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Warming effects on lizard gut microbiome depend on habitat connectivity

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24 **Abstract**

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

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41

42 **Keywords**

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

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49 **Introduction**

50 Contemporary climate change is a major threat to biodiversity with an expected extinction
51 rate of 15-37% of species by 2050 [1]. Species may respond to climate change through two
52 compensatory processes. First, individuals can avoid extreme climatic conditions by
53 dispersing towards more suitable thermal environments over small distances [2], a process
54 that offsets climate impacts on populations and can lead to species range shifts. Second,
55 species can adjust their phenotype to new environmental conditions through the selection of
56 more adapted phenotypes or through intra- and inter-generational phenotypic plasticity [3,4].
57 Both processes strongly rely on the ability of individuals to disperse. Dispersal controls
58 species movement distances and hence ability to track their shifting habitat [5]. It further
59 influences the genetic composition of a population through individual/gene flows [6].
60 Dispersal is however hampered by the increasing destruction and fragmentation of habitats
61 [7,8]. This reduces species abilities to track their suitable thermal habitats [9] and influences
62 species adaptation to local climate by reducing gene flows [10]. Assessing the effects of
63 dispersal is much more challenging and requires better understanding of the complex
64 interplay of climate and fragmentation for ecological and evolutionary processes [11].

65 A large body of literature already documented phenotypic changes with climate change and
66 habitat fragmentation, including changes in reproduction phenology [12,13], physiology [14]
67 or body size [15,16], as well as their interplay [16–18]. However, a still largely overlooked
68 aspect is the role of host-associated microbiome responses to climate change. In animals, gut
69 microbial symbionts play a key role in many essential host functions and traits related to e.g.,
70 metabolism, nutrition, immunity, behaviour, and morphology [19,20]. By harbouring its own
71 genes and functions, the microbiota can thus extend both the functional and genetic
72 repertoires of the host. The gut microbiome is therefore increasingly considered as the host's
73 “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.

122 Here, we work on the gut microbiome data sampled during the experiment described in [16].
123 This study was built on a previous one year experiment examining the effect of climate
124 change on the gut microbiome of the common lizard, *Zootoca vivipara* [31] to perform a new
125 experiment where we investigated the dependency of climate effects on gut microbiome
126 diversity to habitat connectivity for three years. The experiment is conducted in a semi-natural
127 experimental set-up composed of connected or isolated mesocosms subjected to climate
128 treatments, a present-day climate and a $\sim 2^{\circ}\text{C}$ warmer climate, following IPCC's projections
129 for southern Europe in 2080 [42]. This design allows us to study the impacts of warmer
130 conditions on microbiome when lizards could move between thermal habitats and have access
131 to a cooler microclimate or when they were facing warmer habitats only. The common lizard
132 is a relevant model species to investigate these questions, because the body temperature, vital
133 functions (e.g. nutrition), and a wide range of life history or extended traits (e.g. growth rate,
134 survival, reproduction, dispersal propensity, gut microbiome) in ectotherms depend on
135 external temperatures (e.g., [5,31,43–46]). We expect the gut bacterial diversity to be lower in
136 warmer climate, in particular through a decrease of Firmicutes abundance, as well as changes
137 in compositional similarity among host individuals. We further expect climate effects to be
138 buffered in more connected habitats through the access to more diverse thermal habitats, food
139 resources and microbial species pool, as observed for the impacts on life history traits [16].
140 Finally, using an extended phenotype viewpoint, we studied whether changes in host
141 microbial diversity resulted from host survival, microbiome plasticity and host dispersal.

142 **Results**

143 **Lizard gut diversity over years**

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

146 connectivity became stronger over time, as shown by the triple interaction between climate,
147 connectivity between mesocosms and centered years effects, with a slightly stronger
148 interaction between climate and connectivity for year 2 (RI = 1, p-value = 0.510 in table S1),
149 and a much stronger interaction in year 3 (RI = 1, p-value = 0.005, table S1).

150 In isolated mesocosms, the gut microbiome diversity was 14.4% lower in warm climate across
151 years (table 1). This negative effect was slightly stronger through time. Indeed, the
152 interactions between warm climate and years 2 and 3 were retained in the best model but not
153 significant (RI = 1, p-values = 0.249 & 0.402, table 1). By contrast, there was no overall
154 effect of warm climate on gut microbiome diversity across years in connected mesocosms.
155 Instead, we observed a strong positive effect in year 3 (RI= 1, p-value = 0.010, table 1).
156 Moreover, we found no significant effects of age, sex and body length in models.

157 Most OTUs belonged to Firmicutes, Proteobacteria (mostly Gamma-, Delta- and Alphaproteo-
158 obacteria), Bacteroidetes, Actinobacteria, Fusobacteria and Epsilonbacteraeota (figure S1).
159 Firmicutes was the only clade whose diversity was strongly affected by climate and connec-
160 tivity treatments and was likely responsible for the diversity patterns observed for the whole
161 community (table 2, figure S2)

162 **Lizard gut composition over years**

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

169 We further analyzed differences in OTUs abundance. Only a few OTUs were identified by
170 ANCOM-BC. These were mainly affiliated to Firmicutes (table S3). Yet, analyzing the distri-
171 bution of log fold changes values from present-day to warm climates, on which is based the
172 ANCOM-BC, suggests an accumulation of small non-significant differences in OTUs abun-
173 dances between climate treatments across years. Indeed, log-fold changes distribution had
174 lower kurtosis in year 1 regardless of the habitat connectivity (figure S4) and exhibited values
175 that were more negative in year 3 for isolated mesocosms and in year 2 and 3 for connected
176 mesocosms.

177 **Host survival, dispersal and microbiome plasticity**

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

183 We also found that the gut microbiome plasticity, defined here as the intra-individual varia-
184 tion in microbiome diversity between two consecutive years, responded negatively to warm
185 climate, warm climate being included in the best averaged model with a strong relative im-
186 portance, despite a non-significant p-value (RI = 1, p-value = 0.476, table S5, figure S6). Gut
187 microbiome plasticity in diversity varied across years in a similar fashion in both climate
188 treatments (table S5, figure S6). However, in connected mesocosms, warm climate had a posi-
189 tive effect on gut microbiome plasticity at the end of the experiment, but with a small sample
190 size in the present-day treatment (year 2 to year 3, table S5, figure S6).

191 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).

196 **Discussion**

197 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.

203 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.

208 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

215 given to taxa frequency. Differences in external climatic conditions may also explain the
216 difference of results, the mesocosms being subjected here to inter-seasonal and inter-annual
217 climatic fluctuations, which influence life history traits and response of lizards to climate
218 warming (environmental data and inter-annual variations are described in [16]) . For example,
219 in Pellerin *et al.* 2022 [16], the impact of warming on life history traits varied across years and
220 could be explained by inter-annual variation in climate treatments or/and by lizards
221 adaptation/acclimation to warming [50]. Our results further suggest that the climate effects
222 become stronger in the long term, consistent with another study that showed that the gut
223 microbiome of the slender anole is resilient to warming in the short term but affected in the
224 long-term [51]. Both observations highlight that climate effects may progressively settle in
225 time and emphasize the importance of long-term experiments when studying the response of
226 the gut microbiome under climate change.

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

232 A higher bacterial diversity index (i.e., Shannon's diversity index) is often associated with
233 positive impacts on host fitness and performances [30–32], favour its own resilience [52] and
234 prevent intestinal dysbiosis [53]. Thus, we could have expected a warming-induced reduction
235 to impair host fitness and heat tolerance. But contrary to our expectations and short-term
236 effects [31], our result suggests that the lower survival of adults [16] and the microbial
237 changes in warmer treatments observed here over three years do not likely result from a
238 relationship between gut microbiome diversity and host survival. This discrepancy can also be
239 explained by differences in diversity metrics used or by temporal variation in climatic

240 conditions, as discussed for diversity changes. The variation in external temperature across
241 years may influence the strength of our climatic treatments and of the relationship between
242 gut diversity and host survival. Moreover, given its correlative nature, this relationship may
243 result from direct effects of diversity on survival or from effects on other traits (as thermal
244 preference or optimal temperature) related to both microbiome and host survival responses to
245 climate. Typically, the impact of warmer climates on the survival of the lizards, whose
246 microbiome is under investigation in the present study has been shown to vary substantially
247 across years [16]. Another possible explanation is that the diversity loss may be buffered by
248 functionally redundant taxa preventing the loss of specific functions central to the host
249 [54,55]. Our functional analysis shows no specific function affected by climate treatments, but
250 many OTUs could not be functionally annotated (see table S7-S8). In addition, we cannot
251 exclude that the phylogenetic resolution of our DNA marker is insufficient to unveil eco-evo
252 dynamics in microbes that would have functional consequences. This would require further
253 functional analyses with e.g. metagenomics.

254 Both stochastic and/or selection processes can generate variation in gut microbial composition
255 in a non-exclusive manner [29,56]. Coupling multivariate analyses and null models, we found
256 that our experimental treatments had weak effects on gut microbiome compositional
257 dissimilarity patterns, which were already large between individuals from a same treatment.
258 As such, about 65% of community changes did not differ from random expectation, the
259 remaining dissimilarities out of the null distribution being potentially driven by drift with
260 limited dispersal, or by selection by our treatments and/or unmeasured environmental
261 parameters [28,57]. These results on community dissimilarity patterns contrast with that of
262 diversity, which suggest an effect of our treatment on the community structure, regardless of
263 the community taxonomic composition. For example, climate may affect the gut community

264 carrying capacity (i.e. number of individuals, and hence potential number of species than can
265 be present through a simple sampling process) without selecting specific bacterial clades [58].
266 Accordingly with the above we could not identify many bacterial OTUs whose abundance
267 significantly changed in warmer climates. This might be due again to the intrinsic high
268 compositional variability of the gut microbiome between lizards at the OTU level, together
269 with the high conservatism of ANCOM-BC to detect subtle differences in abundance between
270 climate treatments, in particular for low-abundance OTUs [59]. More OTUs exhibited small
271 changes in abundance in the third year compared to the previous years, suggesting that small
272 changes in abundance may accumulate over time without significance threshold in the
273 ANCOM-BC. Here again it emphasizes the importance of longer studies when studying the
274 response of the gut composition to climate changes.

275 Focusing back on more emergent properties of the community that are less likely
276 heterogeneous, here the diversity of each phylum, we found that the decrease of gut diversity
277 in warm climate was mainly driven by Firmicutes. This phylum is characteristic of vertebrate
278 gut microbiome [60], and has been repeatedly found to decrease in abundance and/or diversity
279 under warmer condition in many species [30,61]. Firmicutes taxa are known to play a key role
280 in the production of easily absorbable short fatty acids in human guts [62], which are involved
281 in mass gain and metabolic efficiency [63,64]. As such, depletion of this clade has been
282 associated with a decrease in the host digestive capacity in the red-backed salamander [32].
283 This may result from an investment of the host in the maintenance of particular beneficial
284 members of Firmicutes against heat stress at the expense of others [65]. As certain members
285 of Firmicutes, in particular Ruminococcaceae, have been found promoted by high-fat diet in
286 mice [66], this observation might also suggest an increase of metabolic rate and energetic
287 needs of lizards in warm climates [67].

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

302 Individuals leaving warm climates for a present-day climate indeed tended to display a less
303 diverse microbiome than individuals staying in warm climates, with a reversed pattern for
304 individuals leaving present-day for warm climates. This dispersal-microbiome association
305 may therefore counteract the negative effect of warming on the gut microbiome diversity and
306 even reverse its effects, as observed in the third year, because immigrants dispersing to
307 warmer climates had more diverse microbiome and emigrants leaving for present-day
308 climates had less diverse ones. The question remains why dispersal behaviour is related to
309 microbiome and why it varies with climatic conditions. Dispersing individuals often display a
310 range of morphological, behavioural or physiological traits that differs from resident
311 individuals (i.e., dispersal syndrome, [68,69]) because phenotypic specialization reduces the
312 costs of movements, increases the success of movements or is related to individuals' habitat

313 preferences in heterogeneous landscapes [68,69]. The gut microbiome could be related to
314 climate-dependent dispersal because gut microbiome influences the probability or the success
315 of movements or the habitat choice among thermal habitats. For instance, gut microbiome has
316 been shown to relate to hosts exploratory and cognitive behaviours [70,71] as well as
317 locomotor behaviour [72]. Alternatively gut microbiome can be related to other host
318 phenotypic traits [19] involved in or influencing this climate-dependent host dispersal. Gut
319 microbiome diversity and composition can for example be related to host food preferences,
320 metabolism or thermal performances, traits which can influence climate-dependent dispersal
321 choices, as found for thermal preference in common lizards [44]. The dispersal-traits
322 association could therefore carry along the gut microbiome without being directly related to
323 individual performance in and preference for thermal habitats.

324 Regardless of the mechanisms, this climate-dependent relationship between dispersal and mi-
325 crobiome may further influence the spatial differentiation of microbiome composition among
326 habitats and microclimates. Dispersal may favour the introduction of taxa and communities
327 homogenization, modifying the strength of stochastic and selective processes [35,73]. Hence,
328 the effects of local selection may be balanced. However, the mechanism at stake highly relies
329 on the community structure and the dispersal rate [74,75]. Here, it appears that the connectivi-
330 ty among heterogeneous thermal habitats altered the effects of warming on gut diversity likely
331 through a link between microbial diversity and climate-dependent dispersal decisions. How-
332 ever, other factors as changes on prey community or changes on lizard's prey preferences
333 with climate [46] and connectivity may influence the lizards gut communities. A remaining
334 objective will be to integrate the climate- and connectivity-dependent effects on all phenotyp-
335 ic traits and by considering jointly several factors internal or external to the host, including
336 reproductive success, social interactions and diet [71,76,77] in a holistic understanding of
337 global change impacts. [23,46]

338 **Material and Methods**

339 **Experimental system and population monitoring**

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

345 Gut microbiome samples were collected during the experiment described in [16] in semi-
346 natural mesocosms, (Metatron, [78], Ariège, France, figure S7). Each mesocosm (10m x 10m)
347 is a small ecosystem composed of natural lizard habitat with rocks, logs, small water ponds
348 and naturally occurring dense and diverse communities of plants ($45.5 \pm 5.2\text{SD}$ species per
349 mesocosm in 2018) and invertebrates ($36.2 \pm 4.8\text{SD}$ families per mesocosm averaged between
350 2015 and 2018, [45]). The plant and invertebrate communities are naturally present on the
351 Metatron site and are similar within and outside the mesocosms and between our different
352 treatments [45]. To reduce predation and insure hermeticity, mesocosms are delimited by
353 tarpaulins buried 50 cm into the soil and are covered with insect-proof nets, avoiding lizards
354 to escape [78]. Within each mesocosm, temperature, illuminance and hygrometry are recorded
355 every 30 minutes and can be manipulated using motor-driven shutters and a sprinkler system.
356 Mesocosms can be connected through 19 meter-long corridors, matching this species
357 minimum dispersal distance [79]. The climate can be manipulated through shutters that close
358 automatically when ambient temperature exceeds either 28°C to maintain conditions
359 equivalent to the present-day climate, or 38°C to simulate warm climate [78]. The warm
360 climate obtained is on average 1.4 and 2.6°C warmer (mean and maximal summer daily
361 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

387 multiple captures sessions until no further lizard was found. Within connected mesocosms,
388 after one year, lizards were recaptured either within the same or in another mesocosm than the
389 previous year and were classified as residents and dispersers respectively.

390 **Microbiome sampling**

391 Lizard gut microbiome was sampled only on adults and yearling before egg laying and
392 hatching. To sample hindgut bacterial communities, we used a cloacal flushing sampling
393 method. This method allows to easily collect gut bacterial communities in a non-invasive way
394 – a prerequisite for long-term monitoring. Moreover, cloacal sampling are also often
395 considered as relatively good proxy of the hindgut microbiome due to their overall similarity
396 with the lower intestines [80,81]. Prior to sampling, the edges of cloaca were cleaned with
397 alcohol. Samples were then collected by flushing the inside of the hindgut twice with a sterile
398 pipette filled with 50 ml of a sterile saline solution (Phosphate buffer saline, pH 7.4, Sigma)
399 and gently introduced into the cloaca (0.5 mm). At least two flushes were performed on each
400 lizard (range 2-5). Samples were stored at -20°C in sterile 1.5 ml vials. Two types of negative
401 controls of cloacal sampling were also performed: using PBS buffer alone, to check for
402 contaminants in this reagent, and using a saline solution collected with a pipette that remained
403 around 10 seconds in the open air, to control for local contaminants.

404 **Molecular and Bioinformatic Analyses**

405 The diversity, composition and structure of the microbiome were studied through
406 amplification by PCR and high-throughput sequencing of the v5-6 region (ca 250 bp length)
407 of the bacterial 16S rRNA gene. DNA extraction, marker amplification and sequencing
408 protocols were performed as in [31]. Briefly, after a total DNA extraction with the Qiagen
409 DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands), PCR amplification was conducted
410 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 μ L containing 3 μ L of 1/10 diluted DNA extract, 0.25 μ M of each primer, 1U of
414 AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 2.5 mM of
415 $MgCl_2$, 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 `sumacust` 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).

435 We used the SILVA database (release r132) and the taxonomic assignment tool from the
436 SILVAngs pipeline [89] to assign each OTU a taxon, using default parameters. Taxonomic
437 assignments with probability < 80% were considered as unreliable. Finally, we used the
438 metabar R package [90] to curate the data from contaminants and potential tag-jumps based
439 on all experimental blank controls, to exclude sequences assigned to chloroplasts or
440 mitochondria, and to inspect the final dataset quality. At the end, the final data set included 10
441 017 573 reads, 7778 OTUs for 860 lizards sampled.

442 **Statistical analysis**

443 General statistical methodology

444 Statistical analyses were performed using R (version 4.0.3, R Core Team 2020), and mainly
445 consisted of linear mixed models and the following steps. First, using the lme4 package [91],
446 we built a full model including (i) climate treatment and habitat connectivity, the year and
447 their interactions as fixed effects, (ii) age class, sex and snout-vent length as covariates, as
448 these traits are influenced by climate treatment and habitat connectivity and are known drivers
449 of survival and dispersal [16,31], and (iii) lizards and mesocosm identities as random
450 intercepts. To interpret estimates of main climate and connectivity effects across year, years
451 were treated as a categorical variable and then centered as described by [92]. Indeed, the
452 inclusion of interactions in a model prevents from interpreting mean/simple effects of
453 variables/factors involved in the interactions. For example, in a model with an interaction
454 between years and climate treatment, the simple effect of climate treatment is estimated for a
455 single year (*i.e.*, the intercept year) and not across all years. To estimate the mean climate
456 effects across all years, years should be centered as described by [92]. To this end, binomial
457 variables (coded as 0 or 1) were created for each year (year 1, year 2 and year 3). For
458 example, an individual sampled in year 2 was coded 0 for year 1 and year 3 variables and

459 coded 1 for the year 2 variable. The variables for year 1, 2 and 3 were then centered by
460 subtracting the mean value of each year variable. Models could then include the variables for
461 each year and their interaction with treatments, allowing us to interpret simple effects of
462 treatments on top of their year-specific effects. Note that only variables for year 2 and 3 were
463 included, because the effect of year 1 variable is redundant with the additive effects of
464 variables for year 2 and 3 together. The year 0 was before treatments and was hence not
465 included in the analyses. All possible candidate models with the same random structure, from
466 full to intercept only, were ranked by AIC and averaged for models with $\Delta AIC < 2$ [93].
467 Conditional estimates, standard errors, z-value, the relative importance of variables (RI) and
468 p-values of variables kept in best averaged models were obtained using MuMIn package [94].
469 Normality and homoscedasticity were checked graphically on residuals. When the interaction
470 between climate treatment and connectivity was maintained in the best averaged model, we
471 ran separate models for each connectivity treatment. We did so to assess more directly the
472 effect of climate across years in each connectivity conditions, as the full model yielded
473 dealing with triple interactions that are too challenging to interpret. Each computed model is
474 summarized in table S6.

475 Lizard gut diversity over years

476 We first quantified the diversity of the gut microbiome for each lizard at each sampling year
477 with Hill numbers [95,96]. Rarefaction curves indicated a good coverage of sample diversity,
478 in particular for $q=1$, which corresponds to the exponential of Shannon index [$\exp(H)$] (figure
479 S8). This index further down weights the impact of potential remaining rare molecular
480 artifacts in the dataset [49], as well as of insufficient sampling [48]. We hence used this index
481 to quantify OTUs diversity using the `vegan` R package [97]. We ensured that climate
482 treatment and habitat connectivity had no effects on the gut microbiome diversity in year 0 at
483 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

507 survival). We considered three time periods for survival: year 0 to year 1, year 1 to year 2 and
508 year 2 to year 3 (table S6).

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

515 Finally, we investigated how dispersal could explain the effect of climate on the gut microbial
516 diversity. In common lizards, dispersal mostly occurs during the first year of life [100], but
517 the small size of juveniles prevent their microbiome to be sampled. To consider all lizards,
518 including juveniles, we studied the relationship between the dispersal status from year t to
519 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 ($N_{\text{lizards}}=395$) and connected mesocosms ($N_{\text{lizards}}=230$). 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 ($N_{\text{lizards}} = 395$) and connected mesocosms ($N_{\text{lizards}} = 230$). 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 |
| Y e a r 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 |
| Y e a r 2 | Gammaproteobacteria | -0.223 | 0.777 | -0.287 | 0.774 | -0.510 | 0.851 | -0.599 | 0.549 |
| | 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** |
| Y e a r 3 | 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 |
| | 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 |
| Y e a r 3 | 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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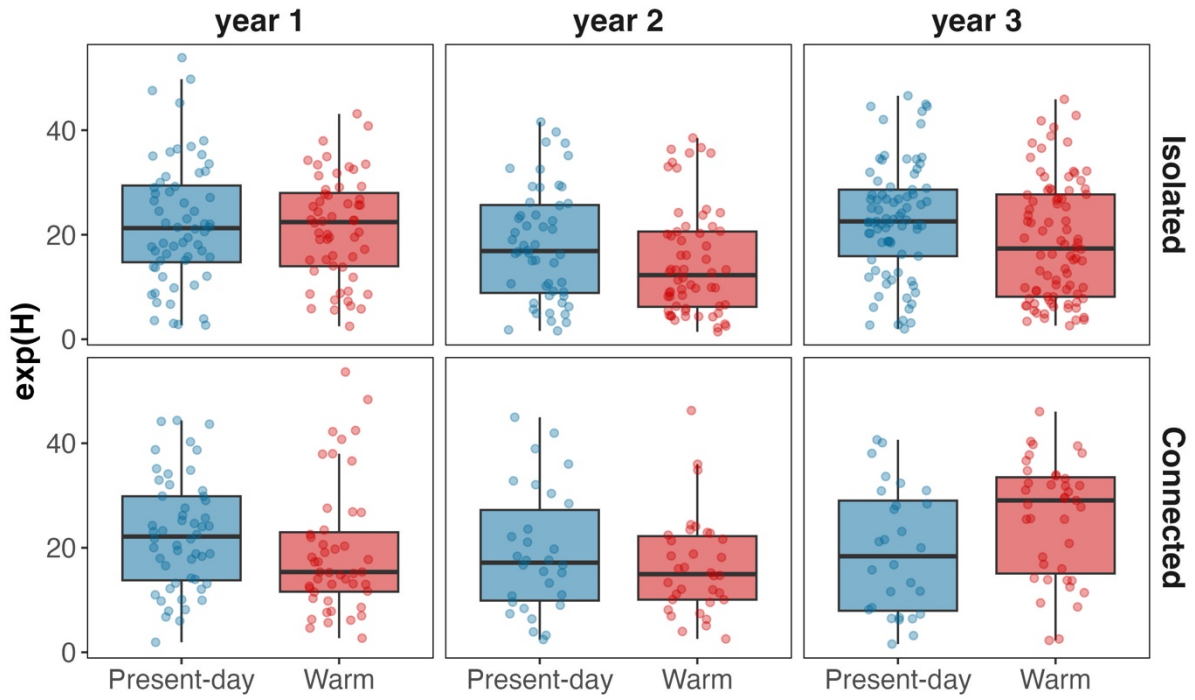
Table 3. Effects of dispersal status, climate treatment, year centered and their interaction on Shannon diversity. Snout-vent length, age, and sex with the initial mesocosm were considered as covariates. Interactions and parameters not shown in the table were excluded from models according to AIC procedure. 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 |

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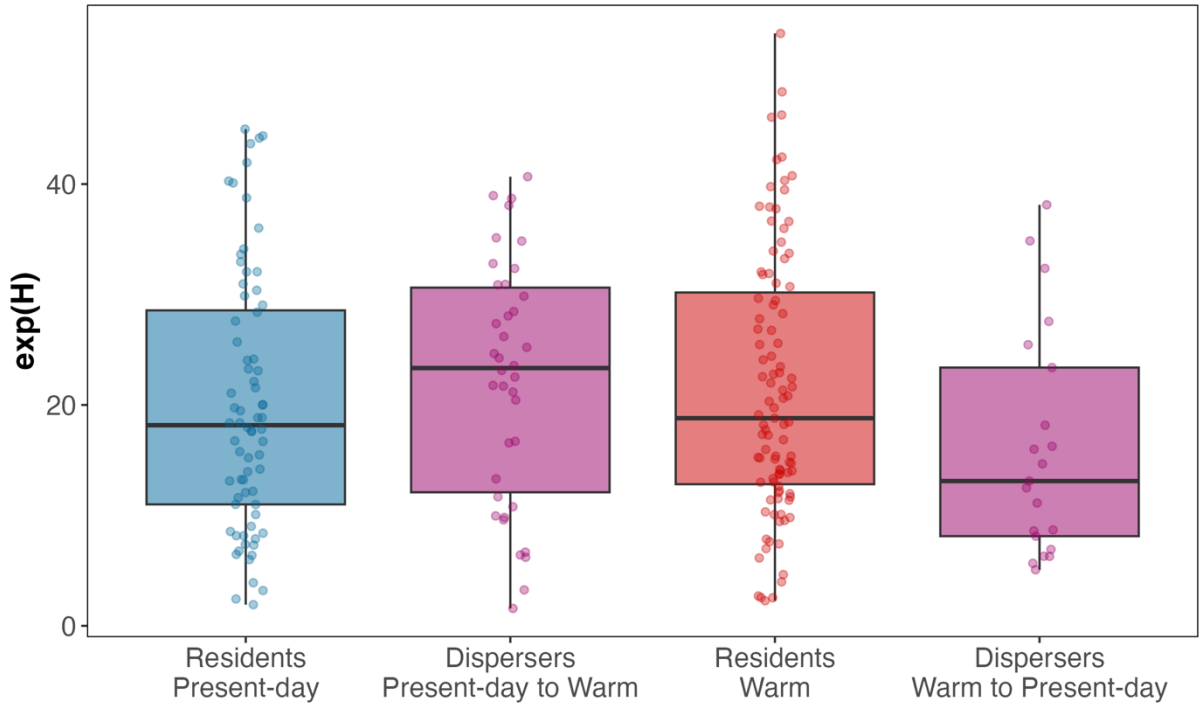
Fig. 1. Gut diversity in each climate and habitat connectivity over time. Gut microbiota diversity, calculated as the exponential of Shannon index [$\exp(H)$], in present-day and farm climates each year for isolated and connected mesocosms.



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

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949

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