimate	SE	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

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Table 3. Effects of dispersal status, climate treatment, year centered 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	SE	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

Fig. 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 farm climates each year for isolated and connected mesocosms.

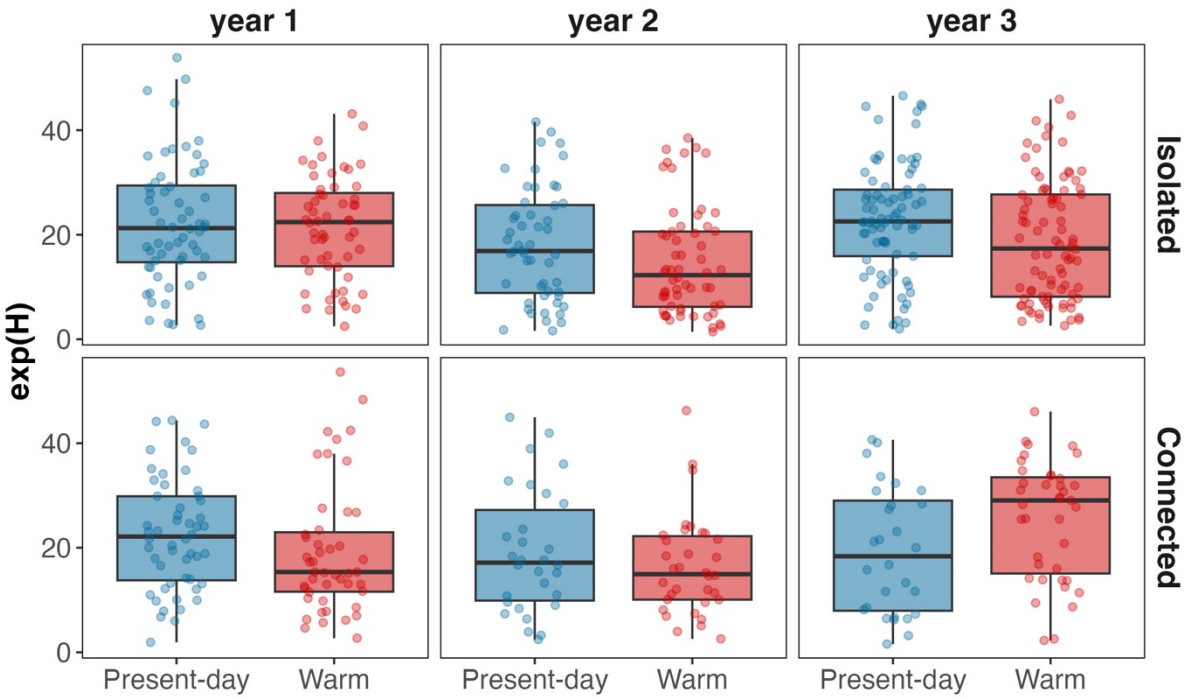
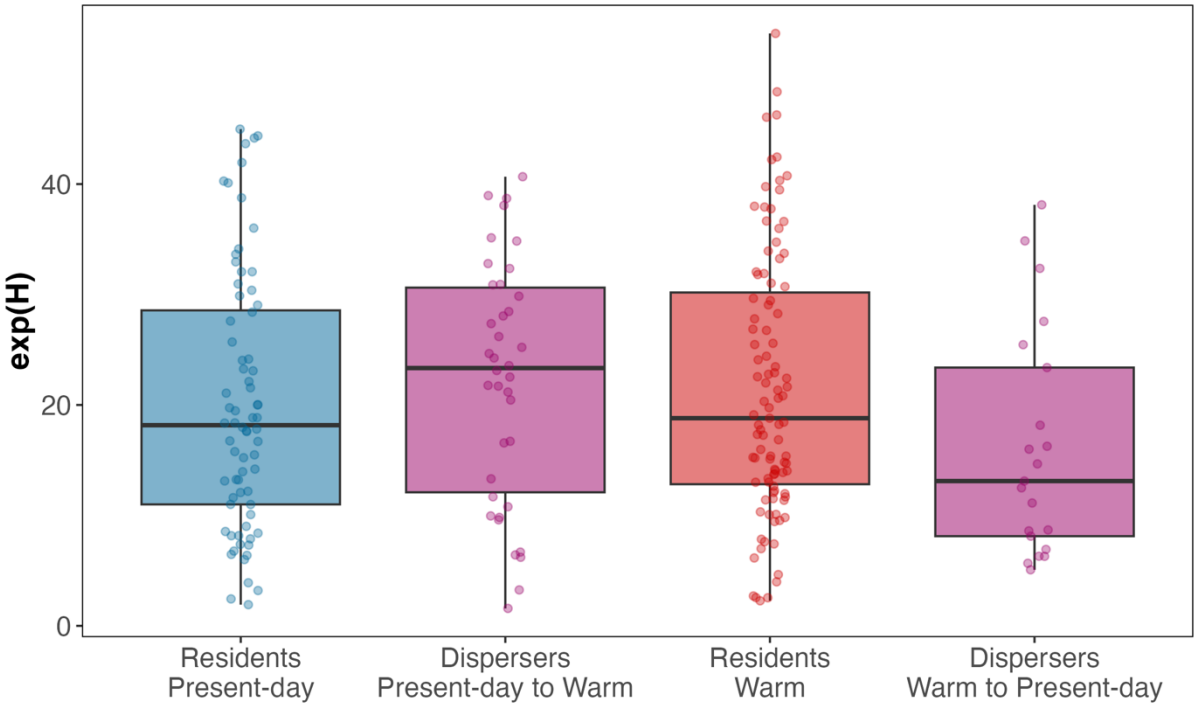


Fig. 2. Gut microbiota diversity, calculated as the exponential of Shannon index [exp(H)] depending on dispersal status and climate over the three experimental years. Residents of present-day and warm climates are respectively in blue and red, and dispersers are in purple.



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