

1 **Temperature increases soil respiration across ecosystem types and soil development,**
2 **but soil properties determine the magnitude of this effect**

3

4 **Running head:** Temperature effect on soil respiration

5

6 Marina Dacal^{1,2*}, Manuel Delgado-Baquerizo³, Jesús Barquero³, Asmeret Asefaw Berhe⁴,

7 Antonio Gallardo³, Fernando T. Maestre^{2,5} & Pablo García-Palacios⁶

8

9 ¹Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey

10 Juan Carlos, C/ Tulipán s/n, 28933 Móstoles, Spain

11 ²Instituto Multidisciplinar para el Estudio del Medio “Ramon Margalef”, Universidad de

12 Alicante, Carretera de San Vicente del Raspeig s/n, 03690 San Vicente del Raspeig, Spain

13 ³Departamento de Sistemas Físicos, Químicos y Naturales, Universidad Pablo Olavide,

14 41704 Sevilla, Spain.

15 ⁴Department of Life and Environmental Sciences; University of California, Merced CA

16 95343, USA

17 ⁵Departamento de Ecología, Universidad de Alicante, Carretera de San Vicente del

18 Raspeig s/n, 03690 San Vicente del Raspeig, Spain

19 ⁶Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Serrano

20 115 bis, 28006, Madrid, Spain.

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23 * Correspondence e-mail: marina.dacal1@gmail.com

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25

26 **Abstract**

27 Soil carbon losses to the atmosphere, via soil heterotrophic respiration, are expected to
28 increase in response to global warming, resulting in a positive carbon-climate feedback.
29 Despite the well-known suite of abiotic and biotic factors controlling soil respiration,
30 much less is known about how the magnitude of soil respiration responses to temperature
31 changes over soil development and across contrasting soil properties. Here, we
32 investigated the role of soil development stage and soil properties in driving the responses
33 of soil heterotrophic respiration to increasing temperatures. We incubated soils from eight
34 chronosequences ranging in soil age from hundreds to million years, and encompassing
35 a wide range of vegetation types, climatic conditions, and chronosequences origins, at
36 three assay temperatures (5, 15 and 25°C). We found a consistent positive effect of assay
37 temperature on soil respiration rates across the eight chronosequences evaluated.
38 However, soil properties such as organic carbon concentration, texture, pH, phosphorus
39 content, and microbial biomass determined the magnitude of temperature effects on soil
40 respiration. Finally, we observed a positive effect of soil development stage on soil
41 respiration that did not alter the magnitude of assay temperature effects. Our work reveals
42 that key soil properties alter the magnitude of the positive effect of temperature on soil
43 respiration found across ecosystem types and soil development stages. This information
44 is essential to better understand the magnitude of the carbon-climate feedback, and thus
45 to establish accurate greenhouse gas emission targets.

46

47 **Keywords:** climate warming, land carbon-climate feedback, microbial biomass, nutrient
48 availability, soil chronosequences, soil texture.

49

50 **Introduction**

51

52 Temperature is a key driver of heterotrophic soil respiration (hereafter soil respiration),
53 –a major process of carbon (C) loss to the atmosphere (Bond-Lamberty, Bailey, Chen,
54 Gough, & Vargas, 2018; Bond-Lamberty & Thomson, 2010; Zhou et al., 2016). Global
55 warming is expected to accelerate the rate of soil respiration (Davidson & Janssens, 2006;
56 Kirschbaum, 2006), reinforcing climate change with a land C-climate feedback
57 embedded in the Intergovernmental Panel on Climate Change (IPCC) projections (Ciais
58 et al., 2014). Despite the recognized importance of an accurate representation of this
59 feedback in Earth System Models to establish appropriate greenhouse gas emission
60 targets (Bradford et al., 2016), the extent to which climate change will increase soil C
61 losses to the atmosphere via soil respiration is still highly uncertain (Arora et al., 2013;
62 Exbrayat, Pitman, & Abramowitz, 2014). Learning more about how and why soil
63 properties regulate the magnitude of soil respiration responses to elevated temperatures
64 is essential to accurately predict the land C-climate feedback in a warmer world.

65 To build confidence in the projected magnitude of the land C-climate feedback,
66 the response of soil respiration to climate warming should be addressed across large
67 spatial scales and encompassing a wide range of soil development stages. Beyond
68 temperature, it is also critical to determine the influence of other key abiotic and biotic
69 factors that regulate soil respiration (Guo et al., 2017; Rustad, Huntington, & Boone,
70 2000; Schindlbacher, Schnecker, Takriti, Borken, & Wanek, 2015). These include key
71 soil abiotic drivers such as organic carbon (SOC), texture (i.e., the percentage of sand,
72 silt, and clay), pH, and phosphorus (P), as well as biotic properties such as microbial
73 biomass (Bradford, Watts, & Davies, 2010; Karhu et al., 2014). For instance, soil texture
74 influences soil respiration by controlling water and nutrient availability (Delgado-

75 Baquerizo et al., 2013) and regulating the potential of soil minerals to physically and
76 chemically stabilize organic carbon (Rasmussen et al., 2018). A previous study showed
77 that soils with higher proportion of clay sized particles also had higher microbial activity
78 due to greater water and nutrient availability, leading to higher soil respiration (Karhu et
79 al., 2014). Further, soil respiration increases as microbial biomass rises (Wang, Dalal,
80 Moody, & Smith, 2003). Despite the knowledge accumulated about soil respiration
81 drivers, much less is known about how soil properties modulate soil respiration responses
82 to warming.

83 Soils are known to develop from centuries to millennia, resulting in important
84 changes in key abiotic properties (Crews et al., 1995; Vitousek, 2004; Wardle, Bardgett,
85 et al., 2004). For example, young soils are known to accumulate organic carbon during
86 soil development from centuries to millennia (Schlesinger, 1990), and older soils are
87 expected to support more acid, and P depleted soils compared with younger substrates
88 (Doetterl et al., 2018; Laliberté et al., 2013). Importantly, although soil properties do
89 change as soil develops over geological timescales, the parent material does not vary.
90 Because of this, soil development has been suggested as a good model system to
91 investigate the role of soil abiotic and biotic properties in driving the responses of soil
92 respiration to disturbances such as increasing temperatures (Orwin et al., 2006). A
93 number of studies performed at individual soil chronosequences have investigated
94 whether soil development stage influences soil respiration rates, showing contrasting
95 results. Whereas some studies found an enhancing effect of soil development on soil
96 respiration (J. L. Campbell & Law, 2005; Law, Sun, Campbell, Van Tuyl, & Thornton,
97 2003), others observed that soil respiration rates decreased as soil develops (Tang et al.,
98 2008; Wang, Bond-Lamberty, & Gower, 2002). These differences are likely due to site-
99 specific variations in soil development trajectories between chronosequences with

100 contrasting parent material and climatic conditions (Alfaro, Manzano, Marquet, &
101 Gaxiola, 2017). Therefore, to gain a more comprehensive understanding of how soil
102 development affects soil respiration and its response to temperature, such effects should
103 be evaluated both within single chronosequences but also across multiple
104 chronosequences occurring in different ecosystem types with contrasting environmental
105 conditions (e.g. climate, parent material, soil origin, etc.).

106 Beyond soil properties and soil development, other mechanisms may also
107 modulate soil respiration responses to temperature. For instance, substrate depletion and
108 thermal acclimation have been demonstrated to alter soil respiration responses to
109 temperature (Bradford et al., 2010; Hartley, Hopkins, Garnett, Sommerkorn, & Wookey,
110 2008). Temperature accelerates microbial activity, leading to an increase in soil
111 respiration (Hochachka & Somero, 2002). However, microorganisms develop several
112 mechanisms to acclimate to the ambient temperature regime such as changes in enzyme
113 and membrane structures. Hence, when subjected to the same temperature range, the
114 microbial activity and soil respiration of acclimated microorganisms would be lower
115 compared to the not acclimated ones (Hochachka & Somero, 2002). Therefore, thermal
116 acclimation to the ambient temperature regime may help to reduce the magnitude of soil
117 respiration responses to temperature (Bradford et al., 2019; Dacal, Bradford, Plaza,
118 Maestre, & García-Palacios, 2019). At the same time, such acceleration in microbial
119 activity with temperature may also cause an important reduction in the availability of
120 readily decomposable C sources, leading to substrate depletion (Cavicchioli et al., 2019;
121 Schindlbacher et al., 2015). Consequently, substrate depletion can limit microbial
122 processes such as soil respiration (Walker et al., 2018). Given that such mechanisms may
123 mitigate soil respiration responses to temperature, they should also be evaluated to
124 improve the accuracy in the predictions of the land C-climate feedbacks.

125 Herein, we used soil development as an ecological model system to test the
126 importance of soil properties in driving the responses of soil respiration to changes in
127 temperature. To such an end, we take advantage of soils collected from eight
128 chronosequences (Delgado-Baquerizo et al., 2019, 2020) located in Arizona (AZ; USA),
129 California (CAL; USA), Colorado (CO; USA), Hawaii (HA; USA), New Mexico (JOR;
130 USA), Chile (CH), Spain (CI) and Australia (WA) to perform an independent laboratory
131 assay based on short-term soil incubations at three assay temperatures (5, 15 and 25°C).
132 These chronosequences range from hundreds to million years and encompass a wide
133 range of vegetation types (i.e., grasslands, shrublands, and forests), climatic conditions
134 (arid, continental, temperate and tropical), and origins (i.e., sand dunes, sedimentary and
135 volcanic; see Table 1 for more details). Further, we addressed whether soil respiration
136 and its response to temperature change over soil development either within or across
137 chronosequences. Finally, we assessed whether thermal acclimation influences soil
138 respiration responses to temperature across contrasting ecosystem types and soil
139 development stages.

140

141 **Materials and methods**

142 *Study design and field soil collection*

143 The environmental conditions of the eight chronosequences used spanned a wide gradient
144 in climatic conditions (MAT from 8.7 to 19.55°C, and MAP from 276 to 1907 mm) and
145 soil properties (SOC from 0.6 to 25.3 and the percentage of clay plus silt from 3.8 to 44.1,
146 Table 1). The selected chronosequences included four to six stages of soil development.
147 Stage number one corresponds to the youngest soil, whereas four, five, or six correspond
148 to the oldest one within each chronosequence. Each chronosequence was considered a
149 site, so the total number of sites and stages surveyed in our study is 8 and 41, respectively.

150 At each stage, we established a 50 m x 50 m plot for conducting field surveys. Three
151 parallel transects of 50 m length, spaced 25 m apart, formed the basis of the plot. The
152 total plant cover and the number of perennial plant species (plant diversity) were
153 determined in each transect using the line-intercept method (Delgado-Baquerizo et al.,
154 2019). All of the sites were surveyed between 2016 and 2017 using a standardized
155 sampling protocol (Delgado-Baquerizo et al., 2019). At each plot, three composite soil
156 samples (five soil cores per sample: 0 – 10 cm depth) were collected under the canopy of
157 the dominant ecosystem vegetation type (e.g., grasses, shrubs, and trees). Soil samples
158 were collected during the same days within each soil chronosequence. After field
159 collection, soils were sieved at 2 mm, and a fraction was immediately frozen at -20°C for
160 soil microbial biomass analyses. The rest of the soil was air-dried for a month and used
161 for biochemical analyses and laboratory incubations.

162

163 *Soil abiotic properties*

164 We measured the following abiotic soil properties in all samples: soil organic C (SOC),
165 texture (% of clay + silt), pH, and available soil phosphorus (soil P). To avoid
166 confounding effects associated with having multiple laboratories performing soil
167 analyses, all dried soil samples were shipped to Spain (Universidad Rey Juan Carlos) for
168 laboratory analyses. The concentration of SOC was determined by colorimetry after
169 oxidation with a mixture of potassium dichromate and sulfuric acid at 150° C for 30
170 minutes (Anderson & Ingram, 1993). Soil pH was measured with a pH meter in a 1:2.5
171 suspensions of dry soil mass to deionized water volume. Soil texture (% clay + silt) was
172 determined on a composite sample per chronosequence stage, according to Kettler,
173 Doran, & Gilbert (2001). Olsen P (soil P hereafter) was determined by extraction with
174 sodium bicarbonate, according to Olsen, Cole, Watanabe, & Dean (1954). Mean annual

175 temperature (MAT) and mean annual precipitation (MAP) values for the soils of each site
176 were obtained using Wordclim version 2.0 (Fick & Hijmans, 2017), which provides
177 global average climatic data for the 1970-2000 period.

178

179 *Soil microbial biomass*

180 We estimated soil microbial biomass by measuring phospholipid fatty acids (PLFAs).
181 These were extracted from freeze-dried soil samples using the method described in Bligh
182 & Dyer (1959), as modified by Buyer & Sasser (2012). The extracted PLFAs were
183 analysed on an Agilent Technologies 7890B gas chromatograph with an Agilent DB-5
184 ms column (Agilent Technologies, CA, USA). The biomarkers selected to indicate total
185 bacterial biomass are the PLFAs i15:0, a15:0, 15:0, i16:0, 16:1 ω 7, 17:0, i17:0, a17:0,
186 cy17:0, 18:1 ω 7 and cy19:0, and the biomarker to indicate total fungal biomass is the
187 PLFA 18:2 ω 6. Using the selected PLFA biomarkers, the biomass was calculated for each
188 soil sample (Frostegård & Bååth, 1996; Rinnan & Bååth, 2009). Total microbial biomass
189 includes the sum of all bacterial and fungal biomarkers plus that of other soil microbial
190 biomarkers such as the eukaryotic C18:1 ω 9.

191

192 *Laboratory incubations and soil heterotrophic respiration measurements*

193 We conducted short-term (10 h) incubations of our soil samples, in accordance with
194 previous studies (Atkin & Tjoelker, 2003; Bradford et al., 2010; Hochachka & Somero,
195 2002; Tucker, Bell, Pendall, & Ogle, 2013), at 5, 15, and 25°C at 60% of WHC. The short
196 timescale used was chosen to prevent acclimation to the assay temperatures used in the
197 laboratory. The incubation temperatures (5, 15 y 25°C) were selected to cover the range
198 spanned by the MAT values of the eight chronosequences studied (from 8.7 to 19.55°C).
199 Additionally, such incubation temperatures are similar to the ones used in previous

200 studies (Bradford et al., 2008, 2019; Dacal et al., 2019). Soil samples were incubated in
201 96-deepwell microplates (1.3 mL wells) by adding *c.* 0.5 g soil per well. All soil samples
202 were run in triplicate (laboratory replicates). Incubations were performed in growth
203 chambers under dark conditions and 100% air humidity. Microplates were covered with
204 polyethylene film to prevent soil drying but to allow gas exchange.

205 Soil respiration rates were measured using a modified MicroRespTM technique (C.
206 D. Campbell, Chapman, Cameron, Davidson, & Potts, 2003). Glucose at a dose of 10 mg
207 C g⁻¹ dry soil was used as a substrate. It was used to avoid substrate limitation on soil
208 respiration rates (Bradford et al., 2010), as the dose used in our study is supposed to
209 exceed microbial demand (Davidson, Janssens, & Luo, 2006). Soils were incubated at the
210 particular assay temperature (5, 15, and 25°C) for ten hours. However, the detection plates
211 used to measure soil respiration were only incubated during the last 5 hours to avoid the
212 oversaturation of the detection solution. The absorbance of the detection plate was read
213 immediately before and after its use. Three analytical replicates were run per sample, and
214 the mean of these repeats per assay temperature was used as the observation of potential
215 respiration rate for each sample.

216

217 *Statistical analyses*

218 We evaluated the importance of soil properties in driving the responses of soil
219 respiration to changes in temperature. To do that, we firstly analysed soil respiration
220 responses to assay temperature within and across chronosequences. For within
221 chronosequences analyses, we built eight linear regression models (LM) including soil
222 development stage, assay temperature, the interaction between both variables, SOC,
223 texture, pH, soil P, and microbial biomass as fixed factors. Soil properties were removed
224 until there is a low collinearity between them and soil development stage (i.e. square-root

225 VIFs <2, Bradford et al., 2017). However, to evaluate the assay temperature effect on soil
226 respiration across chronosequences, we performed a linear mixed-effects model (LMM)
227 with soil development stage (in years), MAT, assay temperature, SOC, texture, pH, soil
228 P, and microbial biomass as fixed factors, and the chronosequence identity as a random
229 factor. We then compared whether there were differences in the magnitude of the effect
230 of assay temperature on soil respiration among chronosequences, using the standardized
231 coefficients of assay temperature obtained in the within chronosequence LMs. Finally,
232 we tested whether biotic and abiotic factors drive the response of soil respiration to
233 temperature. For doing so, we built LMMs that incorporated soil development stage (in
234 years) and assay temperature as fixed factors, and chronosequence identity as a random
235 factor using different subsets of data. Specifically, we grouped the chronosequences in
236 two levels according to each of the environmental conditions and soil properties
237 considered such as the origin of the chronosequence, MAT, SOC, texture, pH, P, and
238 microbial biomass. Then, we ran the model described above separately for each group of
239 data to evaluate how the magnitude of the effect of temperature on soil respiration
240 changes between the models using groups of data with contrasting environmental
241 conditions and soil properties. In most cases, each of the groups of data included four
242 chronosequences each (i.e., half of the chronosequences studied each). We classified each
243 chronosequence by the mean across the whole chronosequence of each of the selected
244 variables to avoid separating different stages of the same chronosequence in different
245 groups. The threshold to distinguish between both groups of each category was
246 established at the value closest to the mean among all observations that allow having the
247 same or almost the same number of chronosequences in each group.

248 On the other hand, to evaluate the effect of soil development on soil respiration
249 and its response to temperature we used the same approach described above for evaluating

250 the effect of assay temperature on soil respiration (LMs within chronosequences and an
251 LMM across chronosequences). Additionally, we used two different approximations for
252 soil development stage depending on the spatial scale. When analysing each
253 chronosequence separately, we used the stage (from 1 to 6) to address the effects of soil
254 development stage (Delgado-Baquerizo et al., 2019; Laliberté et al., 2013; Wardle,
255 Bardgett, Walker, & Bonner, 2009; Wardle, Walker, & Bardgett, 2004), given the high
256 level of uncertainty in assigning precise ages for many of the chronosequences studied
257 (Wardle, Walker, et al., 2004). However, when analysing across chronosequences, we
258 used the estimation of years as a measure of soil development stage (Crews et al., 1995;
259 Tarlera, Jangid, Ivester, Whitman, & Williams, 2008) to compare chronosequences
260 covering contrasting ranges of soil development stages.

261 Finally, to test whether the thermal acclimation of soil respiration to the ambient
262 temperature regime influences the soil respiration responses to assay temperature over
263 soil development, we performed an LMM as that described above. We statistically
264 controlled for differences in soil microbial biomass by including it as a covariate in the
265 model (Bradford et al., 2019, 2010; Dacal et al., 2019). All the statistical analyses were
266 conducted using the R 3.3.2 statistical software (R Core Team, 2015). The linear mixed-
267 effects models (LMMs) were fitted with a Gaussian error distribution using the ‘lmer’
268 function of the lme4 package (Bates, Mächler, Bolker, & Walker, 2015). Response data
269 were transformed by taking the natural logarithm of each value when needed to meet the
270 assumptions of normality and homogeneity of variance.

271

272 **Results**

273 *Effects of abiotic and biotic drivers on soil respiration responses to temperature*

274 First, we found a consistent and positive significant effect of assay temperature on soil
275 respiration both within and across chronosequences ($P < 0.001$ in all cases, Figure 1 and
276 2, Table S1 and S2, respectively). The magnitude of this positive effect varied between
277 chronosequences (Figure 3). For instance, the assay temperature effect in a Mediterranean
278 sedimentary chronosequence from California (CAL) was 84.5% (95% CI= 51.07%-
279 117.96%) and 144.44% (95% CI = 94.63% - 146.63%) greater than in a Mediterranean
280 sandy chronosequence in Western Australia (WA) or a volcanic forest chronosequence
281 from Hawaii (HA), respectively (Figure 3).

282 The effect of assay temperature on soil respiration was consistently positive across
283 all the climatic conditions and soil properties evaluated (Figure 4). However,
284 environmental variables altered the magnitude of the assay temperature effect on soil
285 respiration. For instance, the effect of assay temperature was 12.08% (95% CI = 5.40% -
286 18.77%) lower for the volcanic chronosequences compared with the ones with a
287 sedimentary or a dune origin (Figure 4). However, the greatest differences on the
288 magnitude of such effect were observed in sites with contrasting soil texture (Figure 4).
289 Specifically, soils with $> 20\%$ silt and clay showed a 43.65% (95% CI = 35.18% -
290 52.12%) higher effect of assay temperature on soil respiration compared with soils with
291 $< 20\%$ silt and clay. On the other hand, the effect of assay temperature on soil respiration
292 was 23% (95% CI = 15% - 30%) greater in sites with higher SOC, microbial biomass,
293 and soil P content compared with soils with lower values of such soil properties (Figure
294 4). The magnitude of the assay temperature effect slight differed (i.e., 9% difference; 95%
295 CI = 5% - 17%) between soils with contrasting pH values (Figure 4). On the other hand,
296 the magnitude of the assay temperature effect on soil respiration did not change across
297 soils with contrasting MAT values (Figure 4).

298

299 *Effect of soil development on soil respiration and its response to temperature*

300 When analysing the effect of soil development on soil respiration at every
301 chronosequence separately, we did not observe any significant effect in five out of eight
302 chronosequences (Figure 1, Table S1). We found higher soil respiration rates in older
303 soils than in younger ones in three volcanic chronosequences located in temperate and
304 tropical forests in Chile (i.e., CH, $P = 0.016$, Figure 1, Table S1), Spain (i.e., CI, $P =$
305 0.049 , Figure 1, Table S1) and Hawaii (i.e., HA, $P = 0.009$, Figure 1, Table S1). We also
306 observed a positive effect of soil development on respiration across chronosequences (P
307 $= 0.004$, Figure 2, Table S2). Regardless these results, soil development did not affect
308 respiration responses to temperature neither within nor across chronosequences, as the
309 interaction between soil development and assay temperature was not significant ($P > 0.05$
310 in all cases).

311

312 *Thermal acclimation of soil respiration to ambient temperature regimes*

313 The site MAT did not affect soil respiration ($P = 0.487$, Table S2) nor its response to
314 assay temperature (MAT \times assay temperature, $P = 0.807$), suggesting the absence of
315 acclimation of soil respiration to the ambient temperature regime. The lack of MAT effect
316 on soil respiration was constant across all soil development stages (MAT \times soil
317 development, $P = 0.122$).

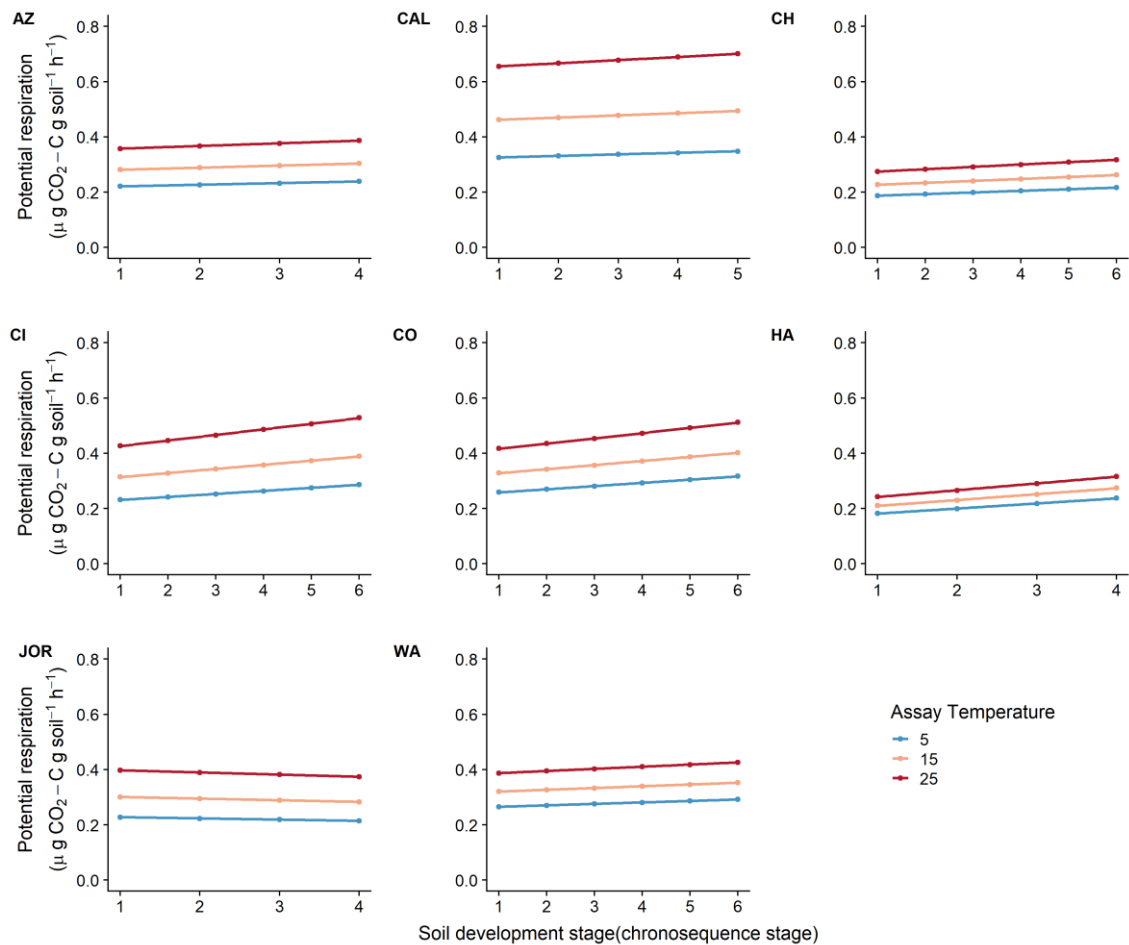
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320 **Table 1. Climate origin, vegetation type, age, and environmental conditions for**
 321 **eight soil chronosequences.** Chronosequence origin describes the major causal agent of
 322 each chronosequence. Climate and vegetation types show the main climatic conditions
 323 and the dominant vegetation for each chronosequence. MAT= Mean annual
 324 temperature, MAP= Mean annual precipitation, SOC= Soil organic carbon, Soil P= Soil
 325 phosphorus, and Microbial biomass= Sum of all bacterial, fungi, and other soil
 326 microbial biomarkers.

Chronosequences								
Label	AZ	CAL	CH	CI	CO	HA	JOR	WA
Country	USA	USA	Chile	Spain	USA	USA	USA	Australia
Name	SAGA	Merced	Conguillio	La Palma	Coal Creek	Hawaii	Jornada Desert	Jurien Bay
Age	0.9-3000ky	0.1-3000ky	0.06-5000ky	0.5-1700ky	5-2000ky	0.3-4100ky	1.1-25ky	0.1- 2000ky
Chronosequence origin	Volcanic	Sedimentary	Volcanic	Volcanic	Sedimentary	Volcanic	Sedimentary	Sand dunes
Climate	Arid	Temperate	Temperate	Temperate	Continental	Tropical	Arid	Temperate
Vegetation type	Forests	Grasslands	Forests	Forests	Grasslands	Forests	Forblands	Shrublands
MAT (°C)	10.4±1.4	16.3±0.3	8.7±0.8	13.8±1.6	9.3±0.5	15.9±0.5	15.43±0.0	19.6±0.1
MAP (mm)	421±57	378±64	1907±16	451±34	482±7	1895±380	276±4	558±4
SOC (%)	2.6±1.9	4.9±2.9	3.8±3.5	5.1±5.5	3.7±1.0	25.3±12.5	0.6±0.2	1.2±0.6
Texture (% clay+silt)	40.4±28.1	44.1±17	8.3±2.6	23.1±11.7	34.6±3.3	14.3±3.8	18.9±3.5	3.8±1.4
pH	7.2±0.3	6±0.8	5.8±0.4	6.7±0.4	6±0.3	4.2±0.6	8.1±0.4	7.3±1.2
Soil P (%)	0.09±0.02	0.06±0.03	0.02±0.01	0.20±0.05	0.06±0.01	0.07±0.03	0.05±0.01	0.02±0.02
Microbial biomass (nmolPLFA/g soil)	356±371	1733±886	1293±1752	622±738	667±289	5991±1784	126±52	112±63

327



328

329 **Figure 1.** Estimated effects of assay temperature and soil development stage
330 (chronosequence stage) on potential respiration rates at a controlled biomass value and
331 with substrate in excess within chronosequence. The effects were estimated using
332 coefficients from the linear model used for each chronosequence (Table S1). Three
333 outcomes of this model are shown, one for each temperature assayed (i.e. 5, 15, and 25°C).
334 Specifically, we estimated soil respiration rates using the unstandardized coefficients of
335 the model, along with the mean value of the soil properties included in the model of each
336 chronosequence, one of the assay temperatures and one of the soil development stages
337 observed in each chronosequence.

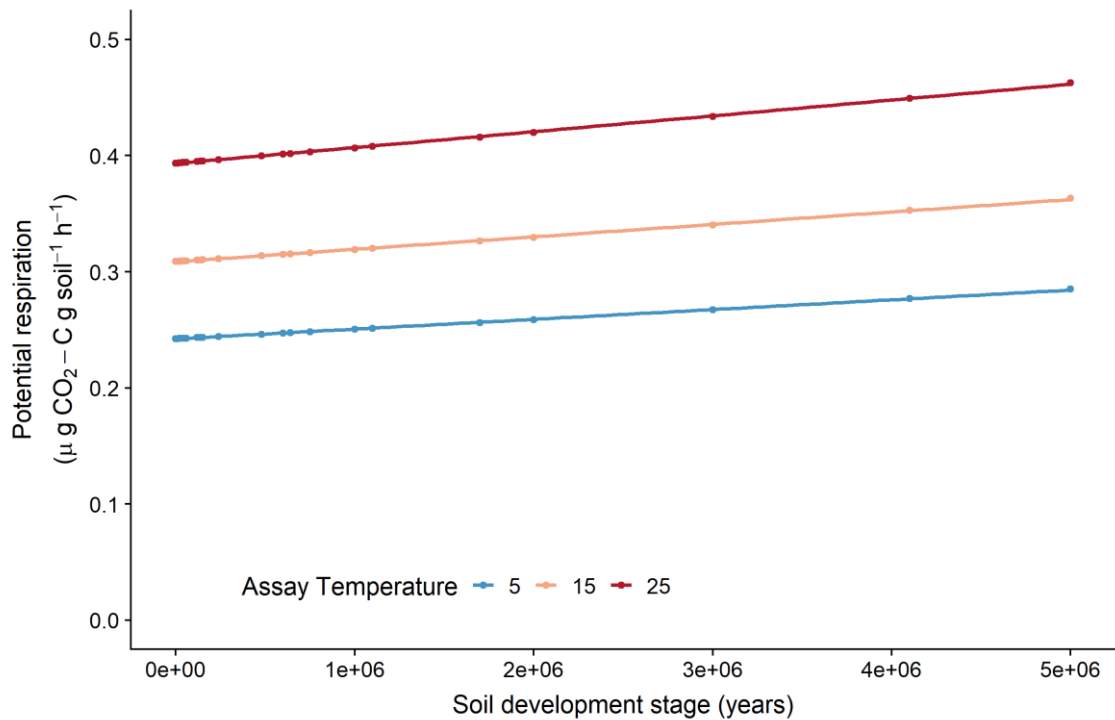
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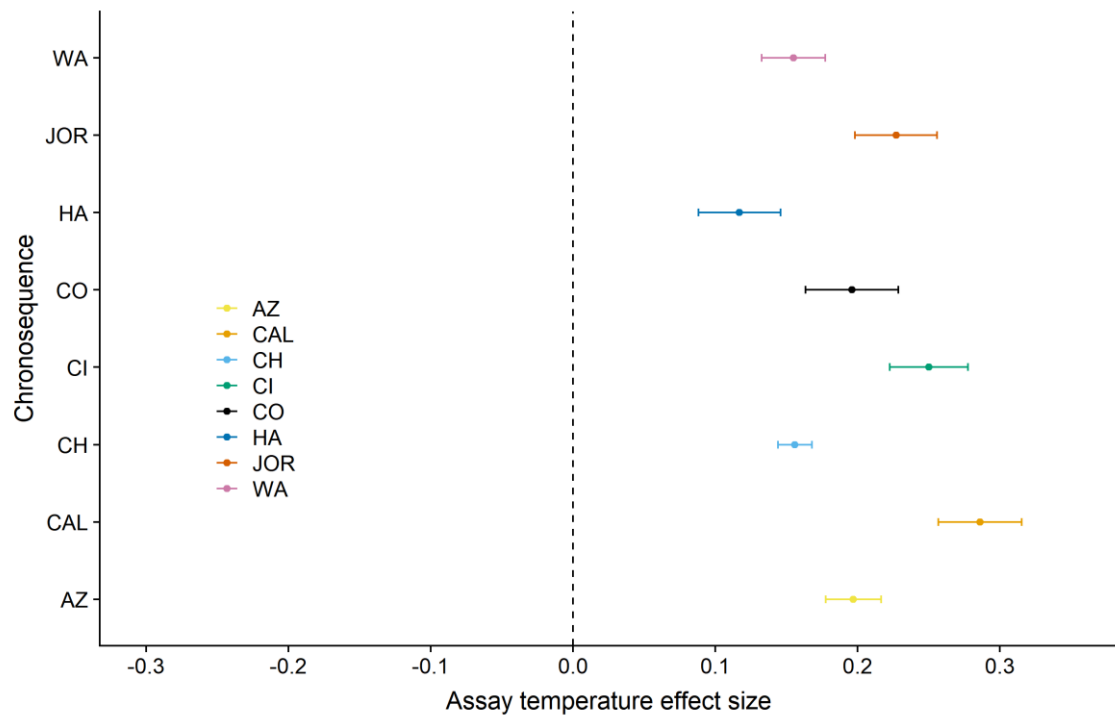
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344 **Figure 2.** Estimated effects of assay temperature and soil development stage (years) on
345 potential respiration rates at a controlled biomass value and with substrate in excess across
346 chronosequences. The effects were estimated using coefficients from the linear mixed-
347 effects model (Table S2). Three outcomes of this model are shown, one for each
348 temperature assayed (i.e. 5, 15, and 25°C). Specifically, we estimated soil respiration rates
349 using the unstandardized coefficients of the model, along with the mean value of the soil
350 properties included in the model of each chronosequence, one of the assay temperatures
351 and one of the soil development stages observed across all sites.

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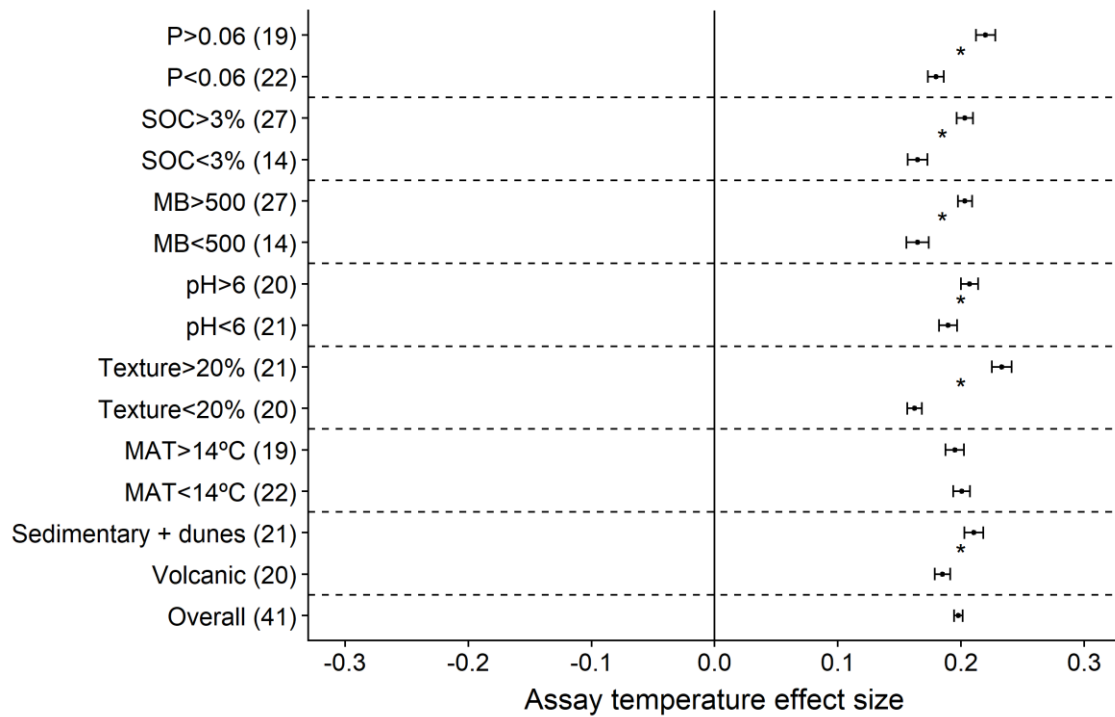


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356 **Figure 3.** Comparison on the magnitude of the effects of assay temperature on soil
357 respiration among the eight chronosequences studied. The points represent the mean and
358 the error bars correspond to the 95% CI. AZ, JOR, HA presented four stages (n=4), CAL
359 had five stages (n=5) and the rest showed six stages (n= 6).

360

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362

363 **Figure 4.** Comparison of the effects of assay temperature on soil respiration among
 364 different environmental conditions. The points represent the mean and the error bars
 365 correspond to the 95% CI. Asterisks denote significant differences at $p < 0.05$. The total
 366 n was shown in brackets and it was the result of the number of stages within the
 367 chronosequences x the number of chronosequences included in each level of the
 368 classification. MAT= mean annual temperature, Texture=% of clay + silt, MB= total
 369 microbial biomass, SOC= soil organic carbon, and P= soil phosphorus. Volcanic and
 370 sedimentary + dunes refer to the different origins observed across the eight
 371 chronosequences studied.

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377 **Discussion**

378 Our study shows that elevated temperatures consistently increased soil heterotrophic
379 respiration rates across contrasting soil chronosequences. Although older soils tended to
380 support higher soil respiration—especially in volcanic, temperate, and tropical forests—,
381 our findings indicate that soil development did not alter the relationship between
382 heterotrophic respiration and temperature. Conversely, soil properties such as SOC, the
383 amount of clay and silt, pH, microbial biomass, and P content had a significant control
384 on the magnitude of positive temperature effects on soil respiration. Overall, these
385 findings provide new insights into the role of soil properties in driving soil respiration
386 responses to temperature, which are essential to project the magnitude of the land C-
387 climate feedback accurately.

388

389 We observed a consistent positive effect of assay temperature on soil respiration
390 within and across chronosequences. Such results agree with previous literature addressing
391 the effects of temperature on soil organic matter decomposition and soil respiration rates
392 (Davidson & Janssens, 2006; Kirschbaum, 2006; Lloyd & Taylor, 1994; Min et al., 2020).
393 The enhancing effect of temperature on soil respiration is largely driven by the
394 acceleration of microbial metabolic rates (Hochachka & Somero, 2002). Importantly, the
395 effect of elevated temperatures on soil respiration was positive in all chronosequences
396 studied, suggesting that this enhancing effect, at least in our study, is independent of the
397 ecosystem type. However, certain chronosequences showed differences in the magnitude
398 of the assay temperature effect between them. That could be explained by our results
399 indicating that environmental conditions and soil biotic and abiotic properties have the
400 ability to determine the magnitude of the consistently positive effect of temperature on
401 soil respiration. For instance, soil respiration responses to assay temperature differed

402 depending on the origin of the chronosequence considered. Such results suggest that
403 parent material also influences soil respiration responses to temperature. An explanation
404 for these observed differences could be that soil develops differently according to several
405 factors such as soil parent material (Alfaro et al., 2017; Carlson, Flagstad, Gillet, &
406 Mitchell, 2010; Jenny, 1941). Moreover, we found that the magnitude of the effect of
407 assay temperature was lower in sites with less soil P available. Such results indicate that
408 this nutrient is necessary to sustain microbial activity (Liu, Gundersen, Zhang, & Mo,
409 2012). Further, we also observed differences in the magnitude of the response of soil
410 respiration to elevated temperatures between sites with contrasting amounts of clay and
411 silt. These differences could be caused by the fact that water availability in the soil is
412 expected to increase when the amount of clay and silt in the soil rises (Delgado-Baquerizo
413 et al., 2013), accelerating microbial activity (Karhu et al., 2014; Luo, Wan, Hui, &
414 Wallace, 2001). However, this effect of the amount of clay and silt on soil respiration
415 responses to temperature could disappear at high amounts of clay and silt, as clay and silt
416 may limit microbial access to SOC. Also, the magnitude of the effect of assay
417 temperature on soil respiration increased in sites with greater soil pH, as the microbial
418 activity is negatively affected by acidification (Reth, Reichstein, & Falge, 2005; Rustad
419 et al., 2000). Finally, our results indicated that soil respiration response to assay
420 temperature increases with substrate availability (i.e., SOC) and microbial biomass. This
421 increase in soil respiration rates in response to temperature under high SOC and microbial
422 biomass conditions may cause the acceleration of microbial activity and, subsequently, a
423 substrate depletion and an important reduction of microbial biomass (Cavicchioli et al.,
424 2019). Thus, our findings provide new insights about how soil properties modulate the
425 magnitude of the consistently enhancing effect of temperature on soil respiration.

426

427 In three out of the eight chronosequences evaluated, we found a significant
428 positive effect of soil development on soil respiration rates. Interestingly, all these
429 chronosequences shared a volcanic origin. The different effect of soil development on
430 soil respiration found across chronosequences may be mediated by contrasting parent
431 material between them, leading to variations in the soil development trajectories followed
432 by the eight chronosequences evaluated. The differences in the range of years covered by
433 each of the chronosequences evaluated may also influence the effect of soil development
434 on soil respiration. Such contrasting results observed when analysing each
435 chronosequence separately limits our capacity to draw more general conclusions about
436 how soil C losses to the atmosphere via soil respiration change over soil development,
437 specially under a warming scenario. Such limitations are similar to the ones found in
438 previous studies (J. L. Campbell & Law, 2005; Law et al., 2003; Saiz et al., 2006; Tang
439 et al., 2008; Wang et al., 2002) conducted on a single chronosequence and covering a
440 narrow range of soil development stages (from years to centuries). Therefore, when
441 evaluating soil development effect on soil respiration across chronosequences, we
442 observed a significant enhancing effect of soil development stage on soil respiration. Our
443 findings improve our knowledge about the effect of soil development stage on soil
444 respiration across large spatial scales including different ecosystem types with contrasting
445 environmental conditions and soil properties. Specifically, our results indicated that elder
446 soils have greater soil C losses to the atmosphere than younger ones. Such greater soil
447 respiration rates found in elder soils within some and across chronosequences may be
448 explained by the increase in soil C easily releasable from mineral-SOC associations in
449 soils that had experienced higher weathering (Keiluweit et al., 2015). Conversely, we
450 observed that soil development did not modulate the magnitude of the effect of assay
451 temperature on soil respiration, as the interaction between soil development stage and

452 assay temperature was not significant either within or across chronosequences. These
453 results indicate that, no matter how old soils are, soil carbon stocks are highly sensitive
454 to increases in temperature associated with climate change. Thus, although worldwide
455 soils show contrasting ages (Laliberté et al., 2013; Wardle, Bardgett, Walker, Peltzer, &
456 Lagerström, 2008), they present similar soil respiration responses to temperature. Further,
457 the assay temperature effect was at least three times larger in magnitude than the effect
458 of soil development stage on soil respiration. Such results agree with previous studies
459 showing pronounced soil respiration responses to assay temperature (Bradford et al.,
460 2010), especially across large temperature ranges such as those used in our incubations
461 (i.e. from 5 to 25°C). Consequently, our study supports that soil microbial communities
462 from very different ecosystem types are capable of rapidly responding to increasing
463 temperature, resulting in greater soil respiration.

464

465 A growing body of evidence suggests that thermal acclimation of soil microbial
466 respiration to temperature can be found across large spatial scales (Bradford et al., 2019,
467 2010; Dacal et al., 2019; Ye, Bradford, Maestre, Li, & García-Palacios, 2020). However,
468 we did not find a significant effect of MAT, suggesting that soil respiration is not
469 acclimated to the ambient temperature regime at our sites. This apparent disagreement
470 may be due to the shorter MAT gradient evaluated in our study (i.e., from 8.7°C to
471 19.55°C) compared with previous ones (i.e., from -2 to 28°C; Bradford et al., 2019; Dacal
472 et al., 2019; Ye, Bradford, Maestre, Li, & García-Palacios, 2020). Nevertheless, our
473 results are similar to other cross-biome studies (Carey et al., 2016; Karhu et al., 2014),
474 and may be the result of negligible effects of thermal acclimation on soil respiration when
475 compared with overarching factors such as assay temperature (Hochachka & Somero,
476 2002).

477 In conclusion, we found that assay temperature consistently enhanced soil
478 respiration across contrasting chronosequences. On the other hand, we observed no
479 evidence of thermal acclimation of soil respiration to the ambient temperature regime.
480 Although we observed a positive effect of soil development on soil respiration, it did not
481 change the magnitude of the assay temperature effect. Despite the clear and positive effect
482 of assay temperature on soil respiration observed, soil properties such as SOC, texture,
483 pH, P content, and microbial biomass significantly modified the magnitude of this
484 positive soil respiration response to temperature. Our findings emphasize the role of biotic
485 and biotic soil properties as drivers of soil respiration responses to temperature across
486 biomes and provide new insights to better understand the magnitude of the land C-Climate
487 feedback and to establish accurate greenhouse emission targets.

488

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506

507 **Authorship**

508 M.D., M.D.-B. and P.G.P developed the original idea of the analyses presented in the
509 manuscript. M.D.-B. designed the field study and wrote the grant that funded the work.
510 J.B. conducted the laboratory work with inputs from M.D.-B and A.G. M.D. performed
511 the statistical analyses, with inputs from M.D.-B., F.T.M and P.G.P. All authors included
512 A.A.B. contributed to data interpretation. M.D. wrote the first version of the manuscript,
513 which was revised by all co-authors.

514

515 **Competing interests**

516 The authors declare no competing financial interests.

517

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