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8 **The toughest animals of the Earth vs global warming: effects of long-term experimental**
9 **warming on tardigrade community structure of a temperate deciduous forest**

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28 **Running head**

29 Leaf litter tardigrades under global warming

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31 **Summary statement**

32 Tardigrade communities inhabiting upper soil horizon (leaf litter) are not affected by a long-
33 term experimental warming simulating different global warming scenarios.

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43 **Abstract**

44 Understanding how different taxa respond to global warming is essential for predicting future
45 changes and elaborate strategies to buffer them. Tardigrades are well known for their ability
46 to survive environmental stressors, such drying and freezing, by undergoing cryptobiosis and
47 rapidly recovering their metabolic function after stressors cease. Determining the extent to
48 which animals that undergo cryptobiosis are affected by environmental warming will help to
49 understand the real magnitude climate change will have on these organisms. Here, we report
50 on the responses of tardigrades within a five-year long, field-based artificial warming
51 experiment, which consisted of 12 open-top chambers heated to simulate the projected effects
52 of global warming (ranging from 0 to 5.5 °C above ambient temperature) in a temperate
53 deciduous forest of North Carolina (USA). To elucidate the effects of warming on the
54 tardigrade community inhabiting the soil litter, three community diversity indices
55 (abundance, species richness, and Shannon diversity) and the abundance of the three most
56 abundant species (*Diphascon pingue*, *Adropion scoticum*, *Mesobiotus* sp.) were determined.
57 Their relationships with air temperature, soil moisture, and the interaction between air
58 temperature and soil moisture were tested using Bayesian Generalized Linear Mixed Models.
59 Despite observed negative effects of warming on other ground invertebrates in previous
60 studies at this site, long-term warming did not affect the abundance, richness, or diversity of
61 tardigrades in this experiment. These results are in line with previous experimental studies,
62 indicating that tardigrades may not be directly affected by ongoing global warming, possibly

63 due to their thermotolerance and cryptobiotic abilities to avoid negative effects of stressful
64 temperatures, and the buffering effect on temperature of the soil litter substrate.

65

66 **Key words**

67 Tardigrades, global warming, climate change, experimental, water bears

68

69 **Introduction**

70 Soil organisms such as nematodes, tardigrades and rotifers — a microfauna that needs
71 a film of water surrounding the body to be active — are generally abundant in most terrestrial
72 biomes. They are relatively poorly studied, especially regarding their response to ongoing
73 climatic changes. Most of the studies concerning the effect of experimental warming on this
74 type of fauna are mainly focused on Antarctic nematodes communities (e.g. Convey &
75 Wynn-Williams, 2002; Simmons *et al.*, 2009; Andriuzzi *et al.*, 2018; Prather *et al.*, 2019;
76 Newsham *et al.*, 2020) and contrasting results on their community have been found according
77 to species and temperature increase (see Discussion). On one hand, these organisms might be
78 particularly susceptible to changes in climate because their surface area relative to volume is
79 great. On the other hand, some of these organisms have life stages that could buffer them
80 from climatic changes. For example, tardigrades (water bears; Fig. 1A) have the ability to
81 enter ametabolic physiological states in all phases of their life cycle, allowing them to survive
82 harsh environmental stressors such as desiccation (anhydrobiosis) and freezing (cryobiosis)
83 (Guidetti *et al.*, 2011). The ability of tardigrades to tolerate chemical and physical extremes
84 in an anhydrobiotic state is so extraordinary (Rebecchi *et al.*, 2007) that tardigrades have
85 been called the “toughest animals on the Earth” (Copley, 1999). Though their densities in soil
86 and leaf litter can vary several orders of magnitude (from 300 up to 33600 animals/m²;
87 Guidetti *et al.*, 1999; Hohberg, 2006), they are commonly detected in leaf litter (Guil &
88 Sanchez-Moreno, 2013) on all continents (Guidetti *et al.*, 1999).

89 Previous studies on the impacts of climate changes on tardigrade communities have
90 found somewhat surprising results: no effect of increasing temperature on tardigrade
91 communities were detected (Briones *et al.*, 1997; Sohlenius & Boström, 1999a). To better
92 understand the effects of increasing air temperature on tardigrade communities of soil litter,
93 we sampled a tardigrade community within an extensive set of experimental warming arrays
94 in the eastern USA, performed in the Duke Forest in the Piedmont region of North Carolina
95 (Pelini *et al.*, 2011). Experimental warming has the potential to analyse the response of the
96 entire tardigrade community compared to transplant experiments because the warming is

97 performed *in situ* without the need to move the animals. Moreover, the use of open-top
98 chambers minimizes the substrate disturbance and allows for long-term, consistent warming
99 over a sustained period (Pelini *et al.*, 2011). Previous studies on the experimental warming
100 array used in this study (Fig. 1B) found that warming had differential effects on ants, other
101 ground-living arthropods, and microbial communities, with some species benefiting from
102 warming and others declining. In particular, ants have species-specific responses to
103 temperature increase with some species benefiting from warming and others declining
104 probably linked to physiological traits that differ between species (Diamond *et al.*, 2013,
105 2016; Pelini *et al.*, 2014; MacLean *et al.*, 2017; Penick *et al.*, 2017) and the same is true for
106 other ground-living arthropods (Fitzgerald *et al.*, 2021). While the effect of warming on
107 microbial community structure and function may become more pronounced as soil
108 temperatures increase and carbon substrates are depleted through time at the Duke Forest site
109 but were not affected at a more northern site where this experiment was replicated (Cregger
110 *et al.*, 2014).

111 The experimental design allowed the opportunity to test for the interactive effects
112 between air warming and other environmental variables, such as soil moisture. We
113 hypothesized two possible outcomes of this experiment: i) we predicted that tardigrade
114 community (or part of it) would be influenced by both increasing temperature and soil
115 moisture with regards to abundance (positive correlation between increased temperature and
116 moisture on tardigrade abundances) and community composition (change in dominant species
117 and/or diversity); ii) alternatively, we predicted that tardigrade community (or part of it) will
118 show no change in responses to the warming treatment, because they escape negative impacts
119 of warming due to anhydrobiosis (avoiding period of substrate drying) or due to an extreme
120 tolerance of high temperatures (see Li & Wang, 2005; Rebecchi *et al.*, 2009a; Giovannini *et*
121 *al.*, 2018, Neves *et al.*, 2020). Those predictions come from the general trend observed for the
122 effect of artificial warming on other terrestrial organisms belonging to the hydrobios, as the
123 soil nematodes, that share the same habitat with tardigrades (Bakonyi & Nagy, 2000; Hiltbold
124 *et al.* 2017; Yan *et al.*, 2017).

125

126 **Materials and methods**

127 *Experimental design and warming chambers*

128 The experimental facilities used in this study are described in Pelini *et al.* (2011). These
129 facilities were established in a relatively warm site at Duke Forest near Hillsborough
130 (35°52'00'' N, 79°59'45'' W, 130 m a.s.l.) in the Piedmont region of North Carolina (USA).

131 The site is located near the center of the deciduous forest biome in eastern North America.
132 The experimental site at Duke Forest was in a ca. 80-year-old oak-hickory stand within the
133 Eno River Unit. The mean annual temperature at Duke Forest is 15.5 °C, and the mean
134 annual precipitation is 1140 mm. The site had 12 octagonal open-top chambers (each one 5 m
135 in diameter with eight walls each 1.9 m wide and 1.2 m tall with an area of 17.4 m², coded
136 with numbers from 1 to 12, that warmed the forest floor year-round with thermostat-
137 controlled forced air passed over hydronic heaters (Fig. 1B). Nine chambers were warmed
138 with a different target temperature each (from 1.5 to 5.5 °C above ambient temperature with
139 increments of 0.5 °C). The heating of the chambers was dynamically adjusted based on the
140 external environment temperature to obtain a constant increment over the environment
141 temperature (Fig. 2A). The highest temperature increase of 5.5 °C was included to mimic the
142 worst scenario predicted for the year 2100 (RCP 8.5; IPCC, 2013). Three chambers
143 considered as controls had air blown at environment temperature (no heat). The experiment
144 lasted for five years (2010-2015).

145

146 *Leaf litter sampling and tardigrade extraction*

147 Leaf litter sampling was conducted on May 15th 2015. Two samples (each one of a volume of
148 about 0.5 dm³) of the superficial layer of leaf litter (~10 cm of depth, composed by a variety
149 of leaves of tree plant species but dominated by *Quercus alba* L.) were collected from two
150 random points from each of the 12 chambers (nine chambers with increasing air temperatures
151 and three control chambers), for a total of 24 samples. Samples were air-dried immediately
152 after collection and shipped in zip-locked plastic bags to the Department of Life Sciences of
153 University of Modena and Reggio Emilia (UNIMORE) in Modena (Italy). The leaf litter was
154 kept desiccated for 2 years until tardigrade extraction. Before animal extraction, desiccated
155 leaf litter was kept at 45% relative humidity (RH) at 20 °C for 24 h, and then 10 g of each
156 sample (pseudoreplicate) for each chamber were rehydrated in tap water for 30 min. We then
157 sieved the leaf litter and water using two sieves of different mesh size: 500 µm and 37 µm.
158 The leaf litter left in the 500 µm sieve was re-submerged in water and sieved a second time
159 after 2 h to be sure to extract all tardigrades and their eggs. The debris collected from the 37
160 µm sieve was resuspended in distilled water and tardigrades along with their eggs were
161 collected manually with a glass pipette under a stereomicroscope.

162

163 *Species identification and data analysis*

164 The tardigrades were fixed in Carnoy's fixative ($\frac{3}{4}$ methanol, $\frac{1}{4}$ acetic acid) and mounted on
165 microscopic slides in Faure's mounting medium. Animal observations were carried out with
166 light microscopy (LM) under phase contrast (PhC) and differential interference contrast
167 (DIC) up to the maximum magnification (100x oil objective) with a Leica DM RB
168 microscope, at the Laboratory of Evolutionary Zoology (UNIMORE). Eggs were not
169 included in the analyses due to their low number. Tardigrade classification was mainly done
170 by following Ramazzotti & Maucci (1983) and Bingemer & Hohberg (2017).

171 Tardigrades are very sensitive to the moisture of the substrates being aquatic animals
172 (although able to live in terrestrial substrates); soil moisture is one of the main environmental
173 factors that could be effected by the air temperature increase. For these reasons, in addition to
174 chamber air temperature, soil moisture was chosen as an additional variable. In this specific
175 experimental setup, soil temperature is correlated with air temperature (Cregger *et al.*, 2014)
176 but soil moisture is not correlated to the air temperature measures (Burt *et al.*, 2014, Fig. 2B).
177 We chose to present the analysis done with air temperature as independent variable instead of
178 soil temperature because within the experimental setup air temperature was the directly
179 manipulated variable and soil temperature was then causally dependent on it. We also
180 performed the analysis with organic soil temperature instead of air temperature and the
181 results were very similar (analysis with soil temperature is available as supplementary
182 materials SM.01 and SM.02). Average air temperature and soil moisture were calculated
183 from the raw data file in Ellison and Dunn (2017); for statistical analysis, the 5-years average
184 of each chamber was used.

185 Three community indices considering all tardigrade species (Abundance, Species
186 richness, and Shannon index), and the abundance of the three most abundant tardigrade
187 species [*Diphascon pingue* (Marcus, 1936), *Adropion scoticum* (Murray, 1905), *Mesobiotus*
188 sp.] were examined and related to the artificially increased air temperatures and soil moisture
189 (singly and in pair). Diversity index calculations and variance partitioning were performed
190 with R software (base package, stats package, vegan package; Oksanen *et al.*, 2018).
191 Variance partitioning was performed to compare the variance contribution of the
192 pseudoreplicates within samples by means of the "aov" function from the R base package
193 with a nested design, without considering the significance testing. Differences in specimen
194 abundances (of all species and the three individual species), number of species (Species
195 richness), and Shannon index were tested against air temperature, soil moisture and their
196 interaction using a Bayesian Generalized Linear Mixed Models (GLMMs) implemented in
197 JAGS with the R package R2jags (Su & Yajima, 2012). In all models, chamber identity (*i.e.*

198 each individual chamber code) was used as a random effect. Predictors (air temperature and
199 soil moisture) were scaled and centered before model fitting. For all three response variables,
200 a log link function was used. The error families used were Negative Binomial, Poisson, and
201 Gamma for number of specimens, number of species, and Shannon index, respectively. From
202 the posterior distributions of the model parameters estimates, a Bayesian p-value and
203 standardized effect sizes were calculated according to Makowski *et al.* (2019) and Cohen
204 (1988). All of the analyses performed with their code are reported in SM.01.

205

206 **Results**

207 Each of the 24 samples of leaf litter contained tardigrades. A total of 1762 tardigrades,
208 belonging to 13 taxa, were collected, for a mean of 14.6 (min 6, max 40) tardigrades/g of dry
209 leaf litter (Table 1). Three species, namely *Diphascon pingue*, *Adropion scoticum* and
210 *Mesobiotus* sp., were found to be numerically dominant, accounting for more than 95% of all
211 observed individuals (41.03%, 40.69%, and 13.96%, respectively). One species
212 (*Pseudechiniscus* sp.) was a singleton, found as a single individual in a single chamber
213 (SM.03).

214 The variance partition (Table 2) between pseudoreplicates within each chamber and among
215 chambers showed that most of the variance for all examined indexes was best explained by
216 the differences among pseudoreplicates of the same chamber than the difference among
217 different chambers (e.g. for *D. pingue* individuals, this difference explains almost 100% of
218 the variation). These high values of variance shown by pseudoreplicates indicate a very high
219 small-scale heterogeneity (at grains less than 5 m) in the distribution of tardigrades. The
220 Bayesian GLMM models converged with good Rhat and autocorrelation values (see SM.01).
221 Bayesian p-values and Effect sizes (Table 2) suggest the absence of an effect for all the
222 combinations of predictors and response variables. The effect of the interaction of
223 temperature and moisture on *Diphascon* has a p-value slightly below 0.05, however the effect
224 size of 0.049 suggests that even if the effect exists, it would be so small to not be of
225 biological relevance. Inversely, the effect of the interaction of air temperature and moisture
226 on the Shannon index shows a high absolute value of effect size (-0.472), however it is not
227 possible to confirm if this effect exists as the Bayesian p-value is 0.255. Additionally, in all
228 posterior estimates of the regression parameters, the 95% Highest Density Interval (HDI)
229 comprises the value 0 (SM.01).

230 This can be translated in the absence of a significant effect of the predictors tested (*i.e.* air
231 temperature, soil moisture and their interaction). In other words, none of the three diversity

232 indexes (Number of specimens, Species richness, and Shannon index), nor the abundances of
233 the three species tested individually were correlated with mean chamber measurements of air
234 temperature values, soil moisture, or air temperature and soil moisture when considered
235 together (*i.e.* the synergic/cross related effects of these environmental parameters) after the 5
236 years of the experiment. To summarize, no significant influence of the long-term warming
237 environmental treatments was found on the examined ecological indexes of the tardigrade
238 community.

239

240 **Discussion**

241 We found that 5 years of experimental warming had no detectable effect on the tardigrade
242 community (nor on a specific component of it) inhabiting the soil litter. While warming has
243 generally affected community composition of those taxa (*i.e.* bacteria, ants, and other ground
244 arthropods) studied within the context of the Duke Forest warming experiment (Cregger *et*
245 *al.*, 2014; Pelini *et al.*, 2014; Diamond *et al.*, 2016; Fitzgerald *et al.* 2021), our results for
246 tardigrades are unique in that we observed no effects of the warming treatment. This was true
247 even in the warmest chambers in which temperatures were raised to mimic the worst scenario
248 predicted (RCP 8.5; IPCC, 2013) for the year 2100.

249 Our results are in line with other studies that have failed to find an effect of warming
250 or other manipulations on tardigrade communities. Sohlenius and Boström (1999a), for
251 example, found no differences in tardigrade abundance between the treatments (samples
252 transplanted to other climatic conditions). Their work was based on short-term transplant
253 studies lasting around one year during which temperature and rainfall changed but also the
254 surrounding substrate. On the other hand, in another short-term transplant study, Briones *et*
255 *al.* (1997) found that tardigrade population oscillations were positively correlated with
256 temperature, though only in the colder sites.

257 There are difficulties in comparing the results of the studies related to the effects of
258 environmental warming on terrestrial microfauna (e.g. tardigrades *vs* nematodes) due to the
259 differences in the experimental designs used in those studies. For example, the studies on
260 nematodes, that are those performed more extensively, have been conducted: in different
261 geographic areas, ecotypes, and habitats, e.g. bryophytes (Newsham *et al.*, 2020), acid soil
262 (Stevnbak *et al.*, 2012), temperate-semiarid soil (Bakonyi & Nagy, 2000; Yan *et al.*, 2017),
263 temperate-boreal forest soil (Thakur *et al.*, 2014), and tundra (Sohlenius & Bostrom, 1999b),
264 moreover most of them were performed in Antarctic soils (Convey & Wynn-Williams, 2002;

265 Simmons *et al.*, 2009; Knox *et al.*, 2017; Andriuzzi *et al.*, 2018) where the effects of warming
266 are difficult to separate from those of the freeze-thaw cycles (Simmons *et al.*, 2009; Knox *et*
267 *al.*, 2017); using different experimental approaches, e.g. transplants (Sohlenius & Bostrom,
268 1999a,b), greenhouses (Convey & Wynn-Williams, 2002; Stevnbak *et al.*, 2012), open-top
269 chambers (Prather *et al.* 2019; Newsham *et al.*, 2020), or different heating systems [infrared
270 (Yan *et al.*, 2017), ceramic and cable heaters (Thakur *et al.*, 2014), sun reflection (Bakonyi &
271 Nagy, 2000)]; to last for different times, e.g. months (Bakonyi & Nagy, 2000) or years
272 (Newsham *et al.*, 2020); and targeting different taxa (e.g. Convey & Wynn-Williams, 2002;
273 Andriuzzi *et al.*, 2018). These studies performed in different conditions showed some
274 contrasting results of the effects of warming on nematodes communities: e.g. reduction
275 (Simmons *et al.*, 2009; Stevnbak *et al.*, 2012; Yan *et al.*, 2017) and/or increasing (Sohlenius
276 & Bostrom, 1999b; Blankinship *et al.*, 2011) in abundances, or different responses according
277 to the species and/or trophic level (see Hiltbold *et al.*, 2017). Therefore, soil nematode
278 communities show responses to climate change that are context dependent and are moderated
279 by ecosystem characteristics such as vegetation type and/or nutrient levels (Hiltbold *et al.*,
280 2017; Prather *et al.*, 2019). Moreover, for most of the microfauna species considered in this
281 and previous studies (e.g. tardigrades and nematodes), very little is known about their
282 ecology and microhabitats conditions.

283 Our findings come from a single tardigrade community from a single location, they
284 may indicate that tardigrades are resilient to the increases in temperature. The comparisons of
285 our results with those obtained with nematodes, with which share similar size, anhydrobiotic
286 capabilities, and often existing in similar substrates, can be difficult due to the above
287 mentioned reasons. Anyway, in an experiment conducted in a similar habitat (i.e. temperate
288 forest), the effects of 2 years increased soil temperature recorded on nematode community
289 were similar to those found in our experiment: no effect on total or trophic group abundances
290 (Thakur *et al.*, 2014).

291 We have three hypotheses, not necessarily alternative one each other, as to the lack of
292 response of tardigrades to extreme experimental warming. First, the tardigrades at the study
293 site may have unusually high thermal optima and maxima, such that they thrive even as
294 conditions warm. Even tardigrade species living in cold regions appear to have relatively high
295 thermal limits. For example, the Antarctic species *Acutuncus antarcticus* can withstand short
296 heat shocks up to 33 °C (Giovannini *et al.*, 2018). Similarly, the boreo-alpine tardigrade
297 species *Borealibius zetlandicus* showed a lethal temperature (LT50) of 33 °C (Rebecchi *et*

298 *al.*, 2009a). It seems plausible that the thermal limits of tardigrades living in the far warmer
299 conditions of the southern part of North America would be higher and perhaps high enough
300 to allow success even in light of the warming studied here.

301 Second, the resistance of tardigrades to warming may be further explained by the
302 characteristics of their habitat. The top layer of soil, the upper horizon, is made up of living
303 and decomposed materials (e.g. leaf litter), the presence of wide air-filled spaces and its
304 heterogeneity can buffer the increase of external temperature and allows animals to select the
305 most suitable microhabitat. Tardigrade responses to small-scale habitat heterogeneity
306 (patchiness) could explain the high variability among pseudoreplicates as shown to be the
307 predominant source of variance for all six examined indexes (Table 2). Leethman *et al.*
308 (1982) and Hohberg (2006) also found a high intra-site variance in the number of tardigrades
309 in soil habitats, and a similar level of patchiness has been shown for other substrates as well
310 (Sohlenius *et al.*, 2004; Meyer, 2006; Tilbert *et al.*, 2019). In our experimental setup, soil
311 moisture was not correlated to air temperature, and was instead correlated with air relative
312 humidity (Burt *et al.*, 2014). The difference between soil moisture and air humidity is likely
313 to create a moisture gradient in the leaf litter, generating microhabitats with different
314 moisture levels. The potential indirect effect of warming (moisture change) could then be
315 buffered by tardigrades moving to or living in different leaf litter areas/layers with a more
316 suitable moisture, as tardigrades have been shown to be able to differentially colonize
317 different soil and leaf litter layers according to the seasons (Briones *et al.*, 1997; Guidetti *et al.*
318 *al.*, 1999, respectively).

319 Third, the ability of tardigrades to enter anhydrobiosis (Guidetti *et al.*, 2011).
320 The thermal tolerance of tardigrades in their desiccated state is known to be higher than when
321 they are in their active state (Rebecchi *et al.*, 2009b; Neves *et al.*, 2020). It is possible that
322 tardigrades escaped the negative effects of high temperatures in our study, especially during
323 summer, because they were in a desiccated state of anhydrobiosis, then they could
324 compensate the larger amount of time spent in anhydrobiosis in summer by taking advantage
325 of a reduced frozen period in winter. During summer when temperatures peak in temperate
326 environments, water availability is usually reduced in substrates (leaf litter, mosses and
327 lichens) inhabited by tardigrades, which dry out quickly. It is thus possible that, in our study
328 site, tardigrades are most active in cooler times of year and spend the hottest time of the year
329 in a state of anhydrobiosis, a state in which their thermal tolerance is increased (Neves *et al.*,
330 2020). The co-occurrence of warmer temperatures with dry conditions could be an additional
331 factor that increases tardigrade survival to warming in specific climatic areas. The

332 experimental data obtained in this work represent a small light to illuminate the effects of
333 climate changes in the dark world of soil microfauna. It highlights that tardigrades in soil
334 litter are part of those tolerant species able to survive the increase in air temperature due to
335 global warming, and this may be related to their ability to enter anhydrobiosis or a higher
336 tolerance of thermal extremes.

337

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472
 473 **Supplementary material**

474 **Supplementary SM.01:** Data analysis performed in R with air temperature as predictor.
 475 Results include explained variance between and within chamber by the different response
 476 variables, model parameters estimates (both values and figures) and plots of recorded
 477 temperature and soil moisture in the different chambers during their whole activity period.

478 **Supplementary SM.02:** Data analysis performed in R with soil temperature as predictor.
 479 Results include explained variance between and within chamber by the different response
 480 variables, model parameters estimates (both values and figures) and plots of recorded
 481 temperature and soil moisture in the different chambers during their whole activity period.

482 **Supplementary SM.03:** Individual tardigrades found in the pseudoreplicates of each
 483 chamber. Underline chambers are controls (*i.e.* chambers without heating). Codes of samples
 484 stored in the Laboratory of Evolutionary Zoology (University of Modena and Reggio Emilia,
 485 Italy) are provided.

486
 487

488 **Table 1.** Average temperature and soil moisture of each chamber (from 5 years of measuring)
 489 and the tardigrades abundance in the leaf litter. Underline chambers are controls (*i.e.*
 490 chambers without heating).

<i>Chamber</i>	<i>Average air temperature (°C)</i>	<i>Average soil moisture (v/v)</i>	<i>Tardigrades abundance average (T/ 10 gr)</i>	<i>Tardigrades abundance standard deviation (T/ 10 gr)</i>
1	12.9443	0.1267	32.5	26.16
2	12.2119	0.1290	122.0	56.57

3	10.2466	0.1133	44.5	9.19
4	8.6845	0.1087	84.5	103.95
5	12.6972	0.1196	43.5	31.82
6	8.4891	0.1485	28.5	23.34
7	11.2096	0.1144	47.0	25.46
8	9.9831	0.1158	112.0	131.52
9	10.3387	0.1894	31.5	4.95
10	12.1041	0.1784	79.5	65.76
11	8.4038	0.1445	53.5	65.76
12	11.4322	0.1919	202.0	206.48

491

492

493 **Table 2:** Variance partitioning and Bayesian GLMM results. Existence (Bayesian p-value
 494 (p)) and effect size statistics (directional median Cohen's d (d)) of the effect of the
 495 environmental parameters (Air temperature, Soil moisture, Air temperature * Soil moisture)
 496 on the community structure parameters (Abundance = total number of tardigrades, Taxa =
 497 total number of species, Shannon = Shannon index, *Adropion* = number of animals of
 498 *Adropion scoticum*, *Diphascon* = number of animal of *Diphascon pingue*, *Mesobiotus* =
 499 number of animal of *Mesobiotus* sp.). Statistics are presented as p/d. In bold: $p < 0.05$, $|d| >$
 500 0.1.

	Abundance	Taxa	Shannon	<i>Adropion</i>	<i>Mesobiotus</i>	<i>Diphascon</i>
Variance partitioning:						
Variance between pseudoreplicates / Total variance	94.85%	94.08%	99.20%	91.49%	99.14%	99.91%
Bayesian-GLMM results						
Air temperature	0.369 / 0.003	0.927 / 0.007	0.788 / 0.073	0.715 / 0.004	0.515 / 0.015	0.522 / 0.004

Soil moisture	0.886 /	0.422 /	0.979 / -	0.510 /	0.141 / -	0.753 / -
	0.000	0.059	0.007	0.008	0.039	0.002
Temperature*Moisture	0.086 /	0.778 /	0.255 / -	0.728 / -	0.088 /	0.049 /
	0.008	-0.029	0.472	0.007	0.063	0.021

501

502

503 **Figure legends**

504 **Figure 1.** A) Tardigrade of the genus *Milnesium* (SEM; scale bar = 50 μ m). B) One open-top
505 chamber experimental setup.

506 **Figure 2.** A) Average air temperature by month over 5 years of the experimental chambers.
507 B) Average soil moisture by month over 5 years of the experimental chambers.

508

509 **Data Accessibility Statement**

510 Warming chambers environmental variables are available at:

511 <http://harvardforest.fas.harvard.edu:8080/exist/xquery/data.xq?id=hf113>. Supplementary
512 material and a copy of the raw environmental measurements are also available in Figshare
513 under the DOI: 10.6084/m9.figshare.14742699

514

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522

523 **Competing Interests Statement**

524 The author have not competing interests to declare

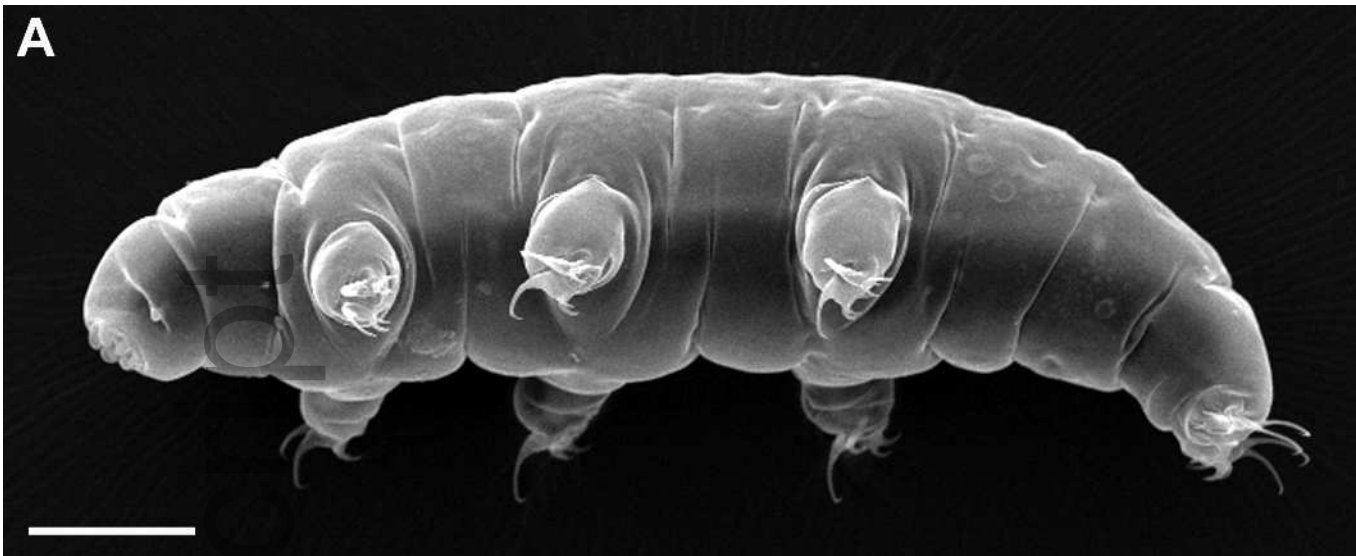
525

526 **Author's contributions**

527 MV, RG, RD, LN, CP, NS conceived the ideas and designed methodology; MV and LA
528 collected the data; MV analysed the data; MV and RG led the writing of the manuscript. MV,

529 RG and LR wrote the first manuscript draft. All authors contributed critically to the drafts and
530 gave final approval for publication.

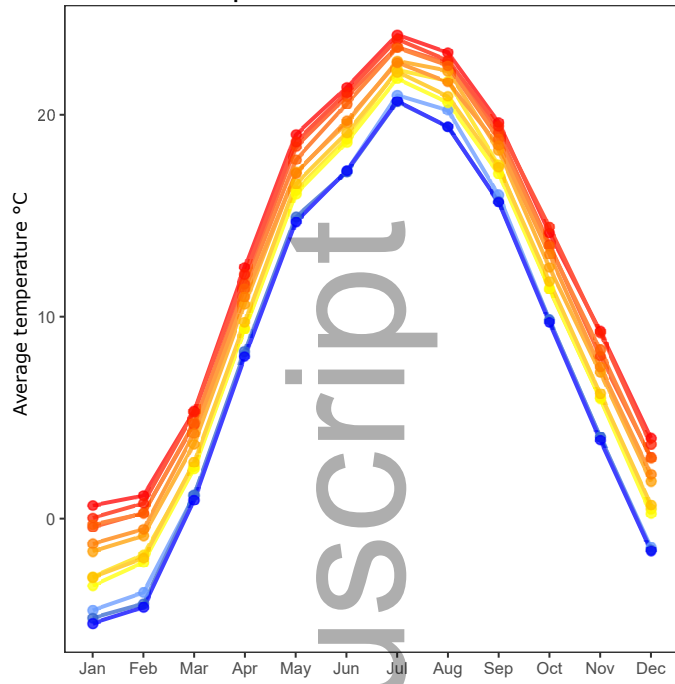
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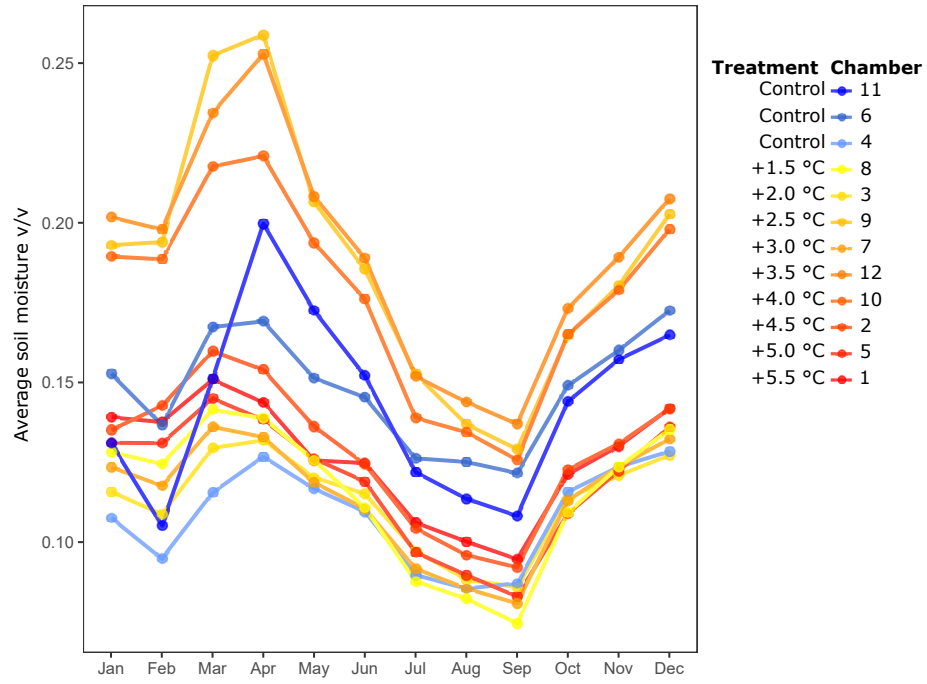
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A - Air temperature



B - Soil moisture



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