

1 **Contrasting mechanisms underlie short- and longer-term soil respiration**  
2 **responses to experimental warming in a dryland ecosystem**

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4 **Running head:** Warming effects on soil respiration

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6 Marina Dacal<sup>1,2\*</sup>, Pablo García-Palacios<sup>3</sup>, Sergio Asensio<sup>2</sup>, Concha Cano-Díaz<sup>1</sup>, Beatriz

7 Gozalo<sup>2</sup>, Victoria Ochoa<sup>2</sup> & Fernando T. Maestre<sup>2,4</sup>

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10 <sup>1</sup>Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey  
11 Juan Carlos, C/ Tulipán s/n, 28933 Móstoles, Spain

12 <sup>2</sup>Instituto Multidisciplinar para el Estudio del Medio “Ramon Margalef”, Universidad  
13 de Alicante, Carretera de San Vicente del Raspeig s/n, 03690 San Vicente del Raspeig,  
14 Spain

15 <sup>3</sup> Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas,  
16 Serrano 115 bis, 28006, Madrid, Spain.

17 <sup>4</sup>Departamento de Ecología, Universidad de Alicante, Carretera de San Vicente del  
18 Raspeig s/n, 03690 San Vicente del Raspeig, Spain

19

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21 \* Correspondence e-mail: [marina.dacal1@gmail.com](mailto:marina.dacal1@gmail.com)

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23

24 **Abstract**

25 Soil carbon losses to the atmosphere through soil respiration are expected to rise with  
26 ongoing temperature increases, but available evidence from mesic biomes suggests that  
27 such response disappears after a few years of experimental warming. However, there is  
28 lack of empirical basis for these temporal dynamics in soil respiration responses, and for  
29 the mechanisms underlying them, in drylands, which collectively form the largest biome  
30 on Earth and store 32% of the global soil organic carbon pool. We coupled data from a  
31 ten-year warming experiment in a biocrust-dominated dryland ecosystem with  
32 laboratory incubations to confront 0-2 years (short-term hereafter) vs. 8-10 years  
33 (longer-term hereafter) soil respiration responses to warming. Our results showed that  
34 increased soil respiration rates with short-term warming observed in areas with high  
35 biocrust cover returned to control levels in the longer-term. Warming-induced increases  
36 in soil temperature were the main driver of the short-term soil respiration responses,  
37 whereas longer-term soil respiration responses to warming were primarily driven by  
38 thermal acclimation and warming-induced reductions in biocrust cover. Our results  
39 highlight the importance of evaluating short and longer-term soil respiration responses  
40 to warming as a mean to reduce the uncertainty in predicting the soil carbon–climate  
41 feedback in drylands.

42

43 **Keywords:** soil respiration, biocrusts, dryland, microbial thermal acclimation, short-  
44 term vs longer-term warming, soil temperature, soil moisture

45 **Introduction**

46 Soil respiration is expected to increase with global warming (Davidson & Janssens,  
47 2006; Kirschbaum, 2006), contributing to enhance atmospheric CO<sub>2</sub> concentration.  
48 Thus, warming-induced soil carbon (C) losses via soil respiration may lead to a positive  
49 C cycle–climate feedback (Tucker, Bell, Pendall, & Ogle, 2013), which is indeed  
50 embedded into the climatic models of the IPCC (Ciais et al., 2014). However, most  
51 experiments conducted to date on this topic have typically lasted less than four years  
52 (Wang et al., 2014), and there is growing evidence showing that elevated soil  
53 respiration rates may gradually be offset towards ambient values after a few years of  
54 experimental warming (Kirschbaum, 2004; Luo, Wan, Hui, & Wallace, 2001; Melillo et  
55 al., 2017, 2002). Multiple mechanisms have been hypothesized to explain such transient  
56 effects of warming on soil respiration. For instance, the thermal acclimation of soil  
57 microorganisms to the ambient temperature regime (Bradford et al., 2019; Dacal,  
58 Bradford, Plaza, Maestre, & García-Palacios, 2019) and the depletion of labile soil C  
59 sources (Hartley, Hopkins, Garnett, Sommerkorn, & Wookey, 2008; Schindlbacher,  
60 Schneckner, Takriti, Borken, & Wanek, 2015) may drive soil respiration responses to  
61 warming over time. Additionally, given that soil temperature and moisture are the most  
62 important controls on soil respiration (Conant, Dalla-Betta, Klopatek, & Klopatek,  
63 2004), warming-induced changes in microclimatic variables may alter soil microbial  
64 activity, leading to shifts in soil respiration rates (Luo et al., 2001). The lack of  
65 consensus on the relative importance of these mechanisms hinders our ability to model  
66 longer-term soil respiration responses to warming, which are fundamental to increase  
67 confidence in soil C projections in a warmer world (Bradford et al., 2016; Zhou et al.,  
68 2012).

69           Beyond heterotrophic microbial CO<sub>2</sub> production, soil respiration is also a  
70 product of plant roots and other autotrophic organisms inhabiting soil surfaces such as  
71 biological soil crusts (biocrusts hereafter; topsoil communities formed by cyanobacteria,  
72 algae, mosses, liverworts, fungi, bacteria and lichens; Weber, Büdel, & Belnap, 2016).  
73 In drylands, which cover 41% of the total land surface (Cherlet et al., 2018) and store  
74 32% of the Earth's soil organic C (SOC) pool (Plaza et al., 2018), up to 42% of the  
75 overall soil respiration comes from biocrust-dominated microsites (Castillo-Monroy,  
76 Maestre, Rey, Soliveres, & García-Palacios, 2011; Feng et al., 2014, 2013). Biocrusts  
77 are particularly relevant for the global C cycle, as it has been estimated that they cover  
78 ca. 12% of the Earth's terrestrial surface (Rodriguez-Caballero et al., 2018) and fix over  
79 2.6 Pg·yr<sup>-1</sup> of atmospheric C globally (Elbert et al., 2012). Given their extent and  
80 importance for the C cycle, biocrusts are a major ecosystem component when  
81 evaluating warming effects on soil respiration in drylands.

82           Biocrust constituents are severely affected by warming; the physiological  
83 performance of biocrust-forming lichens and mosses have been found to decrease with  
84 warming in experiments conducted in Spain, USA, China and South Africa (Grote,  
85 Belnap, Housman, & Sparks, 2010; Maestre, Delgado-Baquerizo, Jeffries, Eldridge, &  
86 Ochoa, 2015; Maestre et al., 2013; Maphangwa, Musil, Raitt, & Zedda, 2012; Ouyang  
87 & Hu, 2017). These responses have been linked to warming-induced reductions in soil  
88 moisture and dew events (Ladrón de Guevara et al., 2014; Ouyang & Hu, 2017). Such  
89 water sources are critical to maintain the photosynthetic activity of biocrust  
90 communities (del Prado & Sancho, 2007; Veste, Littmann, Friedrich, & Breckle, 2001),  
91 and warming-induced alterations on them may lead to dramatic losses in the cover of  
92 biocrust-forming lichens and mosses (up to 40%) after a few years of temperature  
93 manipulation (Darrouzet-Nardi, Reed, Grote, & Belnap, 2018; Escolar, Martínez,

94 Bowker, & Maestre, 2012; Ferrenberg, Reed, Belnap, & Schlesinger, 2015; Maestre et  
95 al., 2013). Given the importance of biocrust cover for soil respiration in drylands  
96 (Castillo-Monroy, Maestre, et al., 2011; Feng et al., 2014, 2013), the heterotrophic  
97 mechanisms (i.e. substrate depletion, microbial acclimation and changes in  
98 microclimatic variables) driving soil respiration responses to warming should be  
99 assessed jointly with the shifts in biocrust cover promoted by elevated temperatures  
100 (García-Palacios et al., 2018; Maestre et al., 2013).

101 In drylands, most studies evaluating soil respiration responses to experimental  
102 warming have been conducted over short-term periods (Darrouzet-Nardi, Reed, Grote,  
103 & Belnap, 2015; Escolar, Maestre, & Rey, 2015; Guan, Li, Zhang, & Li, 2019; Maestre  
104 et al., 2013) and consequently, longer-term warming effects are virtually unknown. To  
105 our knowledge, only Darrouzet-Nardi et al., (2018) have explicitly confronted short- vs.  
106 longer-term soil respiration responses to warming in biocrust-dominated drylands, but  
107 no study so far has addressed the heterotrophic and autotrophic mechanisms underlying  
108 transient soil respiration responses to warming. Given the importance of soil carbon-  
109 climate feedbacks to forecast greenhouse gas emissions globally (Carey et al., 2016;  
110 Crowther et al., 2016), and the extent of drylands worldwide, it is critical to evaluate  
111 both short and longer-term soil respiration responses to warming in these environments  
112 and how these are modulated by biocrusts and soil microbial communities.

113 Here, we confronted 0-2 years (short-term, hereafter) vs. 8-10 years (longer-  
114 term, hereafter) soil respiration responses to experimental warming in a biocrust-  
115 dominated dryland in central Spain. Data from this experiment were coupled to those  
116 from laboratory incubations at four assay temperatures (10, 20, 30 and 40°C), which  
117 allowed us to gain mechanistic insights on the importance of autotrophic and  
118 heterotrophic pathways as drivers of soil respiration responses to warming over time.

119 Using this combination of approaches, which to the best of our knowledge has not been  
120 used before when evaluating soil respiration responses to warming in drylands, we  
121 evaluated: i) short- and longer-term warming impacts on soil respiration and its  
122 temperature sensitivity (objective i), ii) how warming-induced effects on soil  
123 temperature and moisture affect soil respiration responses to elevated temperatures  
124 (objective ii), iii) the role of biocrusts as modulators of short- and longer-term soil  
125 respiration responses to warming (objective iii), and iv) the importance of thermal  
126 acclimation of soil microbial respiration as a driver of soil respiration responses to  
127 longer-term warming (objective iv).

128

## 129 **Materials and methods**

### 130 *Study area*

131 The study was conducted at the Aranjuez Experimental Station, located in central Spain  
132 (40°02'N–3°32'W; elevation = 590 m a. s. l.). Its climate is Mediterranean semiarid,  
133 with mean annual temperature and precipitation values (2008–2018 period) of 16,5°C  
134 and 336 mm, respectively. The soil is a Gypsic Leptosol (IUSS Working Group WRB,  
135 2006). Perennial plant cover is < 40%, and biocrust communities dominated by lichens  
136 such as *Diploschistes diacapsis*, *Squamarina lentigera*, *Fulgensia subbracteata* and  
137 *Buellia zoharyi* and mosses *Pleurochaete squarrosa* and *Didymodon acutus* cover ~32%  
138 of the soil surface (Castillo-Monroy, Maestre, et al., 2011; Maestre et al., 2013).  
139 Cyanobacteria from the genera *Microcoleus*, *Schizothrix*, *Tolypothrix*, *Scytonema* and  
140 *Nostoc* also form part of biocrusts at this site (Cano-Díaz, Mateo, Muñoz-Martín, &  
141 Maestre, 2018).

142 In July 2008, we established a full factorial experiment with two treatments of  
143 two levels each: warming (ambient vs. increased temperature) and initial biocrust cover

144 (low: < 20% vs high: >50%). We installed open top chambers (OTCs) to reach a  
145 warming scenario similar to the temperature increase of 2–3°C forecasted for 2040–  
146 2070 in this region in atmosphere-ocean general circulation models (De Castro, Martín-  
147 Vide, & Alonso, 2005). OTCs present a hexagonal design made of methacrylate sheets  
148 (40 × 50 × 32 cm), a material that according to the manufacturer (Decorplax S.L.,  
149 Humanes, Spain) ensures 92% transmittance in the visible spectrum and very low  
150 emission in the infrared wavelength. To allow air circulation and thus to avoid  
151 overheating, OTCs are suspended 3–5 cm over the ground by a metal frame. Ten plots  
152 (1.25 x 1.25 m) per combination of treatments were randomly distributed and separated  
153 at least 1 m to diminish the risk of lack of independence between replicates (n = 40).  
154 We inserted a PVC collar (20 cm diameter, 8 cm height) in each plot to monitor soil  
155 respiration and biocrust cover over time. See Escolar et al. (2012) and Maestre et al.  
156 (2013) for additional details on the experimental design.

157

#### 158 *Testing the warming effects on soil microclimatic conditions*

159 We focused on warming effects on soil temperature and soil moisture as the two main  
160 drivers controlling soil respiration in drylands (Castillo-Monroy, Maestre, et al., 2011;  
161 Conant et al., 2004; Veste et al., 2001). In parallel to soil respiration measurements, we  
162 monitored soil temperature at 0-2 cm depth with protected diodes at the beginning of  
163 the experiment and since 2012 (i.e. four years after experimental set-up) with a Li-8100  
164 Automated Soil CO<sub>2</sub> Flux System (Li-COR, Lincoln, NB, USA) because the later  
165 measurements were faster and more accurate. Data obtained with the Li-8100 were  
166 corrected using a calibration between both methods (r=0.956, Figure S1). Volumetric  
167 soil moisture was measured monthly at 0-5 cm depth using time-domain reflectometry  
168 (TDR; Topp & Davis, 1985) in every plot at the same time of soil respiration

169 measurements. Additionally, and given that at our study site soil moisture dynamics is  
170 largely driven by specific pulses (Berdugo, Soliveres, & Maestre, 2014; Lafuente,  
171 Berdugo, Ladrón de Guevara, Gozalo, & Maestre, 2018) soil moisture (0-5 cm depth)  
172 was monitored continuously every 2.5 hours using automated sensors (ECH<sub>2</sub>O humidity  
173 sensors, Decagon Devices Inc., Pullman, USA). Specifically, we used four replicates for  
174 control plots (all corresponding to high initial biocrust cover) and six for warming plots  
175 (half of them for each initial biocrust cover level). Sensors to monitor soil moisture  
176 continuously were installed in February 2009.

177

#### 178 *Soil CO<sub>2</sub> efflux measurements and its temperature sensitivity ( $Q_{10}$ )*

179 The soil CO<sub>2</sub> efflux rate of the whole soil column, including both biocrusts and soil  
180 microbial communities, was measured *in situ* once a month in two contrasting periods:  
181 short-term (0-2 years) and longer-term (8-10 years) after the setup of the experiment.  
182 Measurements were conducted with a closed dynamic system (Li-8100). The opaque  
183 chamber used for these measurements had a volume of 4843 cm<sup>3</sup> and covered an area of  
184 317.8 cm<sup>2</sup>. Given the low CO<sub>2</sub> efflux rates typically observed in semiarid ecosystems  
185 (Castillo-Monroy, Maestre, et al., 2011; Maestre et al., 2013; Rey et al., 2011),  
186 sampling period was set-up to 120 s to ensure reliable measurements. We included 45  
187 seconds of purge after each soil CO<sub>2</sub> efflux measurement. Furthermore, we established a  
188 deadband of 15 seconds once the chamber was closed when no flux was recorded to  
189 allow biocrusts to acclimate to dark. In every survey, half of the replicates were  
190 measured in one day (between 10:00 and 13:00 local time), and the other half were  
191 measured over the next day in the same period. Annual plants were removed from the  
192 PVC collars at least 48 hours before soil respiration measurements.

193 We evaluated the temperature sensitivity of soil respiration using  $Q_{10}$ , defined as  
194 the increment in soil respiration when temperature increases by 10°C and calculated at  
195 each plot using the following equations (Luo & Zhou, 2006):

$$196 \quad R_s = R_0 e^{\beta t} \quad (1)$$

197 Where  $R_s$  is soil respiration ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $R_0$  is the basal soil respiration rate ( $\mu\text{mol m}^{-2}$   
198  $\text{s}^{-1}$ ) or intercept of soil respiration at 0°C, and  $t$  is soil temperature (in °C) measured at  
199 the same time as  $R_s$ .  $\beta$  was used to calculate the  $Q_{10}$  (increment in  $R_s$  when  $t$  increases  
200 by 10 °C) as follows:

$$201 \quad Q_{10} = e^{10\beta} \quad (2)$$

202

### 203 *Monitoring changes in biocrust cover with warming*

204 The total cover of the two major visible components of the biocrust community (lichens  
205 and bryophytes) was estimated in each PVC collar at the beginning of the experiment  
206 and on a yearly basis thereafter (except during the second year of the experiment, when  
207 these measurements were not taken). We used high-resolution photographs to assess the  
208 proportion of each collar covered by these biocrust components using the software  
209 GIMP (<http://www.gimp.org/>, to map biocrust-covered areas) and ImageJ  
210 (<http://rsb.info.nih.gov/ij/>, to calculate the size of biocrust-covered areas). Cover  
211 estimates obtained with this method are highly related ( $r^2=0.84$ ) to those observed in the  
212 field with the point sampling method (Ladrón de Guevara et al., 2018). For this study  
213 we only considered biocrust surveys included within our sampling periods. Therefore,  
214 we used the surveys conducted 1 yr and 9-10 yr (average of both surveys) after the setup  
215 of the experiment for the short- and longer-term periods, respectively.

216

217 *Addressing thermal acclimation of soil microbial respiration using laboratory*  
218 *incubations*

219 We sampled soils (0-5 cm depth) in five field replicates per combination of treatments  
220 in 2017, nine years after the setup of the experiment. Biocrust visible components were  
221 removed from the samples, which were sieved at 2 mm mesh and stored at 4 °C for a  
222 couple of days until laboratory incubation. We conducted short-term (10 h) laboratory  
223 soil incubations at four assay temperatures (10, 20, 30 and 40°C) as performed in similar  
224 mechanistic tests of thermal soil microbial acclimation (Atkin & Tjoelker, 2003;  
225 Bradford, Watts, & Davies, 2010; Hochachka & Somero, 2002; Tucker et al., 2013).  
226 Soil incubations were performed at 60% of water holding capacity, dark conditions and  
227 100% air humidity.

228         For each soil sample, we measured soil respiration rates after the addition of two  
229 different substrates: sterile deionized water and glucose (at a dose of 10 mg C g<sup>-1</sup> dry  
230 soil) using the MicroResp<sup>TM</sup> technique (Campbell, Chapman, Cameron, Davidson, &  
231 Potts, 2003). The former substrate was used to determine soil basal respiration, whereas  
232 the latter was used to account for the effect of substrate limitation on soil respiration  
233 rates (Bradford et al., 2010). The glucose dose used in this study is considered to exceed  
234 microbial demand (Davidson, Janssens, & Luo, 2006). The MicroResp<sup>TM</sup> technique  
235 (Campbell et al., 2003) is a high-throughput colorimetric method measuring soil  
236 respiration rates. We used a CO<sub>2</sub> detection solution containing cresol red indicator dye  
237 that experiences a colour change because of the variation in pH occurring when respired  
238 CO<sub>2</sub> reacts with the bicarbonate of the detection solution. Each microplate well was  
239 filled with 150 µl aliquots of the detection solution and was attached to the deep-well  
240 microplates containing the soil samples (0.5 g fresh soil/well). Both plates were  
241 incubated together at the assay temperature (10, 20, 30 or 40°C) during the last five

242 hours of the incubation period to avoid detection solution saturation. The detection plate  
243 colour development was read immediately before and after the last five hours of the  
244 incubation at 595nm. The colour change in the detection solution was calibrated with an  
245 alkali trapping method ( $r^2 = 0.86$ ,  $P < 0.001$ , Lundegardh, 1927, Figure S2). The  
246 absorbance values observed in our study fell into the most flattened part of the  
247 calibration graph (i.e. between 0.3 and 0.6), suggesting that the detection solution was  
248 not saturated.

249         It is necessary to control for microbial biomass to test for thermal acclimation  
250 (Bradford et al., 2010). All available methods to estimate soil microbial biomass have  
251 drawbacks (Bradford et al., 2008, 2009; Hartley, Hopkins, Garnett, Sommerkorn, &  
252 Wookey, 2009), and hence we measured soil microbial biomass using three different  
253 methods to increase the robustness of our results. First, we measured soil induced  
254 respiration ( $\mu\text{g CO}_2\text{-C g soil}^{-1} \text{ h}^{-1}$ ) using autolyzed yeast (Yeast-SIR) as a substrate at  
255 20°C (Fierer, Schimel, & Holden, 2003). Yeast was added at a dose of 1 mL g soil<sup>-1</sup>  
256 (dry weight equivalent) from a solution containing 12 g of yeast L<sup>-1</sup> of water. Second,  
257 we used a chloroform-fumigation extraction (CFE) (Vance, Brookes, & Jenkinson,  
258 1987). Specifically, we prepared two replicates per sample with almost the same amount  
259 of soil: one of the replicates was fumigated with chloroform and the other one remained  
260 as a control. Then, we measured total organic carbon (TOC) with an automated TOC  
261 analyser in K<sub>2</sub>SO<sub>4</sub>-diluted soil samples. The microbial biomass estimation derived from  
262 this technique ( $\text{mg C kg soil}^{-1}$ ) was calculated by the difference between fumigated and  
263 unfumigated samples. Finally, we measured the relative abundance of soil bacteria  
264 (number of DNA copies g<sup>-1</sup> soil) using qPCR. The bacterial 16S-rRNA genes were  
265 amplified with the Eub 338-Eub 518 primer sets as described in Maestre et al. (2015).  
266

267 *Statistical analyses*

268 We conducted a series of statistical analyses to achieve the different objectives of the  
269 study. To achieve objective i (i.e. how short- and longer-term warming affect soil  
270 respiration and its temperature sensitivity), we built linear mixed-effect regression  
271 models (LMMs) that included warming, initial biocrust cover and their interaction as  
272 fixed factors. The temporal dependence of soil respiration measurements across  
273 replicates over time (i.e. repeated measures) was tested by including replicate identity  
274 and sampling date in the model as random factors. To test the effect of warming on  $Q_{10}$ ,  
275 we built linear regression models (LMs) including warming, initial biocrust cover and  
276 their interaction as fixed factors.

277         Soil respiration missing data due to technical issues (11% of the data equally  
278 distributed between both sampling periods) was imputed using the R package  
279 missForest (Stekhoven & Bühlmann, 2012) as it was done in similar studies (Darrouzet-  
280 Nardi et al., 2015, 2018). To confirm that data imputation did not artificially alter the  
281 treatment effects, we repeated the same LMMs using the original data with missing  
282 values. We conducted the analyses testing the effect of warming on soil respiration and  
283 temperature sensitivity ( $Q_{10}$ ) using both the original data with missing values and the  
284 filled data. The missForest is an iterative imputation algorithm based on random forest  
285 models, which are considered ensemble-learning methods (Breiman, 2001). The  
286 algorithm starts filling the missing data with the variable with the fewest gaps and then  
287 iteratively re-fits new imputation models until a stopping criterion is reached. We fit  
288 one separated missForest model for each combination of treatments (i.e. four models in  
289 total) including soil respiration, temperature and moisture, biocrust cover and sampling  
290 date.

291 To achieve objective ii (how warming-induced impacts on soil temperature and  
292 moisture affect soil respiration responses to this climate change driver), we first  
293 evaluated the effects of warming on soil temperature and moisture measured monthly  
294 using LMMs that included warming, initial biocrust cover and their interaction as fixed  
295 factors. The temporal dependence of soil temperature and soil moisture measured  
296 monthly across replicates over time (i.e. repeated measures) was tested by including  
297 replicate identity and sampling date in the model as random factors. Additionally, we  
298 performed a similar analysis for soil moisture measured continuously, but for both  
299 biocrust cover levels together, as we lack enough replicates to separate between low and  
300 high cover. To compare the effect of warming on soil moisture measured monthly and  
301 continuously, we only consider soil moisture monthly measurements conducted once  
302 both sampling methods were available (i.e. since February 2009). We used the monthly  
303 mean of continuous soil moisture data for doing so. We then calculated the effect size  
304 of warming on soil respiration, temperature and moisture measured at each plot on a  
305 monthly basis for each period (i.e. 0-2 yr and 8-10 yr) using the response ratio (RR,  
306 Hedges, Gurevitch, & Curtis, 1999):

$$307 \text{ RR (soil respiration) } = \ln (\text{RSW} / \text{RSC}) \quad (3)$$

308 where RSW is the soil respiration in each warmed plot and RSC is the mean soil  
309 respiration in the control plots. The RRs were estimated separately for each initial  
310 biocrust cover level. To calculate the RRs, we first computed the average across the  
311 sampling dates per period for each plot. Then, to test how warming-induced changes in  
312 soil temperature impacted soil respiration, we evaluated the relationship between the RR  
313 of soil respiration and that of soil temperature using LMs. Similarly, to address the  
314 relationship between warming-induced changes in soil moisture and soil respiration, we

315 evaluated the relationship between the RR of soil respiration and that of soil moisture  
316 using LMs.

317 To achieve objective iii (role of biocrusts as modulators of short- and longer-  
318 term soil respiration responses to warming), we first evaluated the effects of warming  
319 on the biocrust cover using LMs with warming, initial biocrust cover and their  
320 interaction as fixed factors. Then, we evaluated whether warming-induced changes in  
321 biocrust cover control soil respiration responses to warming during short- and longer-  
322 term periods. To do so, we evaluated the relationship between the RR of soil respiration  
323 and that of biocrust cover using LMs. The RRs were calculated as described above for  
324 soil respiration.

325 To achieve objective iv (importance of thermal acclimation by soil microbial  
326 respiration), we tested whether soil heterotrophic microbial respiration acclimates to  
327 elevated temperatures after longer-term warming. To do so, we first evaluate the effect  
328 of warming on soil microbial biomass in the longer-term. For that purpose, we used  
329 LMs that include warming, initial biocrust cover and their interaction as fixed factors.  
330 We fit a separate model for each of the three methods used to estimate microbial  
331 biomass (i.e. Yeast-SIR, CFE and qPCR). Then, we statistically controlled for  
332 differences in microbial biomass by including it as a covariate in the model (substrate  
333 limitation was alleviated in the laboratory incubations using glucose in excess of  
334 microbial demand) as conducted in previous studies (Bradford et al., 2019, 2010; Dacal  
335 et al., 2019). We used this approach to control for microbial biomass instead of the  
336 mass-specific respiration (ratio between soil respiration and microbial biomass), as  
337 ratios may obscure true relationships among variables (Bradford et al., 2019; Jasienski  
338 & Bazzaz, 1999). Additionally, some previous thermal adaptation studies found the  
339 same results using either mass-specific respiration or SIR and microbial biomass as a

340 covariate in the model (Bradford et al., 2008, 2010). This suggests that this covariate  
341 approach is also appropriate to control the effect of changes in microbial biomass when  
342 testing for thermal acclimation of soil respiration. Therefore, we followed the covariate  
343 approach as it was an appropriate method to achieve this objective; by doing so we also  
344 avoided the problems associated with including ratios in statistical models. Specifically,  
345 we ran a separate LM for each soil microbial biomass estimation method (i.e. Yeast-  
346 SIR, CFE and qPCR). These LMs incorporated warming, initial biocrust cover, the  
347 interaction of these two treatments, assay temperature and soil microbial biomass as  
348 fixed factors, and analysed their effects on potential soil microbial respiration. The  
349 interaction between assay temperature and the warming treatment was also tested but  
350 removed because it was not significant ( $p = 0.860$ ). To represent the results of these  
351 thermal adaptation analyses (i.e. Figure 5), we followed an approach that estimates soil  
352 respiration rates considering all the variables included in the models (i.e. warming  
353 treatment, initial biocrust cover, the interaction between these two treatments, assay  
354 temperature and microbial biomass. Specifically, we estimated the relative effect size of  
355 each of these variables on potential soil microbial respiration rates using the  
356 unstandardized regression parameters. These effect sizes depend on the slope coefficient  
357 for the specific variable obtained by fitting the LMM and on the observed variation in  
358 the values of that variable. To discern the effect of warming, we allowed their values to  
359 vary across both levels of this treatment (i.e. control and warming) while holding the  
360 remaining variables constant at the mean of all observations for each variable.

361 All the statistical analyses were conducted using the R 3.3.2 statistical software  
362 (R Core Team, 2015). The LMMs were fit with a Gaussian error distribution using the  
363 ‘lmer’ function of the lme4 package (Bates, Mächler, Bolker, & Walker, 2015). All the  
364 analyses were performed separately for short-term and longer-term sampling periods.

365 Response data were transformed by taking the natural logarithm of each value when  
366 needed to meet the assumptions of normality and homogeneity of variance.

367

## 368 **Results**

### 369 *Short-term and longer-term soil respiration and $Q_{10}$ responses to warming*

370 Warming significantly increased soil respiration during the first two years of the  
371 experiment in the high biocrust cover plots (Figure 1a, Table S1,  $p = 0.029$ ). However,  
372 these positive effects disappeared in the longer-term (i.e. 8 to 10 years after  
373 experimental setup; Figure 1b, Table S3,  $p=0.457$ ). Seasonally, soil respiration rates  
374 were consistently greater in autumn and spring, matching major precipitation events  
375 over both the short- (Figure 2a) and the longer-term (Figure 2b). The  $Q_{10}$  was similar in  
376 warmed and control plots in the short-term (Figure 1c,  $p = 0.818$ ), but this variable was  
377 a 10% lower (95% CI= 9 to 11%) in warmed than in control plots for both biocrust  
378 cover levels in the longer-term (Figure 1d,  $p < 0.001$ ). The effects of warming and  
379 biocrust cover level on soil respiration and  $Q_{10}$  at both sampling periods were similar to  
380 those addressed when using the original data without imputation (Table S2 and S4, Fig.  
381 S3).

382

### 383 *Changes in soil microclimatic variables as a driver of soil respiration responses to* 384 *warming*

385 On average, soil temperature was 2.95°C (95% CI= 2.90 to 2.99 °C) and 1.43°C (95%  
386 CI= 1.39 to 1.48 °C) higher in warmed than in control plots at both short and longer-  
387 term periods, respectively (Figure S4a and b, respectively,  $p < 0.001$  for both periods).  
388 Seasonally, differences in soil temperature between control and warmed plots were  
389 greater in summer (i.e. from July to September) both in the short- (Figure 2c) and

390 longer-term (Figure 2d). On the contrary, differences in soil moisture measured monthly  
391 between control and warming plots were higher in winter (i.e. from December to  
392 March) both in the short- (Figure 2e) and longer-term (Figure 2f). These results can be  
393 also observed when using continuous soil moisture measurements (Figure S5a and S5b  
394 for short- and longer-term, respectively). Indeed, soil moisture showed a similar pattern  
395 independently of the measurement frequency (i.e. monthly or continuous) both in the  
396 short- (Figure S5c and S5e, respectively) and in the longer-term (Figure S5d and S5f,  
397 respectively).

398         When evaluating the effect of warming on soil moisture measured monthly in  
399 the short-term, we found that this variable was 1.5% (95% CI= 0.97 to 1.55%) lower in  
400 warmed than in control plots (Figure S6a,  $p= 0.005$ ); significant warming effects were  
401 not observed when using continuous soil moisture data (1.6% reduction; Figure S6a  $p=$   
402 0.851). We did not find significant differences in soil moisture between control and  
403 warming plots in the longer-term, neither when using monthly nor continuous soil  
404 moisture data (Figure S6b,  $p=0.696$  and Figure S6b,  $p=0.163$ , respectively).

405         The effect size of warming on soil respiration, as measured with the response  
406 ratio, increased when the warming effect on soil temperature was higher under short-  
407 term warming (Figure 3a). Contrarily, the effect sizes of warming on soil respiration  
408 and soil temperature were not related under longer-term warming (Figure 3b). On the  
409 other hand, the effect sizes of warming on soil respiration and moisture were not related  
410 in the short- (Figure 3c) and longer-term (Figure 3d) periods.

411

#### 412 *Changes in biocrust cover as a driver of soil respiration responses to warming*

413 In the short-term, the total biocrust cover was similar in warmed (9.40%, 95% CI= 8.84  
414 to 9.96% and 66.27%, 95% CI= 63.80 to 68.80%, for low and high initial biocrust cover

415 respectively) and control (7.94%, 95% CI= 7.51 to 8.37% and 64.18%, 95% CI=62.43 to  
416 65.92%, for low and high initial biocrust cover respectively) plots (Figure S7a,  
417  $p=0.737$ ). In the longer-term, this pattern changed dramatically (Figure S7b), as  
418 warming significantly ( $p < 0.001$ ) decreased total biocrust cover by 26.78% (95% CI=  
419 25.85 to 27.70%) in plots with low initial biocrust cover and by 27.50% (95% CI=  
420 27.17 to 27.83%) in plots with high biocrust cover. The effect size of warming on total  
421 biocrust cover and soil respiration were unrelated in the short-term (Figure 4a).  
422 However, these effect sizes were significantly and positively related in the longer-term  
423 (Figure 4b), indicating that decreases in biocrust cover with warming matched with a  
424 reduction in soil respiration.

425

426 *Microbial thermal acclimation as a driver of longer-term soil respiration responses to*  
427 *field warming*

428 We did not find any significant differences in soil microbial biomass between warming  
429 and control plots independently of the method used to estimate it (Figure S8;  $p=0.813$ ,  
430  $p=0.810$  and  $p=0.712$  for the Yeast-SIR, CFE and qPCR method, respectively).

431 Although the positive effects of assay temperature on potential soil microbial respiration  
432 rates were the largest in magnitude by far (Figure 5, Table S5,  $p < 0.001$ ), we also found  
433 a negative effect of the warming treatment on soil microbial respiration (Figure 5, Table  
434 S5,  $p = 0.002$ ). This effect was, on average, a 30% lower across all assay temperatures.

435 Importantly, this reduction accounted for potential differences in soil microbial biomass  
436 (models statistically controlled for differences in microbial biomass), and substrate  
437 limitation (incubations were performed with substrate in excess), and were observed  
438 independently of the method used to estimate microbial biomass (Table S5).

439

440 **Discussion**

441 The positive effect of warming on soil respiration observed in the short-term in plots  
442 with high initial biocrust cover disappeared after ten years of experimental warming.  
443 This longer-term response to warming was linked to a decrease in  $Q_{10}$  in the warmed  
444 compared to the control plots. Additionally, we found support for several mechanisms  
445 driving short and longer-term soil respiration responses to warming such as warming-  
446 induced increases in soil temperature, microbial thermal acclimation and changes in  
447 total biocrust cover. These mechanisms are discussed in detail below.

448         Short-term studies have found contrasting soil respiration responses to warming  
449 in drylands, ranging from positive (Darrouzet-Nardi et al., 2015; Shen, Reynolds, &  
450 Hui, 2009) to negative (García-Palacios et al., 2018; Xu, Hou, Zhang, Liu, & Zhou,  
451 2016). The rare dryland studies that have evaluated warming effects for more than five  
452 years have found that soil respiration rates return to control levels after few years of  
453 warming (Darrouzet-Nardi et al., 2018; García-Palacios et al., 2018). We compared soil  
454 respiration responses to warming in the short- vs. longer-term and found a positive  
455 warming effect in areas with high initial biocrust cover after two years of warming. This  
456 positive short-term effect was not sustained through time, and it disappeared after ten  
457 years of elevated temperatures. Accordingly,  $Q_{10}$  values were significantly lower in the  
458 warmed plots compared to the control plots under longer-term warming. The mismatch  
459 between our short-term results and previous studies also conducted in drylands (García-  
460 Palacios et al., 2018; Xu et al., 2016) may be caused by different soil respiration  
461 responses to warming due to changes in the mechanisms driving such responses. Such  
462 changes in the mechanisms underlying soil respiration responses to warming may also  
463 explain the differences between the warming effects on soil respiration observed short-  
464 and longer-term in our study. Therefore, to better understand soil respiration responses

465 to warming both in the short and longer-term, the different drivers that could regulate  
466 such responses must be investigated.

467 Warming-induced increases in soil temperature led to a rise in soil respiration in  
468 the short-term, especially in areas with high biocrust cover. Such warming effects on  
469 soil respiration may be influenced by significant peaks of soil respiration after small  
470 rainfall or dew events in the study area (Cable & Huxman, 2004; Ladrón de Guevara et  
471 al., 2014). For instance, peaks in soil respiration have been observed in a biocrusted site  
472 in the Kalahari Sands (Botswana) after rainfall events of just 1.6 mm (Thomas, Hoon, &  
473 Dougill, 2011). Therefore, increases in soil temperature were the main driver underlying  
474 the short-term soil respiration responses to warming, given that the mean soil moisture  
475 observed (8.5% in the short-term) may be enough to support microbial activity.

476 However, we did not observe this direct effect of warming-induced elevated  
477 temperature on soil respiration in the longer-term. The disagreement between this result  
478 and the expectation that soil respiration should increase with warming (Kirschbaum,  
479 2006) may be a consequence of a longer-term effect of the warming treatment on the  
480 biocrust and soil microbial communities, compensating the direct effect of increased  
481 temperatures.

482 Experimental warming reduced soil moisture when measured monthly by 1.5%  
483 in the short-term, whereas it did not have any effect in the longer-term (0.2% reduction).  
484 However, no differences in soil moisture were observed when it was continuously  
485 measured neither in the short- nor in the longer-term. The difference in the results  
486 observed between both soil moisture sampling frequencies may be due to singularities  
487 of an specific sampling date, given that there were no differences in soil moisture  
488 measured continuously between warming and control plots during long periods both in  
489 the short- and the longer-term. Additionally, a similar pattern was observed

490 independently of the measurement frequency, suggesting that both methods are  
491 appropriate to measure soil moisture to test its effect on soil respiration responses to  
492 warming. This absence of differences in soil moisture during long periods both in the  
493 short- and longer-term may explain why the soil respiration responses to warming were  
494 independent of changes in soil moisture over both periods. Our results indicate that soil  
495 respiration responses to warming are not a product of a reduction in soil microbial  
496 activity with warming-induced soil drying, which disagrees with the results found in  
497 previous dryland studies (Pendall et al., 2013; Rey et al., 2011). This mismatch between  
498 our results and previous findings may be a consequence of the magnitude of the soil  
499 moisture change induced by warming. For instance, in Pendall et al., (2013) soil  
500 respiration responses to warming were mediated by a 15% decrease in soil moisture.  
501 Therefore, the warming-induced reduction of soil moisture observed in our study may  
502 not be large enough to drive soil respiration responses to warming. Additionally, soil  
503 respiration in drylands is not only controlled by rainfall events but also by dew  
504 generated in the early morning (Rey et al., 2011), as dew-like water inputs were enough  
505 to stimulate the respiration of biocrust-forming lichens and the soil microorganisms  
506 associated to them (Delgado-Baquerizo, Maestre, Rodríguez, & Gallardo, 2013; Ladrón  
507 de Guevara et al., 2014). Therefore, the increased activity of biocrusts, which are a  
508 major contributor to soil respiration in our study area, due to water inputs derived from  
509 dew events may explain why soil moisture was not driving soil respiration responses to  
510 warming either in the short- or in the longer-term. For all these reasons, we  
511 acknowledge how a more detailed temporal evaluation of air and soil moisture and  
512 temperature data (as well as dew events) will be needed in future studies to help  
513 disentangle the abiotic component of warming effects on soil respiration. According to  
514 our soil temperature and moisture results, warming-induced changes in soil

515 microclimatic variables do not seem to be the main mechanism underlying observed soil  
516 respiration responses to elevated temperatures in the longer-term. Therefore, we tested  
517 whether changes in biocrust cover and thermal acclimation of soil microbiota could be  
518 driving such responses.

519         We observed that soil respiration was larger in the plots with high initial biocrust  
520 cover (compared with those with a low initial biocrust cover) during both warming  
521 periods, albeit temporal trends of soil respiration were similar at both biocrust cover  
522 levels. These results agree with those observed in previous studies showing greater  
523 respiration rates in areas with visible and well-developed biocrusts (Castillo-Monroy,  
524 Maestre, et al., 2011; Feng et al., 2013). Accordingly, higher soil respiration rates have  
525 also found in areas with lichen-dominated biocrusts than in those dominated by mosses  
526 or algae (Feng et al., 2014). Therefore, the differences in soil respiration between low  
527 and high biocrust cover plots observed in our study may be a result of the biological  
528 activity of the mosses and lichens directly or through their effect on soil microbial  
529 communities (Castillo-Monroy, Bowker, et al., 2011). On the other hand, our results  
530 showed that soil respiration responses to short-term warming were independent of  
531 changes in biocrust cover, as biocrusts were not affected by the warming treatment in  
532 the short-term. Contrary, we observed that soil respiration responses to warming were  
533 mediated by warming-induced reductions in biocrust cover in the longer-term. The  
534 observed decrease in biocrust cover with warming may not seem consistent with  
535 previous findings showing that lichens are well adapted to elevated temperatures and are  
536 resistant to desiccation (Green, Sancho, & Pintado, 2011). However, it agrees with other  
537 studies conducted in drylands which found an important reduction in biocrust cover  
538 after some years of warming (Ferrenberg et al., 2015; Maestre, Escolar, et al., 2015).  
539 Although clarifying the physiological mechanisms underlying this dramatic decrease in

540 biocrust cover under longer-term warming is not a goal of this study, we speculate that  
541 warming effects led to a significant reduction of C fixation and subsequent mortality of  
542 lichens (Ladrón de Guevara et al., 2014). This biocrust cover reduction would,  
543 therefore, lower the autotrophic soil respiration that, may explain the decreased soil  
544 respiration rates observed in the warmed plots in the longer-term. To sum up, our results  
545 suggest that biocrusts modulate soil respiration rates and that warming-induced changes  
546 in their cover are one of the main drivers governing observing soil respiration responses  
547 to longer-term warming.

548         Finally, we found a negative effect of field warming on soil microbial respiration  
549 at a common biomass and excess substrate in the laboratory incubations. This result  
550 highlights that soil microbial respiration acclimated to warming conditions in the  
551 dryland ecosystem studied and suggests that thermal acclimation may drive the lack of  
552 warming effects on soil respiration over the longer-term. Importantly, this result was  
553 observed regardless of the method used to measure microbial biomass, suggesting that  
554 substrate-induced respiration is an appropriate estimate of microbial biomass to test for  
555 thermal acclimation of soil respiration (Bradford et al., 2008, 2009). The negative field  
556 warming effect on soil microbial respiration observed is consistent with biochemical  
557 acclimation to different thermal regimes reached through evolutionary trade-offs  
558 (Hochachka & Somero, 2002). However, we cannot state that biochemical acclimation  
559 is the only mechanism operating to explain our results as they may be caused by shifts  
560 at the individual, population and community levels due to the ‘aggregate’ respiratory  
561 activity of soil microbial communities and the spatial scale we were analysing.  
562 Although the observed negative effect of warming on potential soil microbial  
563 respiration rates may seem incompatible with the expected positive link between  
564 temperature and soil microbial respiration rates (Davidson & Janssens, 2006;

565 Kirschbaum, 2006; Lloyd & Taylor, 1994; Tucker et al., 2013), such positive  
566 relationship was supported by the conspicuous positive effect of assay temperature on  
567 respiration rates observed in our study. The thermal acclimation of soil respiration  
568 observed in this study provides empirical support to previous global extrapolations  
569 showing that soil C losses to the atmosphere via soil respiration with elevated  
570 temperature may be lower in drylands than in other biomes (Crowther et al., 2016).  
571 Indeed, in a global study analysing data from 27 different temperature manipulation  
572 experiments, spanning nine biomes, drylands and boreal are the only ecosystems where  
573 differences in temperature sensitivity between warmed and control plots have been  
574 found (Carey et al., 2016). Therefore, they only found evidence for thermal acclimation  
575 of soil respiration in drylands and boreal forests, agreeing with our results.

576         In conclusion, we found that short-term increases in soil respiration with  
577 warming disappeared after ten years of continuous warming in a biocrust-dominated  
578 dryland. This pattern was associated with a longer-term decrease in temperature  
579 sensitivity of soil respiration ( $Q_{10}$ ). Our results suggest that the main driver regulating  
580 short-term soil respiration responses to warming was the increase in soil temperature,  
581 whereas both thermal acclimation and a dramatic loss of biocrust cover drove soil  
582 respiration responses to warming in the longer-term. Our results highlight the need to  
583 evaluate the effects of warming at both the short- and longer-term to better understand  
584 soil respiration responses to this climate change driver and the important role that  
585 longer-term experiments play for doing so. They also emphasize the need to include  
586 both thermal acclimation and biocrust communities in models aiming to forecast soil  
587 greenhouse gas emission predictions in drylands, as this would improve our capacity to  
588 forecast future temperatures and expand our understanding of C - climate feedbacks.

589

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601 **Competing interests**

602 The authors declare no competing financial interests.

603

604 **Author**

605 F.T.M. designed the field study and wrote the grant that funded the work. F.T.M, P.G.P  
606 and M.D. developed the original idea of the analyses presented in the manuscript. M.D.  
607 performed the statistical analyses, with inputs from F.T.M and P.G.P. M. D., S.A., C.C.-  
608 D., B.G. and V. O. conducted the field and laboratory work. All authors contributed to  
609 data interpretation. M.D. wrote the first version of the manuscript, which was revised by  
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611

612 **Data Sharing and Data Accessibility**

613 The data that support the findings of this study and the R code are openly available in  
614 Figshare at <http://doi.org/10.6084/m9.figshare.11536989>.

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890 **Figure legends:**

891 **Figure 1.** Warming effects on soil respiration rates (a-b) and the temperature sensitivity  
892 of soil respiration ( $Q_{10}$ , c-d) in the short-term (0 – 2 years after experimental set-up, a  
893 and c) and longer-term (8- 10 years after experimental set-up, b and d) at both biocrust  
894 cover levels (i.e. low and high). Box plots represent medians, 25th and 75th percentiles  
895 (n=210 and 240 per combination of treatments at the short and longer-term, respectively  
896 for soil respiration measurements and n =10 per combination of treatments at each  
897 period for  $Q_{10}$ ). Error bars represent 10th and 90th percentiles. Asterisks denote  
898 significant differences at  $p < 0.05$ . These analyses were performed with an 11% data  
899 imputation. The same results performed using original data without imputing missing  
900 values are shown in Fig. S3.

901

902 **Figure 2.** Soil respiration, temperature and moisture measured across short- (after  
903 experimental set-up, a, c, e) and longer-term (8 – 10 years after experimental set-up, b,  
904 d, f) periods. Data are means  $\pm$  SE (n=10). WA = warming. Low and high refers to  
905 initial biocrust cover  $< 20\%$  and  $>50\%$ , respectively.

906

907 **Figure 3.** Relationship between the effect size of warming (RR) on soil respiration and  
908 soil temperature in the short- (a) and longer-term (b), and between RR of soil respiration  
909 and RR of soil moisture in the short- (c) and longer-term (d). RR data are in ln- scale.  
910 The solid line denotes a significant linear model fitted between both variables. ( $R^2$  and p  
911 values on the graph).

912

913 **Figure 4.** Relationship between the effect size of warming (RR) on soil respiration and  
914 on biocrust cover in the short- (a) and longer-term (b). RR data are in ln- scale. The

915 solid line denotes a significant linear model fitted between both variables ( $R^2$  and  $p$   
916 values on the graph).

917

918 **Figure 5.** Estimated effects of longer-term warming on potential soil respiration rates at  
919 a common soil microbial biomass value and with substrate (glucose) in excess of  
920 microbial demand. Effect sizes were estimated using coefficients from the ‘Yeast-SIR’  
921 model (Table S5). Specifically, the unstandardized coefficients were used in a  
922 regression equation, along with the mean of the observed values for microbial biomass,  
923 one of the treatments (i.e. control or warming) and one of the assay temperatures (i.e.  
924 10, 20, 30 or 40°C). Given that there were no differences between both initial biocrust  
925 cover levels, we only represent the data at the low initial biocrust cover. Error bars show  
926 the standard deviation. Asterisks denote significant differences at  $p < 0.05$ .

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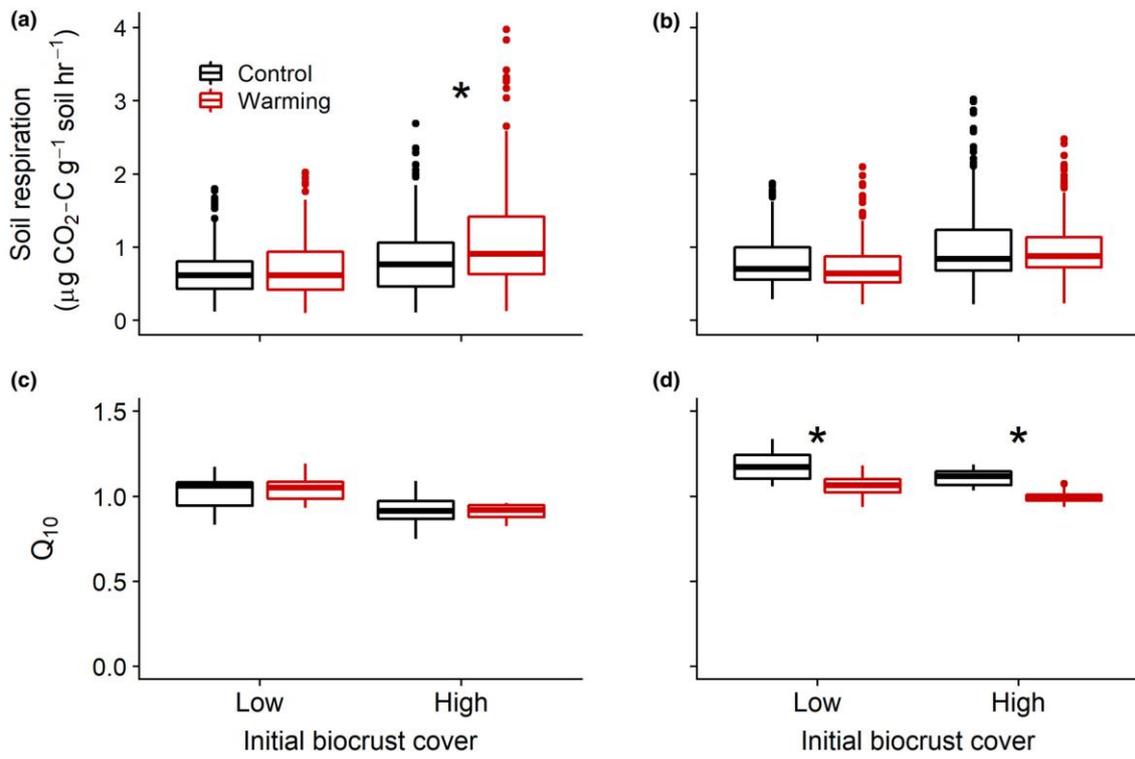
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942 **Figure 1**

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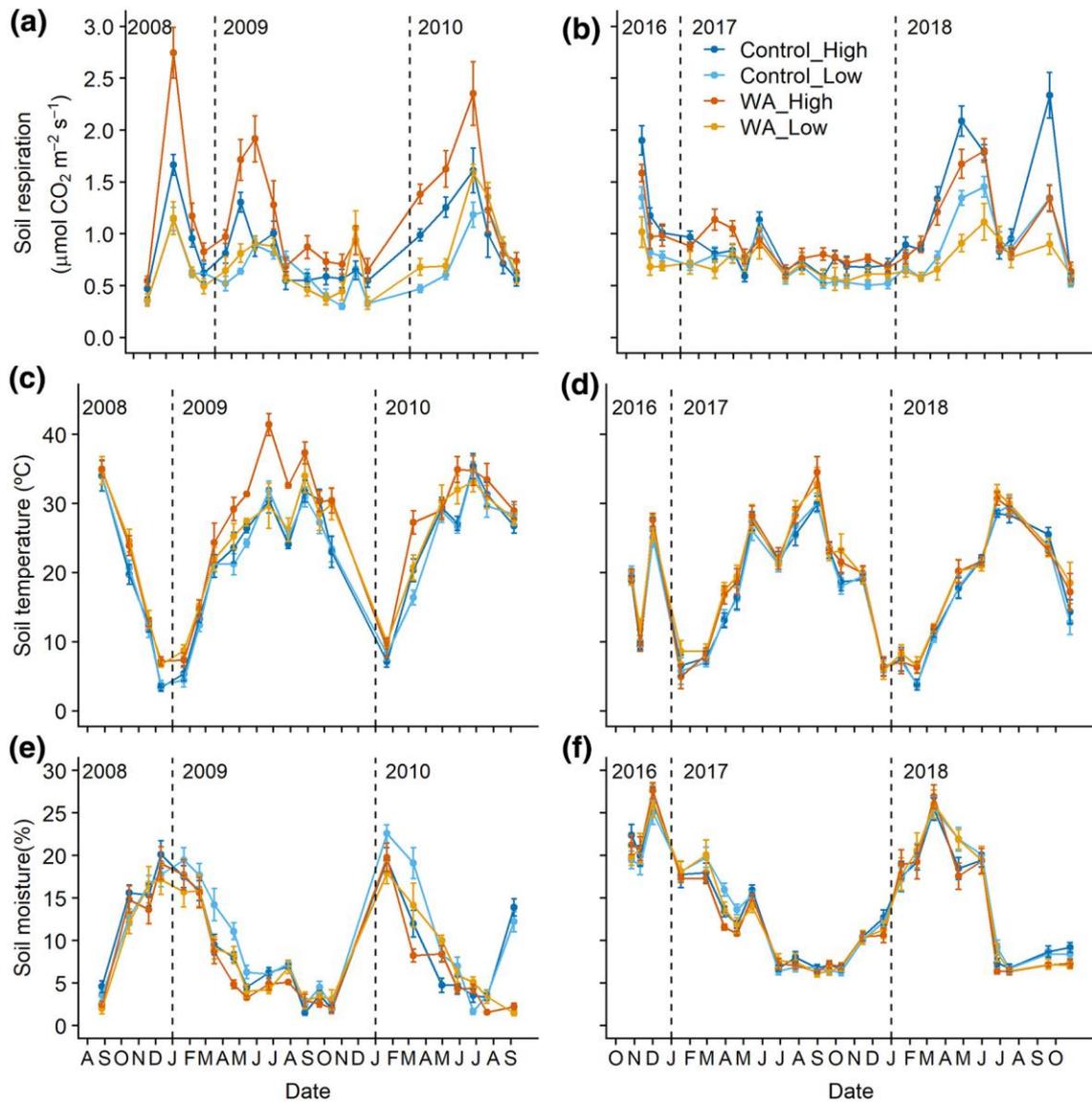
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957 **Figure 2**

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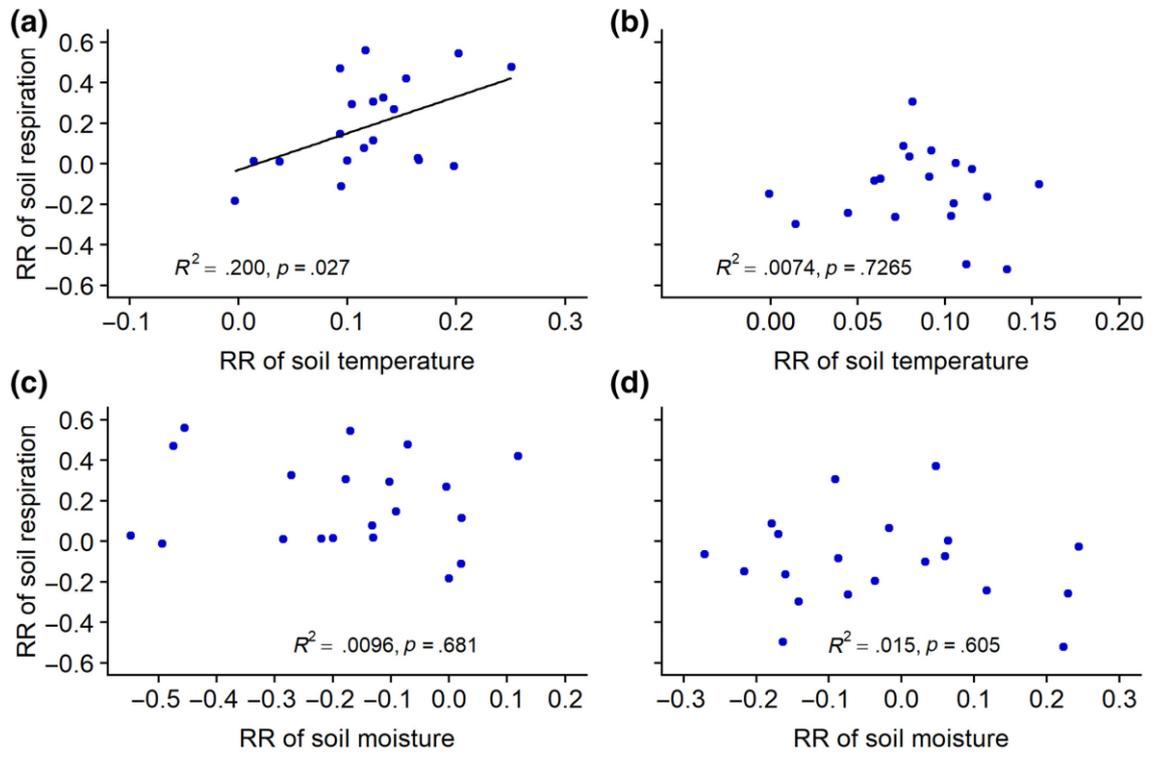
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967 **Figure 3**

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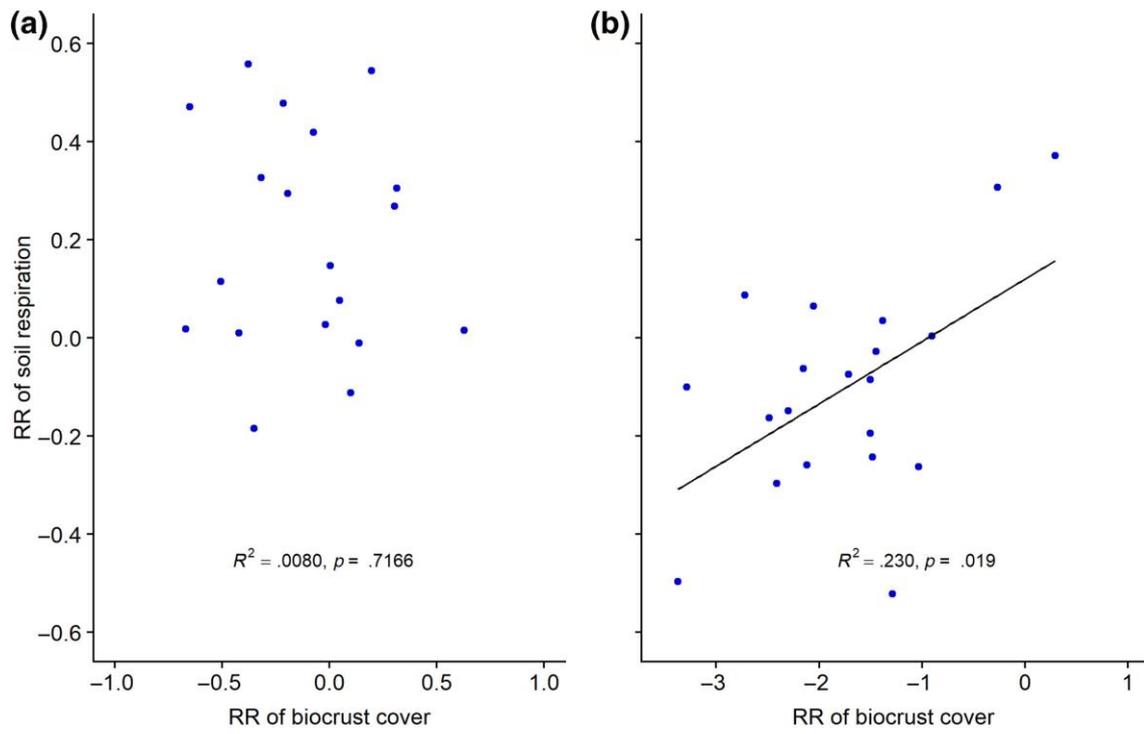
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983 **Figure 4**

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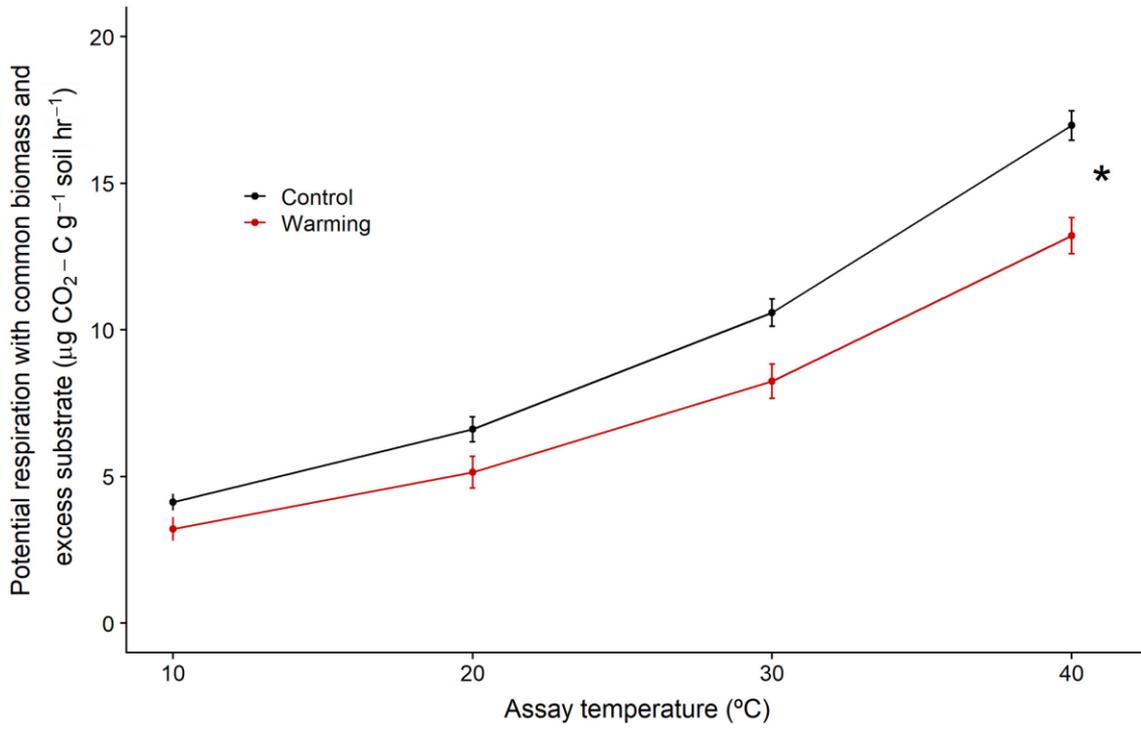
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999 **Figure 5**

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