Published in final edited form as:
Nat Ecol Evol. 2019 February ; 3(2): 232–238. doi:10.1038/s41559-018-0770-5.

Soil microbial respiration adapts to ambient temperature in global drylands

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Abstract

Heterotrophic soil microbial respiration – one of the main processes of carbon loss from soils to the atmosphere – is sensitive to temperature in the short-term. However, how this sensitivity is affected by long-term thermal regimes is uncertain. There is an expectation that soil microbial respiration rates adapt to the ambient thermal regime, but whether this adaptation magnifies or reduces respiration sensitivities to temperature fluctuations remains unresolved. This gap in our understanding is particularly pronounced for drylands as most studies conducted so far have focused on mesic systems. Here, we conducted an incubation study using soils from 110 global drylands encompassing a wide gradient in mean annual temperature. We tested how mean annual temperature affects soil respiration rates at three assay temperatures while controlling for substrate depletion and microbial biomass. Estimated soil respiration rates at the mean microbial biomass were lower in sites with higher mean annual temperatures across the three assayed temperatures. The patterns observed are consistent with expected evolutionary trade-offs in the structure and function of enzymes under different thermal regimes. Our results therefore suggest that soil microbial respiration adapts to the ambient thermal regime in global drylands.

Carbon (C) losses to the atmosphere through heterotrophic soil microbial respiration are expected to increase with climate warming1–3, representing a positive C cycle–climate

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Data availability

Data in the support of these findings and the R code for the statistical models are available in Figshare (DOI: 10.6084/m9.figshare.5777940; https://figshare.com/s/18186211f9f259c4e2b0).

Authorship

F.T.M. designed the field study and wrote the grant that funded the work. P.G.P and M.D. developed the original idea of the analyses presented in the manuscript. M. D. and C. P. conducted laboratory work. M. D. conducted the statistical analyses with the help of M.A.B. All authors contributed to data interpretation and manuscript writing.

Competing interests

The authors declare no competing financial interests.

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feedback4 embedded into the climatic models of the IPCC5. The magnitude of this soil feedback can sum up to 12–17% of the expected global anthropogenic emissions by 20506. Despite the importance of addressing this feedback for establishing accurate greenhouse gas emission targets7, there are multiple gaps in our understanding of the responses of soil microbial respiration to warming. For instance, some studies suggest that elevated soil respiration rates initially found under experimental warming gradually recover to ambient values due to substrate depletion and/or microbial thermal adaptation8–16. Soil microbial respiration adapts to temperature through physiological adjustment of individuals, evolutionary adaptation of populations, and/or species turnover17,18. Recent studies evaluating the thermal adaptation of soil microbial respiration have generated contrasting results4,18–22. These results mostly derive from field warming experiments9,15 or short-term laboratory incubations18,19, whereas much of the work assessing thermal adaptation in animals and plants has evaluated mass-specific respiration rates of organisms occurring across pronounced latitudinal climatic gradients23. We do this here, with the expectation that patterns in thermal adaptation across latitudinal gradients should have had ample time for ecological and evolutionary processes to play out, permitting us to discern whether soil microbial respiration adapts to thermal regimes as would be expected under evolutionary theory17.

At the global scale, the magnitude of soil microbial respiration responses to temperature may be biome-specific. A global study spanning from the Arctic to the Amazon only found strong evidence of microbial thermal adaptation in cold climates20. However, the total number of sites evaluated was low (22), especially in dryland areas, which include arid, semi-arid and dry-subhumid ecosystems. Thus, we barely know whether soil respiration adapts to the ambient thermal regime in drylands, a biome covering about 45% of the total land surface24 and storing 32% of the global soil organic C pool25. Drylands are extremely sensitive to ongoing warming, and their global extent is expected to increase by 11-23% during the 21st century due to forecasted increases in aridity26. Further, a global synthesis of field warming experiments identified drylands as the only biome where the temperature sensitivity of soil microbial respiration is lower in warmed than in ambient temperature plots27, which is indicative of thermal adaptation. Thus, addressing the thermal adaptation of soil microbial respiration in drylands may be of major importance for evaluating possible offsets to the soil C cycle-climate feedback in this widespread biome28.

Given their global distribution, drylands span a wide range of mean annual temperatures (MAT)29. Carbon dynamics in drylands are highly spatially variable because drylands are heterogeneous environments in which soils have greater concentrations of organic C (SOC) and other nutrients under plant canopies compared to adjacent areas devoid of perennial vascular vegetation30–32. Given the heterogeneous distribution of soil resources typically found in drylands, analysing the behaviour of these microsites (open and vegetated areas) is of paramount importance for understanding how these ecosystems function. Therefore, to understand how temperature affects soil microbial respiration in drylands33,34, one of the main processes of soil C loss in these systems28,35, we must follow approaches that account for variability in controlling factors other than just temperature, such as microsite, SOC and microbial biomass.

To test for latitudinal patterns in the thermal response of soil microbial respiration in drylands, we collected soils from 110 globally distributed dryland ecosystems and then conducted a short-term laboratory incubation experiment to measure the response of soil microbial respiration to three assay temperatures (10, 20 and 30°C). The sites studied span a gradient of MAT from -1.8 to 28°C (Supplementary Table 2) and include the most representative vegetation types that can be found in drylands worldwide (grasslands, shrublands and open forests/savannahs). Our experimental design was sensitive to the patchiness of ecosystem functioning in drylands30, with soils sampled separately from vegetated and open microsites (giving 220 soils in total). Following the approach of previous microbial thermal adaptation studies17,18,36, we removed the substrate and moisture limitation of respiration by incubating soils at excess substrate and plentiful moisture in the laboratory incubations (see Methods). In our multiple regression models, we statistically controlled for differences in soil microbial biomass estimated using three different assessment techniques (substrate-induced respiration, chloroform-fumigation extraction and qPCR). Then, the results derived from those models were used to estimate respiration rates at the mean microbial biomass across all soils to show the effect of MAT and microsite on soil respiration rates (Figures 2-3 and Figure 4, respectively).

Prior findings in other biomes suggest three alternative hypotheses regulating potential soil respiration responses to the *in situ* thermal regime (MAT in our study, Figure 1). The first hypothesis is ‘no adaptive response’, where respiration rates for a common microbial biomass value and a common measurement temperature (i.e. each of the assay temperatures: 10, 20 and 30°C), should be unrelated to MAT. As with the other two hypotheses, rates would however be expected to be related to other variables such as SOC content21. In other words, soil microbial respiration rates will increase with assay temperature and be related to soil properties, but respiration rates estimated at a common biomass (and when other soil properties are also standardised) will be unrelated to the site MAT within each assay temperature evaluated (Figure 1a). The second hypothesis is an enhancing response where respiration rates, again when expressed at a common biomass (and common values of other soil properties), will be higher in soils sampled from sites with warmer as opposed to colder MAT values (Figure 1b). A mechanism proposed to explain this response is more intense competition for soil C and nitrogen resources under warmer conditions20. It has been suggested that such enhancing responses may lead to a positive C cycle-climate feedback4. The third is a ‘compensation’ hypothesis, where potential respiration rates at a particular assay temperature will be greater for soils from colder than warmer MATs (Figure 1c). That is, there will be a negative effect of MAT on soil respiration rates. Such compensatory responses would be consistent with evolutionary trade-offs in enzyme and cell membrane structure and function as a consequence of microbial adaptation to different thermal regimes17.

Results and Discussion

We tested for thermal adaptation of soil microbial respiration in dryland ecosystems worldwide. Our results highlight the role played by ambient thermal regime (i.e. MAT) as a determinant of soil microbial respiration rates at consistent assay temperatures, as we observed a negative effect of MAT on such rates. Specifically, we built regression models

including important controls on soil microbial respiration rates: assay temperature, MAT, SOC content, soil texture (as percentage sand) and soil pH20,37. We also included microsite as a term given the known differences between vegetated and open areas on soil biogeochemistry in drylands38. In all the regression models, potential respiration rates – where glucose was added in excess of demand to remove the influence of substrate limitation – was used as the metric of soil microbial respiration. Further, given that the different soils had differences in soil microbial biomass, we needed to include this variable in our regression models to estimate respiration rates at a controlled biomass18, which is conceptually analogous to evaluating mass specific respiration rates. Doing so avoids the confounding effect of differences in microbial biomass when testing for thermal adaptation of soil microbial respiration18. Many of the explanatory variables evaluated (i.e. SOC, sand content, and soil pH) were strongly correlated ($\rho > 0.5$ for all variables) with soil microbial biomass (see Methods), meaning that the effect sizes derived from the regression models including all variables were not reliable. Therefore, we built two regression models that included the critical variables needed to evaluate thermal adaptation of soil microbial respiration (i.e. Assay Temperature and MAT) but that differ in the rest of the variables retained in the model. The former includes microbial biomass (Table 1), whereas the latter retains SOC, sand content, and soil pH but excludes microbial biomass (shown in Supplementary Table 1). The effects found in this model without microbial biomass (Supplementary Table 1) agree with previous results from other biomes19,20. However, given that previous studies observed that controlling microbial biomass was of paramount importance to test for thermal adaptation15,16,18,21, we then used the model with microbial biomass in subsequent analyses.

Using the model that included soil microbial biomass, we tested for the effects of ambient thermal regime (i.e. MAT) on potential soil respiration rates. Interactions among the variables were not included in this model because they were both non-significant and had very small coefficients. The main effects model captured a large part of the variation found in the data ($r^2 = 0.80$; Table 1). Potential respiration rates were negatively related to MAT (Table 1). This result was observed regardless of the method used to measure microbial biomass (Table 1), suggesting that substrate induced respiration (SIR) is a suitable estimate of microbial biomass – at least in our study – for assessing thermal adaptation responses15,39. The effect of MAT was at least four times smaller than that of the assay temperature in all models, and smaller than that of microbial biomass (compare standardised coefficients in Table 1). However, this is unsurprising because the responses of soil respiration rates to ambient temperature (i.e. assay temperature in this instance) and community biomass tend to be very pronounced18, especially across large temperature ranges such as those used here (i.e. from 10 to 30°C). In contrast, the influence of adaptive physiological responses (to MAT in this instance) typically only partially compensate for such changing conditions, and so would be expected to be smaller than the assay temperature and biomass effects17. Regardless, the negative effect of MAT on potential respiration rates was consistent with the ‘compensation’ hypothesis (Figure 1c), and with observed patterns for the metabolic rates of individual plants, animals and microbes17,18.

To visualise the effect of MAT, we estimated potential respiration rates after controlling for the other variables (Table 1). Specifically, we used the unstandardised coefficients of the

regression model (Table 1) together with the mean value across all 110 sites for the microbial biomass, the open microsite value (i.e. 0 as opposed to 1 that corresponds to vegetated areas), and the relevant assay temperature (i.e. 10, 20 or 30°C). We next plotted the MAT effect by multiplying its coefficient by the observed range of MAT values across the 110 sites studied. A pronounced and negative effect of MAT on potential respiration rates, at the mean microbial biomass, was observed regardless of the assay temperature used for both vegetated and open microsites (Figure 2). This negative MAT effect is consistent with expectations of evolutionary trade-offs observed during biochemical adaptation to different thermal regimes17. For instance, enzymes adapted to lower temperatures are less rigid than those adapted to higher temperatures, leading to more rapid catalytic rates under controlled conditions for cold-adapted enzymes17. Given that much of the research on biochemical thermal adaptation is focused on enzymes of respiratory pathways, it seems reasonable to assume that such trade-offs underlie the patterns we observed for soil microbial respiration, as they likely also do for plant and animal respiration17,36. However, given that the 'aggregate' respiratory activity of soil microbial communities is likely influenced by individual, population and species shifts, we cannot state that biochemical adaptation is the only mechanism operating to explain the negative MAT-respiration relationship that we observed regardless of assay temperature (Table 1 and Figure 2). However, this consistently negative relationship is not congruent with the expectation that the amount of substrate assimilated that is allocated to respiration rises with temperature40. Thus, the patterns observed in our study do not seem to arise through differences in the physiology of the microbial community related to such phenomena as microbial C use efficiency (CUE)41. Measurements of microbial growth rates, and how they are related to respiration rates, would be necessary to better evaluate these possibilities42. Despite these limitations, our results show that soil microbial communities appear adapted to different environmental thermal regimes (i.e. MAT) across global dryland ecosystems. Additionally, similar results were observed in other biomes41, suggesting that thermal adaptation of soil microbial respiration is a common phenomenon across ecosystem types.

We observed a consistent negative MAT effect on potential soil respiration rates (at the mean biomass and excess substrate), a pattern that seems incompatible with the expected positive relationship between temperature and heterotrophic soil respiration rates4,43–45. However, this positive link was supported by the positive effect of assay temperature on respiration rates observed in our study (Table 1 and Figure 2). Further, when estimating potential respiration rates for each soil at its respective MAT using our regression model, we found a positive effect of assay temperature on potential respiration rates, which was dampened by the observed thermal adaptation (i.e. the negative MAT effect; Figure 3). The dampening effect of thermal adaptation is greater as MAT rises. The changing magnitude of this dampening effect may point to the effects of other variables, such as microbial community composition, on potential respiration rate responses to temperature. For example, soil microbial diversity varies across our network of dryland sites due to changes in climatic conditions, plant cover and SOC46. Further, there are also differences in functional traits of soil microorganisms across space47, and hence different life-history strategies can be expected among different environmental conditions47,48. For example, some organisms invest relatively more energy toward plasticity than they do toward growth because of life-

history trade-offs between competitiveness and stress tolerance, which in turn affect the functional potential of soil microbial communities47,48. The idea that different functional traits may affect potential respiration rates agrees with the microsite effect observed here. Specifically, vegetated microsites had greater potential respiration rates (again, at the mean biomass and with substrate in excess) than open microsites when all other variables were the same for all soils (Figure 4, Supplementary Table 2). Vegetated microsites in drylands are considered more benign environments because they contain a greater amount of nutrients and have a milder microclimate than open areas. As such, soil microorganisms within vegetated microsites should be more plastic and hence have higher potential respiration rates. This is what we observed (Figure 4), suggesting that adaptation to both the thermal regime and other environmental factors likely shape the respiration rates of soil microbial communities across global drylands.

Our results suggest that there is adaptation of soil microbial respiration to the ambient thermal regime in global drylands. While these patterns match those expected because of biochemical trade-offs in enzyme and membrane structure and function, they could also result from microbial population and/or community-level shifts. However, our finding that the MAT-respiration relationship was consistently negative regardless of assay temperature suggests that the observed patterns were not the result of differences in important physiological attributes such as CUE. Additionally, our result supports model expectations that negative responses arising through shifts in enzyme and membrane properties have effects on respiration rates that are independent of changes in CUE40. Regardless of the mechanism(s) involved, our results fill a critical gap in our understanding of thermal adaptation of soil microbial respiration in drylands. These ecosystems cover an important portion of the total land surface that is expected to increase in the future given forecasted increases in aridity due to ongoing climate change26. Therefore, the process-level understanding generated by our study can be used to inform the assumptions of the climatic models used to estimate soil C cycle-climate feedbacks.

Methods

Study design and soil collection

We sampled 110 dryland ecosystems located in 19 countries across all continents except Antarctica between June 2006 and December 2013 (see Maestre et al.49 for details of the field survey). These 110 sites are a subset of the 236 sites used by Ochoa-Hueso et al.38. Briefly, the sites represented an assemblage of the environmental conditions found in global drylands, with Aridity Indices ranging from arid to dry-subhumid and MAT and mean annual precipitation (MAP) values from -1.8°C to 28°C and 66 mm to 1177 mm, respectively. Soils were sampled during the dry season at each site. Five replicated soil samples (0- to 7.5-cm depth) were randomly taken at two different microsites: under the canopy of the dominant perennial plant species (vegetated microsites) and in adjacent areas devoid of perennial vascular vegetation (open microsites). When more than one dominant plant species was found, samples were also collected randomly under the co-dominant species. The analysis of vegetated and open microsites is fundamental to the study of ecosystem functioning in drylands as they have marked differences in nutrient contents,

microclimate and soil microbial abundance/composition and activity31,38. Soils were bulked and homogenized to get two composite samples per site, totalling 220 soil samples. These soil samples covered a wide range of vegetation, soil and climatic conditions (Supplementary Table 2). A fraction of each soil sample was immediately frozen at -20°C for real-time PCR (qPCR) analysis, whereas another fraction was air-dried for a month in the laboratory and stored until physicochemical analyses and respiration assays. Soils in dryland ecosystems are usually dry under field conditions for a prolonged period, until they are rewetted with seasonal rain pulses32. Therefore, previous studies have found that air drying and further storage of dryland soils has small or inconsequential effects on soil biogeochemistry50.

Field climatic and soil conditions

The coordinates and elevation of each site were recorded *in situ* and were standardised to the WGS84 ellipsoid by using Google Earth (https://www.google.com/earth/). Mean annual temperature values for soils of each site were obtained using Worldclim51, which provides the average of MAT for the 1950-2000 period. Soil conditions for each site varied, and were represented in our analysis as total SOC concentration, sand content (for texture) and pH because they play key roles in the availability of water and nutrients in drylands52 and are major drivers of microbial activity20 and community composition53. The concentration of SOC was determined by colorimetry after oxidation with a mixture of potassium dichromate and sulfuric acid at 150° C for 30 minutes54. Relative sand, silt and clay contents were determined according to Kettler et al.55 and added up to 100% in each sample, so only the sand content was used in further analyses. Soil pH was measured with a pH meter, using a 1: 2.5 mass: volume soil and water suspension. Other soil variables were measured, such as total nitrogen and available phosphorus content, but they were not shown in this study as we only assessed the effect of soil variables that, such as SOC, are known to exert a strong control on soil microbial respiration rates33.

Soil respiration at three assay temperatures

Soil laboratory incubations—We tested for soil microbial adaptation to MAT by conducting short-term (10 h) incubations, with the short timescale chosen to prevent acclimation to the assay temperatures. This approach is similar to those used in plants, cultured microbes and other soil studies4,17,18,36. Moreover, we did not conduct a pre-incubation phase because our objective was to test for thermal adaptation to the MAT of the sites where the soils were collected. Soil incubations were performed at 10, 20 and 30°C, and soil moisture was adjusted using sterile deionized water to 60% of their water holding capacity (WHC), which is optimal for microbial activity18. Soil sub-samples were incubated in 96-deepwell microplates (1.3 mL wells). Microplates were filled with *ca.* 0.5 g soil (dry-weight equivalent) per well. The 220 soil samples were run in triplicate (laboratory replicates). Incubations were performed in growth chambers under dark conditions at the three assay temperatures and 100% air humidity. Microplates were covered with parafilm to prevent soil drying but to allow gas exchange. We measured two soil respiration metrics at each assay temperature using the MicroResp™ technique56. First, we determined soil basal respiration by adding sterile deionized water. Then, and to account for the effect of substrate limitation on soil respiration rates18, we also determined potential respiration rates using

glucose at a dose of 10 mg C g^{-1} dry soil, which is considered to exceed microbial demand across the assay57. We ran 3,960 assays in total (220 soils × 3 temperatures × 2 substrates × 3 laboratory replicates).

Soil respiration measurements—The MicroResp™ technique56 is a high-throughput colorimetric method measuring soil respiration rates after the addition of different substrates (in our study, water and glucose). We used a CO_2 detection solution containing cresol red indicator dye that experiences a colour change because of the variation in pH occurring when respired CO_2 reacts with the bicarbonate of the detection solution. Each microplate well was filled with 150 μl aliquots of the detection solution and were attached to the deep-well microplates containing the soil samples. The two plates were incubated together at the particular assay temperature (10, 20 or 30°C) during the last 5 h of the 10 h-incubation period. The detection plate colour development was read immediately before and after the last 5 h of the incubations. The colour change in the detection solution was calibrated with an alkali trapping method58 (r^2 =0.86, P <0.001). We used the mean of the three analytical repeats per assay temperature as the observation of potential respiration rate for each soil. Additionally, and to assess the uncertainty of our measurements, we calculated the coefficient of variation among analytical repeats, which was below 10% in all cases.

Soil microbial biomass—To assess the thermal adaptation of soil microbial respiration at the different assay temperatures, it is necessary to control for soil microbial biomass, as biomass is itself a factor that regulates soil respiration rates and adaptation is tested for on a mass-specific basis18,59. Given that we could not control soil microbial biomass experimentally, as we aimed to test for thermal adaptation of the microbial communities present in the soil, we controlled for the effects of soil microbial biomass on respiration in the statistical models (see Statistical analyses). For that purpose, we used three different methods to estimate soil microbial biomass. All available methods to estimate soil microbial biomass have drawbacks15,39,60, and consequently assessing the relationship between MAT and soil respiration using multiple methods increases the robustness of our results15,39. First, we used a modified SIR procedure with autolyzed yeast as a substrate18,59 at 20°C. Yeast was added at a dose of 1 mL g $soil^{-1}$ (dry weight equivalent) from a solution containing 12 g of yeast L^{-1} of water18,59. Second, we used a chloroform-fumigation extraction (CFE) technique61 with some modifications62–64, and measured total organic carbon (TOC) with an automated TOC analyser in K_2SO_4-diluted soil samples. Lastly, we measured the relative abundance of soil bacteria using qPCR46, which was used as an estimate of microbial biomass. The latter was obtained on composite samples of each microsite and site. The bacterial 16S-rRNA genes were amplified with the Eub 338-Eub 518 primer sets as described in Maestre et al.46. We could not use the three methods to estimate microbial biomass across all the soil samples due to logistical limitations. Therefore, the number of sites measured was 110, 103 and 65 for the ‘yeast-SIR’, ‘CFE’ and ‘qPCR’ method, respectively. Although the three methods were performed in a different number of sites, all of them encompassed a wide representation of the vegetation, soil and climatic conditions found in drylands across the globe. The range, mean and SD observed using the three methods can be found in Supplementary Table 2. We tested for thermal adaptation of potential respiration rates using separate regression models for each method of microbial

biomass. The negative effect of MAT on potential respiration rates was found regardless of the method used to quantify microbial biomass (Table 1).

Statistical analyses

We built a linear mixed-effect regression model (LMM) to test for the effect of MAT on potential respiration rates, where microbial biomass was statistically controlled (i.e. included as a covariate in the model), and substrate limitation was alleviated. Notably, microbial biomass can drive the effects of elevated temperatures on soil respiration22,65, and hence thermal adaptation studies usually calculate mass-specific respiration as the ratio between soil respiration and microbial biomass18,59. However, ratios are problematic for statistical analyses because they can obscure true relationships among variables18,66. Thus, to control for microbial biomass in our analyses, we used our regression model to estimate potential respiration rates at a fixed value of microbial biomass equivalent to the mean microbial biomass across all soils. We ran a different model for each microbial biomass method (yeast-SIR, CFE and qPCR), and confirmed that MAT effects were independent of the microbial biomass method used. Then, we focused on yeast-SIR to represent the effects sizes graphically because we had an observation for every site. The selection of additional variables to include in the model was based on the approach of Hobbs et al.67, where only variables with a well-established biological foundation are included68. This approach rejects model selection techniques based on philosophical and operational grounds68,69. Thus, our full models only included variables known to exert a strong control on soil microbial respiration. As such, in addition to MAT and soil microbial biomass, we included assay temperature, SOC, sand content and soil pH. We also incorporated microsite in the model because it has a strong effect on soil respiration rates in drylands31,38. In the final models, we dropped SOC, pH and sand content because they correlated strongly with microbial biomass68, giving square-root variance inflation factors (VIFs) >2. Once removed, the remaining terms had VIFs < 2, indicating low collinearity.

We confirmed that the results found with the previous approach based on biological foundation were also robust when considering model selection techniques. To do that, we checked that the former model was among the best models according to AICc values (Supplementary Table 3), regardless of the method used to estimate microbial biomass. The best models are those with the lowest AIC_c values. Models showing a difference in AICc values lower than 2 were considered equally good70. Although MAT was not selected in the model with the largest difference in AICc when comparing the best models using Yeast-SIR as microbial biomass, MAT was retained in all the best models selected across the three microbial biomass methods.

Then, we estimated the relative effect size of each of the variables of the models that included the three microbial biomass methods. These relative effect sizes of the retained terms depend on the slope coefficient for the specific variable obtained by fitting the LMM and on the observed variation in the values of that variable. Hence, we estimated the effect sizes of the different variables on potential soil microbial respiration rates using the regression parameters. To discern the effect of any given variable (e.g. MAT), we allowed its value to vary across the range of observed values while holding the remaining variables

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constant at the mean of all observations for each variable. That is, we used the coefficients of the LMM, the range of values of the variables under study and the mean of the remaining variables to plot the regression equation for the model. We used this approach to statistically control for microbial biomass. Specifically, we tested for thermal adaptation of soil microbial respiration at a common microbial biomass value for all the soils. This fixed microbial biomass value is the mean of the microbial biomass observed across our soils at the site level (Supplementary Table 2). This approach is conceptually analogous to using mass-specific respiration, but avoiding inferential issues related to using ratios in the model66. The LMMs were fit with a Gaussian error distribution using the 'lmer' function of the lme4 package69 of the R 3.3.2 statistical software71. Respiration data were transformed by taking the natural logarithm of each value to meet the assumptions of normality and homogeneity of variance. Although reported *P*-values are quasi-Bayesian, they retain the same interpretation as frequentist *P*-values72. We considered significant coefficients to have *P* values < 0.05. We also determined the r^2 value for each model to verify that they could explain a substantive degree of the variance in potential soil respiration rates. These values were calculated following Nakagawa & Schielzeth73.

Finally, we tested the sensitivity of our results to data exclusion. Specifically, we randomly excluded different percentages of our 110 sites (i.e. 5, 10, 15, 20 and 25% of the sites) and rerun our LMM analyses. We repeated this procedure 100 times for each percentage of sites excluded. Then, the mean estimated coefficient and its confidence interval (2.5-97.5%) of the 100 repetitions were obtained and represented graphically for each predictor variable included in the model. Importantly, for the three models tested (i.e. one for each microbial biomass method: Yeast SIR, CFE and qPCR) coefficients estimated for each variable fall within the confidence intervals and are quite close to the mean regardless of the percentage of data excluded (Supplementary Figures 1, 2 and 3, respectively). Hence, we can confidently say that the results obtained are not sensitive to variation in the identity and number of sites included in our analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the European Research Council (ERC)-funded projects BIOCOM (ERC Grant agreement n° 242658) and BIODESERT (ERC Grant agreement n° 647038), and by the Spanish Ministry of Economy and Competitiveness (BIOMOD project, CGL2013-44661-R). M. D. is supported by an FPU fellowship from the Spanish Ministry of Education, Culture and Sports (Ref. FPU-15/00392). P.G.P. acknowledges the Spanish Ministry of Economy and Competitiveness for financial support via the Juan de la Cierva Program (IJCI-2014-20058). C.P. acknowledges support from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement n° 654132. We thank Dinorah Mendoza for her help in the laboratory.

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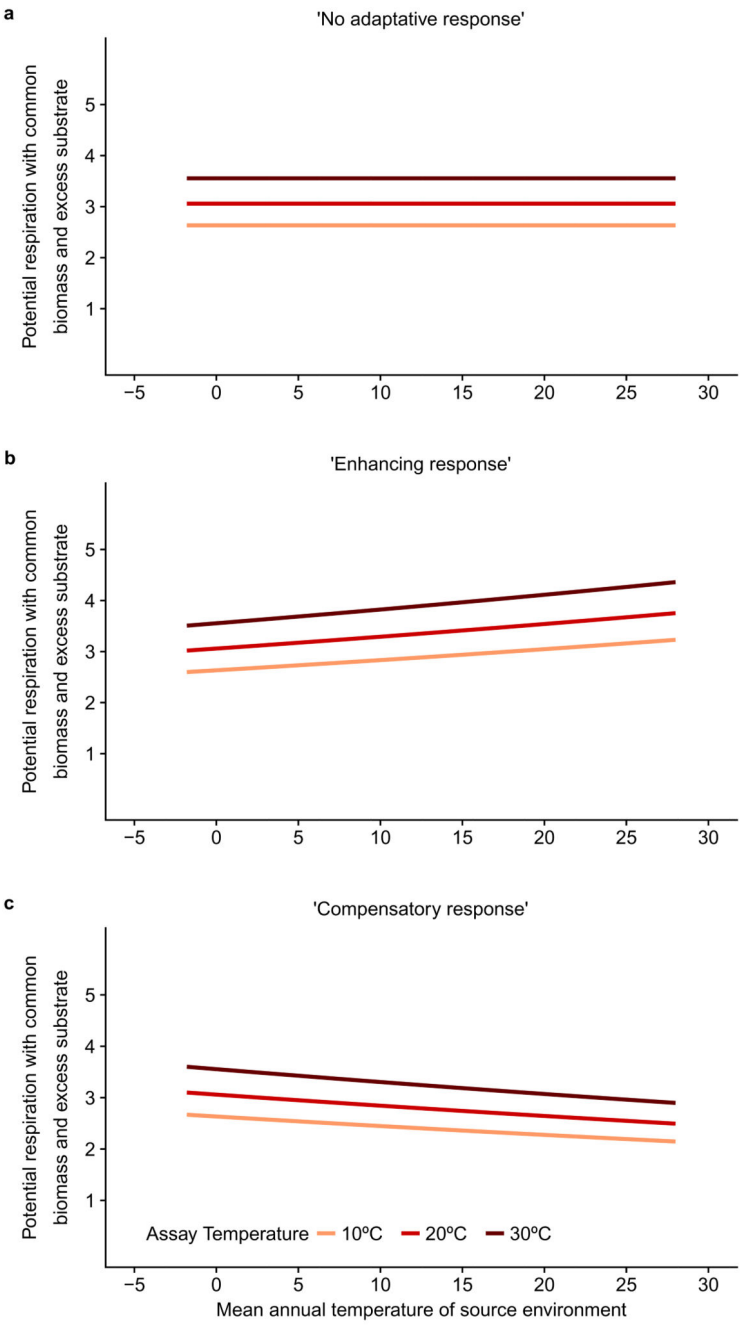

Figure 1. Expected outcomes of the effect of mean annual temperature (MAT) of the source site on potential soil microbial respiration rates at three assay temperatures under three competing hypotheses.

The plots show the hypothesized effects of MAT on potential soil microbial respiration rates at each of the three assay temperatures (i.e. 10, 20 and 30°C) under controlled substrate availability and for the mean microbial biomass across samples. a. 'No adaptive response' hypothesis, where soil microbial respiration is not related to MAT. Under this hypothesis, soil microbial respiration rates are greater with higher assay temperatures, but within each assay temperature respiration rates at a common biomass will be unrelated to the site MAT.

b. The ‘enhancement’ hypothesis, which would suggest more intense competition for soil carbon and nitrogen resources under warmer conditions. Under this hypothesis, soil microbial respiration rates will increase with assay temperature; for each assay temperature respiratory rates (at a common biomass and with substrate in excess) will be higher in warmer than in cooler environments. c. The ‘compensation’ hypothesis, which would be consistent with evolutionary trade-offs in enzyme and microbial cell membrane structure and function. Under this hypothesis, soil microbial respiration rates (again at a common biomass and with excess substrate) will be greater for cooler than for warmer sites regardless of the assay temperature. Although soil respiration responses to temperature can be non-monotonic, we show them as monotonic to represent the competing theoretical outcomes.

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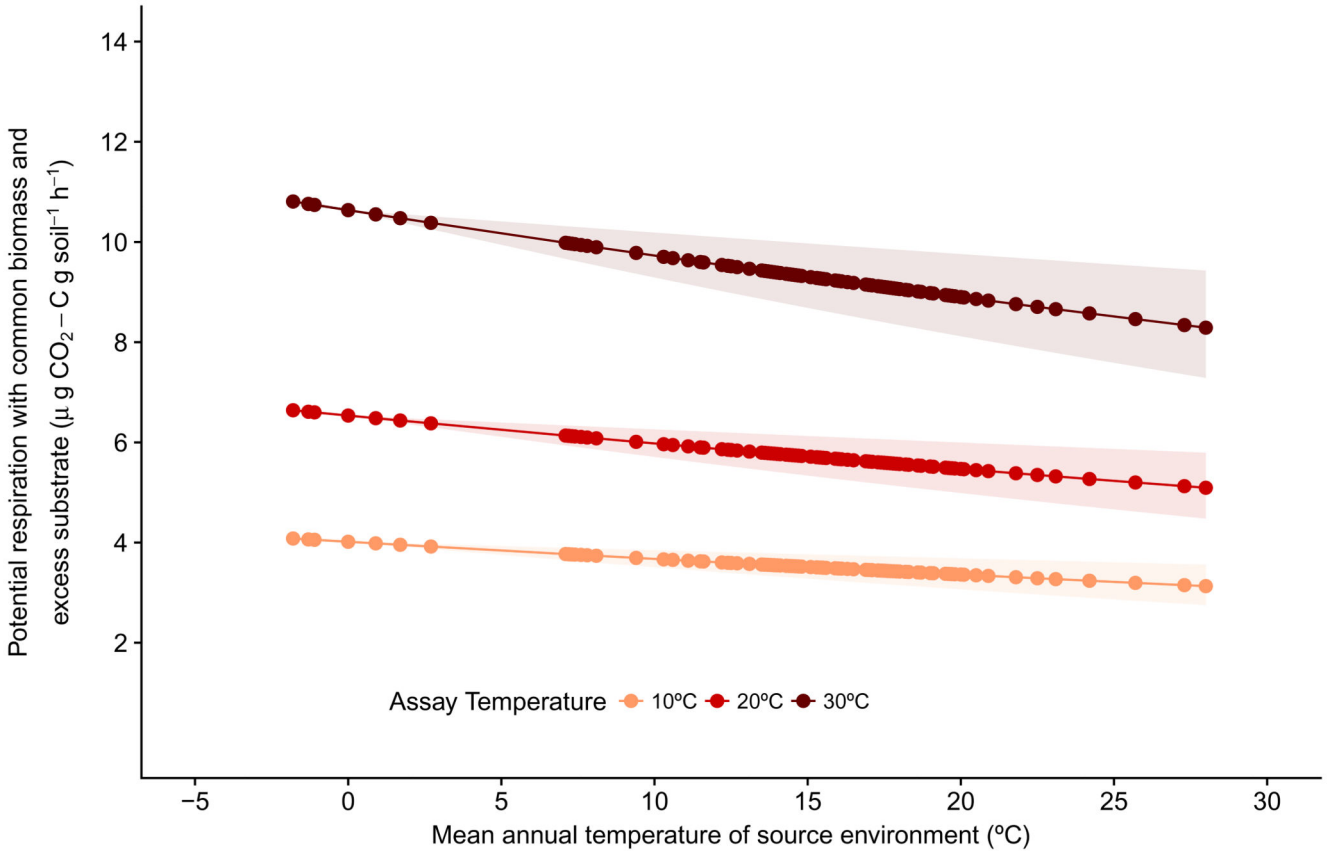

Figure 2. Estimated effects of mean annual temperature (MAT) on potential respiration rates at a common microbial biomass value and with substrate in excess.

Effect sizes were estimated using coefficients from the 'yeast-SIR' model (Table 1). Three outcomes of this model are shown, one for each temperature assayed (i.e. 10, 20 and 30°C). Specifically, the unstandardised coefficients were used in a regression equation, along with the mean value across all 110 sites for the microbial biomass, one of the assay measurement temperatures and then for MAT by systematically increasing the control from the lowest to highest observed values across all sites. All estimates were obtained using soils from open microsites, but the negative relationship is also apparent when using soils from vegetated microsites. The shaded areas show the standard deviation of potential soil microbial respiration rates at each assay temperature (determined using the SD of the MAT coefficient).

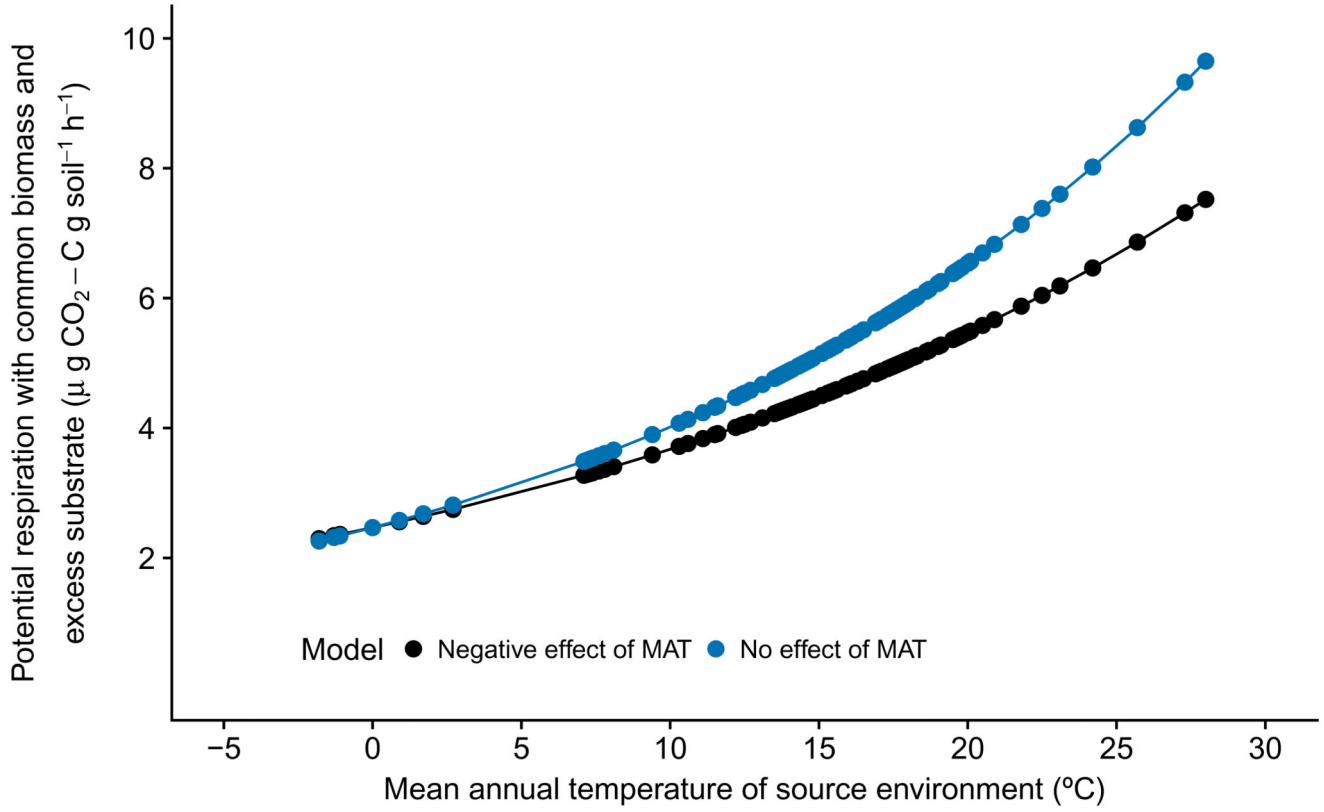

Figure 3. Comparison of the estimated effects of mean annual temperature (MAT) on potential respiration rates, at a common microbial biomass value and with substrate in excess, between our model and a model assuming no MAT effect.

Effect sizes were estimated using unstandardised coefficients from the 'yeast-SIR' model, presented in Table 1, as in Fig. 2. To have a model without MAT effect we set its coefficient to zero. To evaluate the difference in the response between both models, we estimated potential soil respiration rates for each soil assuming an assay temperature that matched a site's MAT value (therefore n = 110 estimates).

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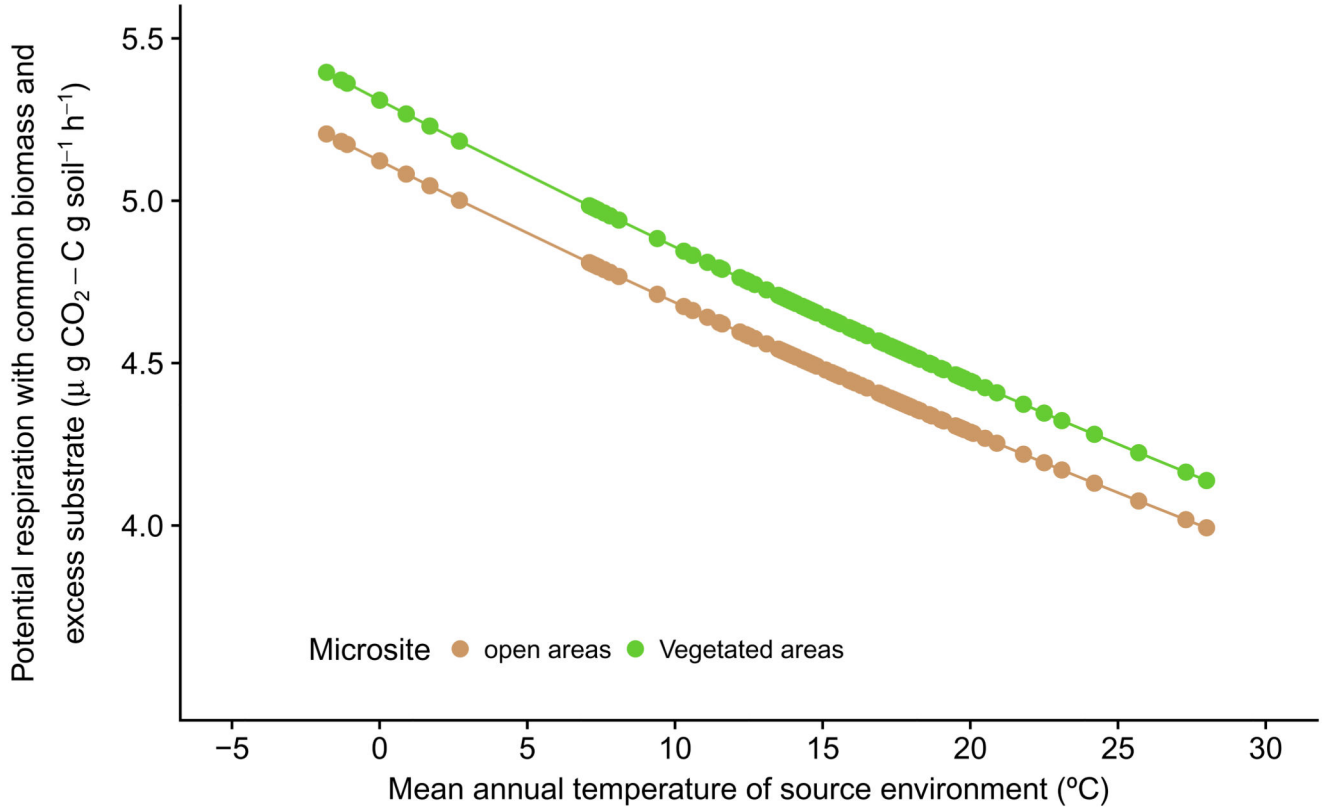

Figure 4. Estimated effects of microsite (vegetated vs. open areas) on potential respiration rates at a common microbial biomass value and with substrate in excess.

Effect sizes were estimated using unstandardised coefficients from the 'yeast-SIR' model, presented in Table 1, using the same approach used in Fig. 2. To evaluate the difference in the response between the two microsites, we estimated potential soil respiration rates for each soil assuming an assay temperature that matched a site's mean annual temperature (therefore n = 110 estimates per microsite) and set the microsite coefficient to 0 (open areas) or 1 (vegetated). Data presented correspond to the inverse natural logarithm of the respiration rate estimates.

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Table 1

Coefficients (mean ± SD) and r^2 values for the linear mixed-effects models used to assess thermal adaptation of potential soil microbial respiration measured with substrate in excess.

The table shows the unstandardised and the standardised coefficients of the model. Respiration rates were ln-transformed to meet normality assumptions. The negative effect of mean annual temperature (MAT), an effect consistent with thermal adaptation, is observed regardless of the method used to estimate microbial biomass: yeast-substrate induced respiration (SIR), chloroform-fumigation extraction (CFE) and bacterial abundance using qPCR. Unstandardised coefficients were used when plotting Figs. 2, 3 and 4. The number of soils is 110, 103 and 65 for the 'yeast-SIR', 'CFE' and 'qPCR' models, respectively, giving an n of 660, 618 and 390 for these models. Significant ($P < 0.05$) coefficients are shown in bold. Model r^2 values were calculated using a method that retains the random effects structure (see Methods). In all the models, all square-root variance inflation factors (VIFs) were < 2.

Variables	Model			
	Unstandardised coefficients	Standardised coefficients		
	Yeast-SIR	Yeast-SIR	CFE	qPCR
Intercept	0.3166 ± 0.0979	1.7704 ± 0.0253	1.7674 ± 0.0411	1.8540 ± 0.0487
Assay temperature	**0.0487 ± 0.0012**	**0.7960 ± 0.0199**	**0.7993 ± 0.0211**	**0.8009 ± 0.0253**
Mean annual temperature	**-0.0089 ± 0.0046**	**-0.1021 ± 0.0525**	**-0.2975 ± 0.0841**	**-0.2328 ± 0.0976**
Microsite	**0.0358 ± 0.0205**	**0.0358 ± 0.0205**	**0.1004 ± 0.0219**	**0.1074 ± 0.0253**
Microbial biomass	**0.1092 ± 0.0083**	**0.6260 ± 0.0474**	0.0215 ± 0.0417	0.0277 ± 0.0345
Model r^2 fixed effects	0.68	0.68	0.43	0.46
Model r^2 fixed + random effects	0.83	0.83	0.84	0.84