# **SI Appendix Temperature response of soil respiration largely unaltered with experimental warming Authors:** Joanna C. Carey, Jianwu Tang, Pamela H. Templer, Kevin D. Kroeger, Thomas W. Crowther, 6 Andrew Burton, Jeffrey S. Dukes, Bridget Emmett<sup>,</sup> Serita Frey, Mary Heskel, Lifen Jiang, Megan Machmuller, Jacqueline E. Mohan, Anne Marie Panetta, Peter B. Reich, Sabine Reinsch, Xin Wang, Steven D. Allison, Chris Bamminger, Scott D. Bridgham, Scott L. Collins, Giovanbattista de Dato, William C. Eddy, Brian J. Enquist, Marc Estiarte, John Harte, Amanda Henderson, Bart R. Johnson, Klaus S. Larsen, Yiqi Luo, Sven Marhan, Jerry Melillo, Josep Peñuelas, Laurel Pfeifer-Meister,Christian Poll, Edward B. Rastetter, Andy Reinmann, Lorien L. Reynolds, Inger K. Schmidt, Gaius R. Shaver, Aaron L. Strong, Vidya Suseela, Albert Tietema **Supporting Methods** *Dataset Generation and Description* A literature search was conducted on September 22, 2014 using Web of Science, which produced five studies presenting non-aggregated instantaneous data that were extractable (Table S1). Published datasets (16-17) and unpublished values make up the majority of the data in the dataset. We obtained unpublished data by first creating a list of all known experimental warming studies globally and asking the principal investigators to supply soil respiration data with corresponding soil temperature and moisture values. Because of widely variable experimental designs across studies, we averaged all plot-scale values for 23 each sampling event to obtain one average  $(\pm SD)$  for each treatment for each sampling event ('sampling events' typically refer to a single day of sampling, although several studies complete full suites of sampling (i.e., 'sampling events') from all plots in both morning and afternoon). Only soil respiration values with corresponding soil moisture and soil temperature values from experimental warming studies

were included in our analysis. Only observations from single-factor treatments (i.e., warming) were used,

- excluding values that combined warming with other treatments (e.g., precipitation or nitrogen
- 29 manipulation). Four studies included more than one level of warming treatment (e.g., both 1.5 and  $3^{\circ}$ C
- warming treatments); in these cases, data from all levels of warming were used for our temperature
- 31 response function analyses. All data were reported as instantaneous change in  $CO<sub>2</sub>$  efflux over a fixed

 area, with belowground (i.e., roots and rhizomes), but not aboveground vegetation, included. Thus, soil respiration values presented here include both heterotrophic and autotrophic soil respiration.

 Experiment locations ranged from 33.5 to 68.4 °N latitude (Fig. S5) and the duration of warming at experiments ranged from <1 to 22 years (average 5.1 years) (Fig. S6). Depths of soil temperature (1-10 cm) and moisture measurements (5-30 cm) ranged across studies, but were always consistent between warmed and control plots within a particular study. The majority of the observations were taken between 5 and 10 years after warming commenced (n=1534), followed by 2-5 year duration (n=1109), less than 2 years (n=896) and >10 years (n=278). Each site was classified into a particular biome (grassland, northern shrubland (i.e., peatlands and heathlands), southern shrubland (i.e., Mediterranean or sub-tropical shrublands), tundra, desert, meadow, temperate agriculture, temperate forest and boreal forest) by the associated principal investigator. Tropical biomes are not represented in our analysis because no data from experimental warming studies in the tropics are yet available. However, the first known tropical warming experiment, Tropical Responses to Altered Climate Experiment (TRACE), is currently being set up in Luquillo Experimental Forest in Puerto Rico, with heating scheduled to commence during spring 2016.

 Seasonality was defined by principal investigators contributing data as those months that fall into the following categories: growing (plants actively growing), non-growing (plants not actively growing), or shoulder (takes into account months of transition and intra-annual variability) season. Data from the growing season accounted for more than half of our observations (n=1840), followed by shoulder season (n=1112), and non-growing season (n=865). Absolute differences in soil temperature, moisture, and respiration across sites were always calculated as values from warmed plots minus values from control 55 plots for each sampling event: e.g.,  $\Delta T = T_w - T_c$ .

*Evaluating role of Soil Moisture, Seasonality, and Warming Duration in Controlling Soil Respiration*

 We investigated the role of soil moisture in controlling the response of soil respiration in four ways. First, we evaluated the significance of soil moisture as a predictor of soil respiration by adding moisture as an additional continuous variable in a multiple linear regression model (Model e in Table S3, Table S2): 

62 (3) 
$$
\ln(R) = a_0 + a_1 T + a_2 T^2 + a_3 M
$$

64 where *R* is soil respiration (µmol C m<sup>2</sup> s<sup>-1</sup>), *T* is soil temperature (°C), and *M* is soil moisture (cm<sup>3</sup> cm<sup>-3</sup>). In cases where significant differences in the response functions of warmed vs. control treatments were observed (boreal and desert biomes), separate models that included moisture were run for each treatment (Table S2). Because respiration rates are often not linearly related to moisture content, we also conducted our analysis with an additional model (Eq. 4), which resulted in no differences in our conclusions (Table S6). Next, we created partial regression plots (i.e., added-variable plots) for both temperature and moisture (Fig. S7), allowing for visual inspection of the role of moisture compared to temperature in controlling the respiration response. Third, we examined how moisture alters the temperature sensitivity of respiration by running a separate model of respiration as a function of temperature with moisture as the interaction term (Model f in Table S3). To evaluate this response visually, we then partitioned the data into moisture quantiles and plotted the temperature sensitivities of respiration at these four different moisture levels (Fig. S3), reporting the coefficients in Table S4. Finally, we normalized each instantaneous difference in respiration between warmed and control plots (∆R) by ∆T, and binned those values by amount of moisture available in warmed plots as a fraction of control plots (Fig. 3). Moisture bins containing less than 5% of total observations from each biome are not shown (not applicable in Fig. 3, where all bins represent at least 5% total data). This analysis allowed us to understand how differences in the magnitude of respiration between treatments change with moisture availability (Fig. S3).

 We evaluated the influence of warming duration and seasonality on the respiration response between 83 treatments in two ways: 1) by partitioning the observations into categories of warming duration  $\langle 2, 2-5, \rangle$  5-10, and >10 years) and season (growing, non-growing, and shoulder) and running the multivariate regression model shown in Table 1 for each category separately, and 2) by running additional multivariate models (Models h and i in Table S3) that included duration or season as a fixed factor, with an interaction with warming treatment.

#### **Supporting Results**

#### *Magnitudes of Temperature and Respiration Change with Experimental Warming*

 Experimental warming generally stimulated soil respiration, with a larger ∆T significantly correlated to a 92 larger respiration effect size ( $p<0.01$  and  $r=0.66$ ; Fig. S2B, Table S1). Across all sites, experimental 93 warming increased soil temperatures by 1.91  $\degree$ C on average, although average soil warming by biome 94 ranged from  $0^0C$  in southern shrublands to 4.09  $^0C$  in temperate forests, with relatively large inter-biome differences (Table S1). On average, the magnitude of soil warming at many sites was too low (when ∆T  $\leq$ 1.72 °C) to statistically increase respiration rates (Fig. S2B). In turn, the relatively low degree of average warming across many sites resulted in an insignificant grand mean effect size for soil respiration (RR= 0.05 [95% CI: -0.03-0.14], n=26), regardless of season and warming duration, with just five sites (Site IDs 2, 6, 7, 8, 27 Table S1) having a significantly positive response of respiration in the warmed plots. Methodological differences in warming methods resulted in a range of ∆T, and thus, ∆R across sites. In our dataset, experiments that warmed via electric cables observed the greatest average soil warming (∆T 102 =3.6 °C, n=5), compared to infrared ( $\Delta T = 2.3$ °C, n=11) and passive ( $\Delta T = 0.4$ °C, n=11) warming methods. Electric cable was the dominant warming method in the temperate forest (4 out of 5 sites) and temperate agriculture (one site) biomes and in turn, these biomes were the only ones when analyzed individually to display a significant increase in respiration (∆R) with warming using traditional meta- analysis (temperate forest: RR=0.18; 95% CI: 0.06-0.30, temperate agriculture: RR=0.21; 95% CI: 0.06- 0.37).

#### *Standardized Mean Difference of Temperature Sensitivity*

 Beyond investigating differences in the log-quadratic temperature response function (Eq. 1) between warming treatments, we also conducted a traditional meta-analysis on site-level temperature sensitivity parameters using the standardized mean difference (SMD) as our index of effect size, which normalizes raw mean differences by the pooled standard deviation. Examining data from across all sites, the grand 114 mean effect size was not significantly different from zero (SMD= -0.29 [95% CI: -1.21, 0.64], n=27), demonstrating further evidence for the general lack of difference in temperature sensitivities between warmed and control plots with experimental warming (Fig. S8). Although the grand mean effect size was not significantly different from zero, 12 sites showed significantly higher SMDs of temperature sensitivity in warmed plots (Site IDs 5, 8, 9, 13, 14,16, 19, 21, 23, 26-28), while eight sites (Site ID 1, 2, 11, 12, 15, 20, 22, 24) demonstrated significantly lower SMD in warmed plots compared to control plots.

## *Role of Moisture in Controlling Respiration Rates*

 Meta-analysis of soil moisture data reveals that moisture was significantly reduced with warming (RR=- 0.08, [95% CI:-0.12- -0.03]), with 7 out of 27 sites having significantly less soil moisture at the warmed compared to control plots. However, such decreases were only marginally significantly correlated with ∆T (r= -0.32, p=0.08) (Fig. S2A). Multivariate linear regression highlights that moisture typically explains a much smaller fraction (0-8%) of the total respiration response compared to temperature (34- 127 82%), except in the case of southern shrublands, where moisture is a stronger predictor of respiration than 128 soil temperature  $(R^2 \text{ model a or b versus Model e in Table S3, Fig. S7).}$  We used partial regression plots (Fig. S7) to help visualize the effect of adding an additional variable (i.e., soil moisture) to a multiple regression model. Partial regression with temperature and moisture highlight the more important role of temperature in driving the soil respiration response compared to moisture (Fig. S7). This response is demonstrated by the lower slopes on the added-variable moisture plots (right hand panels). An exception to this is southern shrublands, where moisture added-variable plot has a much steeper slope compared to

 other biomes, aligning with the multivariate regression output showing moisture playing a more important role in predicting respiration compared to temperature in the southern shrublands.

 Ambient soil moisture is a critical factor in mitigating the respiration-temperature relationship. For example, a negative ∆R/∆T response with soil drying is only apparent in the desert, grassland, and southern shrubland biomes (Fig. S9), likely because these biomes have the lowest ambient soil moisture content (Table S1) and thus, even minor desiccation with warming suppresses C fluxes. On the other hand, in the forest biomes where soil drying with warming was most severe (warmed plots have on average 84% and 87% of the moisture that was observed in control plots in the boreal and temperate forests, respectively), fluxes were still consistently higher from warmed plots despite drying (Fig. S9), due in part to relatively elevated ambient soil moisture conditions at these sites (Table S1).

 Soil moisture often has a non-linear relationship with soil respiration. In order to determine if our multivariate linear model (Table S2) was a factor influencing our results, we re-ran our analysis using an additional function (Eq. 4, see below), which shows little difference in model fits (Table S6). Our study does not take into account differences in soil type between sites, as differences in soil type between warmed and control plots within a site should be minimal. In addition, soil moisture content largely reflects soil type across sites, as sandier soils hold less water than more clay-type soils. We see this in our 152 data, as average soil moisture content in several biomes was negatively related to percent sand (r=0.98, 0.62, r=0.55 in northern shrublands, grasslands and forests, respectively). Our analyses of soil moisture are based on soil water content (SWC), otherwise known as soil moisture concentrations. However, soil matric potentials are a much better indicator of water availability in soils, as this metric takes into account soil texture and organic matter content, which can affect relative water availability at the site level (1, 2). Because both factors undoubtedly change across sites, soil matric potentials are likely a more sensitive metric to evaluate how differences in moisture availability influence soil respiration rates.

#### *Role of Warming Duration and Seasonality on Soil Respiration Rates*

 Multivariate analysis of respiration that included warming duration as a predictor, with an interaction with warming treatment (Model h in Table S3) revealed a significant interaction between duration and warming treatment in four biomes: desert, boreal forest, temperate forest, and northern shrubland. Except for northern shrublands, the other three biomes displayed significantly depressed soil respiration rates with increasing warming duration. Considering that it is in these three biomes where we observed moderate (temperate forest) to strong (boreal forest and desert) evidence of altered temperature response functions to soil warming, it appears that duration of experimental warming is an important factor in driving these results. We also evaluated how duration of warming changes the temperature response function of respiration in warmed versus control treatments by re-running our analysis shown in Table 1 with data partitioned into the following groupings of years of warming duration (<2, 2-5, 5-10, and >10). This analysis continues to support prior conclusions, with no significant differences in the temperature response function in any biome regardless of warming duration, except the boreal forests and desert, and 173 moderate (p=0.06) differences from 2-5 years of warming duration in temperature forest.

 We investigated how season influenced soil respiration rates in a similar fashion to duration. First, we added season as a predictor to our multilinear regression model, with an interaction with warming treatment (Model i in Table S3). Here we found a significant interaction between season and warming treatment in the desert and boreal forest biomes only, indicating that in these two biomes respiration from warmed and control plots responds differently to temperature depending on the time of year. Next, we re- ran our analysis shown in Table 1 with data partitioned into season (non-growing, growing, shoulder) and found a similar result; for all biomes except the desert and boreal forests, no differences in temperature sensitivity were observed when analyzing any particular season in isolation. In the boreal forest, differences in temperature sensitivity were driven by growing season data, which make up the majority of the data (70%) for the boreal forest biome. On the other hand, the differences in sensitivity observed in the desert biome are driven by data from the non-growing season; this was the only season, when

 examined in isolation, where significant differences in the temperature sensitivity of respiration from warmed versus control plots are observed in the desert biome.

*Model Choice*

 We used several different multivariate models (Table S3) to answer specific questions during our analysis. To address our first objective (i.e., determine whether respiration response from warmed plots paralleled that from control plots), we used a temperature-treatment interaction model (Models c or d in 193 Table S3, depending on whether the  $2<sup>nd</sup>$ -order temperature term was significant when including the treatment interaction term). We also compared the fits (specifically AICs) of Models c or d with models excluding warming treatment as a predictor (Models a or b) to determine if warming treatments had an effect on the respiration response (Table S3). Lower AICs in Models a or b (Table S3) compared to Models c or d (Table S3) provides further evidence that experimental warming does not alter the shape of the curve to a large degree in those biomes. Parameter values for Models a and b (Table S3) also shown in Table S5. Next, to evaluate our second objective (i.e., investigate the role of soil moisture in influencing how respiration responds to temperature across treatments), we included soil moisture as a predictor, with an interaction term with temperature in our multivariate models (Models e and f in Table S3). Finally, to determine how warming duration and seasonality were influencing our results, we ran three additional models with these terms as predictors (Model g in Table S3), with an interaction term with warming treatment (Models h and i in Table S3).

206 We did not use the traditional exponential model (the  $Q_{10}$  model) or the Arrhenius model to fit our data as these models cannot adequately reflect our findings that the temperature sensitivity decreased when 208 temperature is above  $\sim$  25 $^{\circ}$ C. The inability of these models to represent varying temperature sensitivities across the temperature gradient has been discussed previously (3, 4). This study focused on understanding the temperature response of soil respiration with experimental warming, rather than modeling soil respiration. However, we also simulated our data using the following equation (5):

$$
R = e^{\alpha (T - T_o)} \left( \frac{T_m - T}{T_m - T_o} \right)^{\alpha (T_m - T_o)} \left( \frac{M}{k_m + M} \right)
$$

214

215 With R = non-transformed soil respiration rate, T = soil temperature ( $\degree$ C), T<sub>0</sub> = optimum soil temperature 216 (°C), T<sub>m</sub> = maximum soil temperature (°C), M = soil moisture concentration (cm<sup>3</sup> cm<sup>-3</sup>). T<sub>o</sub>, T<sub>m</sub>, k<sub>m</sub><sup>3</sup> and α 217 were solved individually for each biome. Irrespective of having a similar or better overall performance 218 ( $R^2$  in Table S6), we selected the log-linear or log-quadratic equations to fit our data (Table 1, Eq. 1, 219 Models c and d in Table S3) because it facilitated use of the binary categorical variable to evaluate 220 differences in temperature response functions with warming treatment.

221

#### 222 *Cross-Biome Differences*

223 Temperature response functions of soil respiration were not equal across biomes; not only were the

224 temperature sensitivities different ( $\gamma_1$  and  $\gamma_2$ , Table 1), but the magnitudes of respiration ( $\gamma_0$ , Table 1) also

225 differed, with highest fluxes from boreal forests and lowest fluxes from deserts (Fig. S4). Multivariate

226 regression output highlights these across-biome differences, as adding 'biome' as a predictor to the larger

227 multivariate regression of all non-desert data increased the predictive power of the model by 28% (Model

228 j in Table S3).

229

#### 230 **Supporting References**

231

- 232 1. Reynolds LL, Johnson BR, Pfeifer-Meister L, Bridgham SD (2015) Soil respiration response to 233 climate change in Pacific Northwest prairies is mediated by a regional Mediterranean climate 234 gradient. *Glob Chang Biol* 21(1):487–500. 235
- 236 2. Vicca S, et al. (2012) Urgent need for a common metric to make precipitation manipulation 237 experiments comparable. *New Phytol* 195(3):518–22.
- 239 3. Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. *Funct Ecol*:315–



- 16. Reinsch, S., Sowerby, A., Emmett, B.A. (2016) Fortnightly soil respiration data from Climoor fieldsite in Clocaenog Forest 1999 – 2015. DOI: 10.5285/[2]c0822023-0ec2-425f-8bf9- a546ce281ee0
- 17. Reinsch, S., Sowerby, A., Emmett, B.A. (2016) Daily plot level (micro meteorological) data at Climoor field site in Clocaenog Forest 1998-2015. DOI: http://doi.org/10.5285/afb994e5-b33d-48b4-ad29-d374b1f9f3c8

**Fig. S1**.





 Temperature sensitivities for desert calculated as the linear functions describing the derivative of the log-

293 quadratic fit of ln respiration as a function of soil temperatures:  $\frac{\partial y}{\partial t}$  = -0.0014 T + 0.072 (warmed) and  $\frac{\partial y}{\partial t}$ 294  $=$  0.0008 T + 0.019 (control), where y refers to ln of respiration ( $\mu$ mol C m<sup>-2</sup> s<sup>-1</sup>) and T refers to

temperature (°C).











306 Best fit regression lines of natural log (ln) of respiration ( $\mu$ mol C m<sup>-2</sup> s<sup>-1</sup>) as a function of soil temperature  $^{\circ}$  ( $^{\circ}$ C) across biome types, with data partitioned into moisture quantiles: dark red ( $1^{\text{st}}$  (lowest) quartile), red 308  $(2<sup>nd</sup> quartile)$ , light blue (3<sup>rd</sup> quartile), dark blue (4<sup>th</sup> (highest) quartile). For model parameters, see Table S3. Separate fits were calculated for control and warmed treatments where statistically different temperature sensitivities were observed (boreal forest and desert), with dashed lines for warmed data and solid lines for control data. Solid lines on all other plots represent both warmed and control data, as their

- fits were not statistically different from one another. Note the scale of Y-axis are all equal, except for
- desert, which had lower respiration rates compared to all other biomes.



318 Ln respiration ( $\mu$ mol C m<sup>-2</sup> s<sup>-1</sup>) as a function of soil temperature (°C) for all data included in our study. Each dot represents an individual data point, including data from both control and warmed treatments (n=3817). Lines are best-fit regression lines using the log-quadratic temperature response functions for all biomes, except the boreal forest and northern shrublands, where log-linear functions were used (for coefficients, see Table S5). 





325<br>326 Map of study sites. Color refers to mean annual temperature (°C). Map created using 'maps', 'mapdata', 327 and 'raster' packages in R.











332<br>333

333 **Fig. S7.**





Partial regression plots of soil respiration as a function of temperature and moisture across all biomes. Plots created using the 'car' package and

336 AvPlots function in R.

**Fig. S8**



339 Forest plot of first-order temperature sensitivities  $(\gamma_1$  in Eq. 1) at each site. Size of filled squares indicates number of observations. Error bars represent 95% confidence intervals. Error bars that do not cross zero line indicate significant differences in temperature sensitivity between warmed and control plots. Values on right of zero line indicate higher sensitivity in warmed plots, while values on left of zero line indicate lower sensitivity of warmed plots.



348

349 Difference in respiration (µmol C m<sup>-2</sup> s<sup>-1</sup>) between warmed and control plots ( $\Delta$ R) normalized by degree 350 of warming (∆T °C), binned by amount of soil desiccation with warming (soil moisture content in 351 warmed plots divided by soil moisture content in control plots) for each individual biome. X axis values 352 <1 indicate warmed plots have less moisture available than control plots. Y axis values <0 indicate that 353 respiration rates were lower from warmed plots, despite warmer soil temperatures. Respiration data not 354 log transformed. Note the scales of the Y-axes are different. For number of observations by biome see 355 Table S3.

## **Supplementary Tables**

## 

#### **Table S1**.



 $rac{y}{\text{data}}$  from published literature only<br>  $\land$  data from both published and unpublished data<br>
\*\*Years of observations since warming started

359<br>360 Characteristics of each site included in study, including both published and unpublished sources (6–17).

## **Table S2**



365 Parameters for multivariate regression model of soil respiration (natural log, in  $\mu$ mol C m<sup>-2</sup> s<sup>-1</sup>) (R) as a

366 function of soil temperature ( $^{\circ}$ C) (T) and soil moisture (cm<sup>3</sup> cm<sup>-3</sup>) (M). In biomes with significantly

different temperature sensitivities between warming and control treatments (boreal and desert biomes),

368 control and warmed data were run in model separately. n= number of observations,  $R^2$  = coefficient of

369 determination. Parameter units:  $\alpha_0 = \ln \mu$  mol C m<sup>-2</sup> s<sup>-1</sup>;  $\alpha_1 = \text{°C}^{-1}$ ;  $\alpha_2 = \text{°C}^{-2}$ ,  $\alpha_3 = \text{cm}^{-3} \text{ cm}^3$ .



## **Table S3**.





376 Summary of various models and their fits of soil respiration as a function of multiple variables.  $R = soil$ 377 respiration (natural log, in  $\mu$ mol C m<sup>-2</sup> s<sup>-1</sup>), T = soil temperature (°C), M = soil moisture content (cm<sup>3</sup> cm<sup>-2</sup> 378  $\frac{3}{2}$ , W = treatment (control or warmed), df=degrees of freedom, R<sup>2</sup>= coefficient of determination,  $\triangle$ AICc = delta Akaike information criterion, with zero as best and all other model values presented relative to zero. Bold indicates significant predictor of respiration. Asterisk indicates interaction term in model.

# 381 **Table S4.**



382<br>383 383 Parameters for models of natural log (ln) respiration ( $\mu$ mol C m<sup>-2</sup> s<sup>-1</sup>) as a function of soil temperature

384 (°C) by moisture quartile for each biome. Data also shown in Fig. S3.



**Table S5**

387 Parameters for multivariate regression model of soil respiration (natural log, in  $\mu$ mol C m<sup>-2</sup> s<sup>-1</sup>) (*R*) as a function of soil temperature (°C) (*T*), including data from both control and warmed treatments (Models a and b in Table S3). Parameters shown for both the log-linear and log-quadratic temperature response 390 functions. n = sample size,  $R^2$ = correlation coefficient. Parameter units:  $\gamma_0 = \ln \mu$  mol C m<sup>-2</sup> s<sup>-1</sup>;  $\gamma_1 = \text{°C}^{-1}$ ,  $\gamma_2$  $=$  °C<sup>-2</sup>. All models significant (p<0.001). For comparison of model fits, see Table S3. For model parameters of control versus warmed plots, see Table 1.

# **Table S6**.

# 



#### 

Comparison of model fits (Eq. 3, Eq. 4) evaluating role of soil moisture in driving soil respiration.