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Pathways regulating decreased soil respiration with warming in a biocrust-dominated dryland

Running head: Warming effects on soil respiration

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Abstract

A positive soil carbon (C)−climate feedback is embedded into the climatic models of the IPCC. However, recent global syntheses indicate that the temperature sensitivity of soil respiration (R_S) in drylands, the largest biome on Earth, is actually lower in warmed than in control plots. Consequently, soil C losses with future warming are expected to be low compared with other biomes. Nevertheless, the empirical basis for these global extrapolations is still poor in drylands, due to the low number of field experiments testing the pathways behind the long-term responses of soil respiration (R_S) to warming. Importantly, global drylands are covered with biocrusts (communities formed by bryophytes, lichens, cyanobacteria, fungi and bacteria), and thus R_S responses to warming may be driven by both autotrophic and heterotrophic pathways. Here, we evaluated the effects of eight-years experimental warming on R_S, and the different pathways involved, in a biocrust-dominated dryland in southern Spain. We also assessed the overall impacts on soil organic C (SOC) accumulation over time. Across the years and biocrust cover levels, warming reduced R_S by 0.30 µmol CO_2 m^{-2} s^{-1} (95 % CI = −0.24 to 0.84), although the negative warming effects were only significant after three years of elevated temperatures in areas with low initial biocrust cover. We found support for different pathways regulating the warming-induced reduction in R_S at areas with low (microbial thermal acclimation via reduced soil mass-specific respiration and β-glucosidase enzymatic activity) vs. high (microbial thermal acclimation jointly with a reduction in autotrophic respiration from decreased lichen cover) initial biocrust cover. Our eight-year experimental study shows a reduction in soil respiration with warming, and highlights that biocrusts should be explicitly included in modelling efforts aimed to quantify the soil C−climate feedback in drylands.

Introduction

Soil respiration (R_S), via the autotrophic and heterotrophic components, is a major source of carbon (C) losses to the atmosphere (Schlesinger & Bernhardt, 2013). Climate warming-induced effects on R_S are expected to exert a positive soil C−climate feedback that could accelerate climate change (Dorrepaal *et al.*, 2009; Melillo *et al.*, 2011; Frey *et al.*, 2013), which is embedded into the climatic models of the IPCC (Pachauri & Meyer, 2014). Such feedback can represent a C loss around 12–17% of the expected global anthropogenic emissions by 2050 (Crowther *et al.*, 2016). Despite the importance of this feedback for establishing accurate greenhouse gas emission targets to limit climate change (Bradford *et al.*, 2016), we still lack a comprehensive understanding of the responses of R_S to warming. Importantly, there is no consensus on the mechanisms driving such responses, neither in their biome-specific magnitude or direction (Karhu *et al.*, 2014; Carey *et al.*, 2016; van Gestel *et al*., 2018).

A recent global synthesis addressing the temperature response of R_S to experimental warming across nine biomes, found that drylands (e.g., Mediterranean shrublands, North American deserts or African Savannas) are the only ecosystems where the temperature sensitivity of R_S is actually lower in warmed than in control plots (Carey *et al*., 2016). This pattern may be indicative of microbial thermal acclimation to the elevated temperatures of the warming scenario. Consequently, the magnitude of the soil C−climate feedback may be reduced in drylands compared with other biomes, which is in agreement with the low soil C losses estimated for these areas by 2050 (Crowther *et al*., 2016). The importance that these low soil C losses may have for climate change projections is considerable, as drylands cover over 45% of the total land surface (Prăvălie, 2016) and store 33% of the Earth's soil organic C (SOC) pool (Safriel & Adeel, 2005). Nevertheless, such assessments are based on extrapolating empirical relationships obtained from field studies to the global scale, and are thus constrained by the lack of experimental warming studies conducted so far in drylands.

In dryland ecosystems, soil C concentrates in the top soil surface (Thomas, 2012), which is the habitat of biocrusts, communities formed by bryophytes, lichens, cyanobacteria and heterotrophs (bacteria and fungi). Biocrusts are of global importance, as they have been estimated to cover over 12% of Earth's terrestrial surface (Rodríguez-Caballero *et al.*, 2018), and play a key role in regulating C cycle in drylands. This community fix substantial amounts of atmospheric CO_2 (over 2.6 Pg of C pear year globally; Elbert *et al.*, 2012), and are responsible of ~ 40% of the C released annually via the autotrophic and heterotrophic components of R_S (Castillo-Monroy *et al*., 2011). The autotrophic constituents of biocrusts (e.g., bryophytes and lichens) are well adapted to the high temperature and low rainfall conditions typical of drylands, as they can undergo long dormant periods (Green *et al.*, 2011). Despite this adaptation, lichens can experience major mortality events as a consequence of reductions in their photosynthetic activity with warming (Grote *et al.*, 2010, Maphangwa *et al.*, 2012; Maestre *et al*., 2013, 2015). In this line, warming-induced changes in photosynthetic rate, and consequently in biocrust cover, can drive the responses of R_S and soil C stocks in biocrust-dominated drylands (Maestre *et al*., 2013; Darrouzet-Nardi *et al.*, 2015). On the other hand, such autotrophic pathway has not yet been confronted with hypothesized heterotrophic mechanisms determining the effects of elevated temperatures on R_S in mesic biomes.

Several mechanisms have been hypothesized to determine the response of R_S to elevated temperatures in mesic biomes, including the depletion of labile C sources with warming (Hartley *et al.*, 2007), decreased microbial activity and biomass after warming-induced reduction in soil moisture (Luo *et al.*, 2001), and microbial thermal acclimation to the ambient temperature regime (Bradford *et al.*, 2008). Specifically, thermal acclimation may be due to shifts in microbial physiology decreasing carbon-use efficiency and enzymatic activity (Allison *et al*., 2010), or to changes in microbial community structure (e.g., fungal-to-bacterial ratio; Ziegler *et al.*, 2013). However, these mechanisms can co-occur to determine the response of R_S to warming, with changes in their relative importance over time, as indicated in a long-term warming experiment in a North American temperate forest (Melillo *et al*., 2017). In drylands, any attempt to determine how R_S responds to warming should address these heterotrophic mechanisms jointly with the role played by biocrusts.

Here, we evaluated the effects of eight-years experimental warming on R_S and its temperature sensitivity in a biocrust-dominated dryland in southern Spain. These effects were investigated in microsites with low and high initial levels of biocrust cover. We tested a series of hypotheses (H) aimed to address the main pathways driving the responses of R_S to warming: H1) decreased R_S with warming is the result of a reduction in autotrophic respiration (CO_2 efflux from lichens, bryophytes and cyanobacteria) due to decreased biocrust cover, H2) decreased R_S with warming is the result of soil microbial thermal acclimation (lower soil mass-specific respiration and enzyme β-glucosidase activity), H3) decreased R_S with warming is the result of soil drying reducing soil microbial activity (significant relationship between R_S and soil moisture), and H4) decreased R_S with warming is the result of substrate depletion (lower soil carbohydrates concentration). Lastly, we also evaluated how warming-induced changes in R_S ultimately impacted the soil capacity to store C over time (*i.e.*, SOC accumulation rates).

Materials and methods

Study site and experimental design

This study was conducted in Sax, in southeast Spain (38º 32´42´´ N, 0º49´5´´ W, 550 m a.s.l.). The climate is Mediterranean semiarid, with an average annual temperature and precipitation of 14.6 ºC and 315 mm, respectively. Summers are hot and dry, and precipitation events mostly occur in autumn and spring. The soil is derived from gypsum, with pH ~ 7, and is classified as Gypsiric Leptosols (IUSS Working Group WRB, 2006). The vegetation is dominated by sparse *Pinus halepensis* trees, which were planted in the 1950s, but also contains typical semiarid grasses and shrubs such as *Lygeum spartum* and *Helianthemun squamatum*. Open areas devoid of perennial vascular vegetation are colonized by a well-developed biocrust community dominated by lichen species such as *Diploschistes diacapsis*, *Squamarina lentigera*, *Fulgensia subbracteata*, and *Psora decipiens*, and by mosses such as *Tortula revolvens*.

In February 2009, we established a randomized field experiment to evaluate the effects of warming on R_S. To increase the ambient temperatures, we built open top chambers (OTCs) using a hexagonal design of methacrylate sheets (40 × 50 × 32 cm), and compared them with nearby control plots at ambient temperatures. Throughout the experiment, the OTCs achieved an average increase in air and soil temperature of 1.9ºC and 1.3ºC, respectively (Maestre *et al.*, 2015), simulating climate warming predicted by atmosphere–ocean general circulation models for the second half of the 21st century in southeastern Spain (Giorgi & Lionello, 2008). These warming effects were maximized during summer (June–September), where air temperatures were increased by up to 5ºC. Importantly, this increased temperature was achieved without conspicuous effects of methacrylate sheets on rainfall and radiation transmission (see Maestre *et al*., 2015 for a detailed explanation on the OTC design). Warming also decreased soil moisture (5 cm depth) by 1.3%, and the length of periods with relative air humidity of 100% by 44% (Maestre *et al*., 2015). To assess whether the effects of warming on R_S interacted with the proportion of soil covered by biocrusts, we crossed the warming treatment with a biocrust cover treatment: low biocrust cover (areas with cover of mosses and lichens < 15%) vs. high biocrust cover (areas with cover of mosses and lichens > 50%). Ten replicates per combination of treatments were set-up, resulting in a total of 40 experimental plots. A minimum buffer distance of 1 m was established between plots. We inserted one PVC collar (20 cm diameter, 8 cm height) 5 cm into the soil in each of the 40 plots to measure soil CO_2 fluxes and biocrust cover.

Soil respiration measurements and modeling

The soil CO_2 efflux rate was measured in all the PVC collars between 2009 and 2015 to assess the warming effects on R_S. Our measurements correspond to the respiration of the whole soil column, including heterotrophic soil microbial communities but also autotrophic respiration from biocrust components. We used a closed dynamic system (Li-8100 Automated Soil CO2 Flux System, Li-COR, Lincoln, USA). The opaque chamber used for these measurements had a volume of 4843 cm^3, and covered an area of 317.8 cm^2. Sampling period was set-up to 120 s, which is adequate in semiarid ecosystems where CO_2 efflux rates are typically low (Castillo-Monroy *et al*., 2011; Maestre *et al*., 2013). All collars were measured between 10:00 am and 13:00 pm. The frequency of measurements was set at 2-4 months during the first three years to record seasonal variation. After this period, this frequency was reduced to once a year due to logistical limitations, except in 2014, where no measurement was taken. Annual plant roots were not excluded from the PVC collars, but their contribution to measured soil CO_2 fluxes is rather negligible, as annual plants were removed from the collars regularly. The roots of *P. halepensis* may have contributed to measured fluxes, and hence the distance of the PVC collar from the closest tree individual (average distance of 4 m) and its diameter at breast height (average diameter of 0.4 m) were measured to account for this effect in the statistical models. In parallel to the R_S measurements, we measured soil temperature with protected diodes buried at 2 cm depth and soil volumetric water content using time-domain reflectometry between two 5 cm length probes.

The relationship between R_S and soil temperature was assessed to investigate the temperature sensitivity of R_S via two common metrics (β and Q_{10}) calculated at each plot (Luo *et al*., 2001; Crowther & Bradford, 2013): the slope of the relationship (β) was calculated using the following equation:

$$R_S = R_0 e^{\beta t} \qquad (1)$$

where R_S is the soil respiration (μmol m^{-2} s^{-1}), R_0 is the basal soil respiration rate (μmol m^{-2} s^{-1}) or intercept of soil respiration at 0ºC, and t is the soil temperature (in ºC) measured at the same time as R_S. β was used to compute the respiration quotient Q_{10} (increment in R_S when t increases by 10 ºC) as follows:

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

Biocrust cover monitoring

The total cover of the two major and visible components of the biocrust community (lichens and bryophytes) was estimated in each PVC collar at the beginning of the experiment and four years after the set-up of the warming treatment. We used high-resolution photographs to assess the proportion of each collared covered by lichens and mosses by mapping their area with the software GIMP (http://www.gimp.org/) and ImageJ (http://rsb.info.nih.gov/ij/). Cover estimates obtained with this method are highly related to those measured directly in the field with a point-intercept survey (Ladrón de Guevara *et al.*, 2018).

Soil β-glucosidase, carbohydrates and organic carbon accumulation measurements

Soil samples (0 – 1 cm) from the 40 plots were collected at the beginning of the experiment, and then one (2010), two (2011), four (2013) and eight (2017) years after the set-up of the warming treatment from five randomly selected plots per combination of treatments. Soil measurements at this depth (0 – 1 cm) are commonplace in biocrust studies, because the effects of this community on soil C and N cycling, and on soil microbial communities are especially marked right beneath them (Pointing & Belnap 2012). Soil sampling was conducted in early summer (June-July) every year, to allow inter-year comparisons. We obtained a composite soil sample per plot from four samples scattered outside the PVC collars, to avoid perturbing the biocrust and soil microbial community there. In the laboratory, we carefully removed visible biocrust components from the soil, which was sieved at 2 mm mesh and air-dried for one month. The activity of β-glucosidase was measured as described in Maestre *et al*. (2012). This enzyme is involved in starch degradation and facilitates the breakdown of low-molecular-weight carbohydrates (Eivazi & Tabatabai, 1988). A labile C fraction (carbohydrates) was also determined from K_2SO_4 soil extracts by quantifying the concentration of hexoses using colorimetric analyses according to Chantigny *et al.*, (2006). Soil organic carbon (SOC) concentration was determined by colorimetry after oxidation with a mixture of $K_2Cr_2O_7$ and H_2SO_4 (Anderson & Ingramm, 1993). Using SOC concentration, bulk density and thickness of the soil layer (1 cm), we calculated SOC stocks. The accumulation of SOC stocks over time (Mg C ha^{-1} yr^{-1}) were calculated as the ratio between the increase in SOC stocks (from the initial plots in 2009 to eight years after the set-up of the warming treatment in 2017) and the number of years (Gattinger *et al*., 2012; García-Palacios *et al.*, 2018).

Soil mass-specific respiration

A fraction of the soil samples (0 – 1 cm) collected four years after the set-up of the warming treatment was immediately frozen at –20 ºC for microbial analyses. Soil basal respiration and microbial biomass were estimated using a substrate-induced respiration

(SIR) technique (Bradford *et al.*, 2008, 2010; Ananyeva *et al.*, 2011; García-Palacios *et al.*, 2016). The SIR method we employed involved short (6 h) soil incubations at 25 ºC with excess substrate (water and glucose), which may overcome some of the limitations associated with the SIR method if microbes are actively growing (Blagodatsky *et al.*, 2000). We calculated soil mass-specific respiration ($R_{mass,}$, µg C g microbial biomass^{-1} day^{-1}), also called metabolic quotient, by dividing basal respiration from the glucose-induced respiration (our surrogate of microbial biomass). It is important to normalize soil respiration measurements with changes in microbial biomass, as microbial acclimation responses to increased temperature involve changes in R_{mass} (Bradford *et al.*, 2008, Crowther & Bradford, 2013).

Statistical analyses

We performed a series of analyses devoted to test the warming effects on soil respiration, the different pathways driving such response at low vs. high biocrust cover areas, and the ultimate consequences for SOC accumulation. First of all, we evaluated the warming effects on R_S and its temperature sensitivity (β and Q_{10}). R_S was analyzed using a three-way (warming, biocrust cover and time) ANOVA, with repeated measured of one of the factors (time). Both warming and biocrust were considered as fixed factors. As the assumption of multisample sphericity was not met, the Huynh-Feldt adjusted degrees of freedom were used for within-subjects tests (Quinn & Keough, 2002). The same model but without time was used to analyze the responses of β and Q_{10}. Once the effects of warming on soil respiration were investigated, we evaluated a series of hypotheses (H) related with the driving pathways at contrasted biocrust covers:

- H1 (decreased R_S with warming is the result of a reduction in autotrophic respiration) was evaluated by testing the treatment effects (warming *vs.* control and low *vs.* high initial biocrust cover) on the cover of lichens and bryophytes after four years of warming using a two-way ANOVA. The proportion of biocrust cover at the beginning of the experiment was introduced in the analysis as a covariate, but removed when not significant.
- H2 (decreased R_S with warming is the result of soil microbial thermal acclimation) was evaluated by testing the treatment effects on: (a) the activity of the enzyme β-glucosidase at four sampling dates (after one, two, four and eight years of warming), and (b) soil mass-specific respiration rates after four years of warming. H2 was tested using a two-way ANOVA.

- H3 (decreased R_S with warming is the result of soil drying reducing microbial activity) was evaluated by testing the relationship between the effect sizes of warming on R_S and soil moisture. To do so, we calculated the average across the 10 replicates for each temporal measurement. We used the response ratio (RR) as a metric of the warming effect size. RR (R_S) = ln (R_{SW} / R_{SC}), where R_{SW} is the soil respiration in the warming plots and R_{SC} is the soil respiration in the control plots.
- H4 (decreased R_S with warming is the result of substrate depletion) was evaluated by testing the effects of warming and initial biocrust cover on the concentration of soil carbohydrates one, two, four and eight years after the set-up of the experiment. As diverse subsets of five randomly selected plots per combination of treatments were measured at each year to avoid conspicuous soil perturbation, the treatments effects were evaluated at each sampling date by using two-way ANOVAs, with biocrust and warming as fixed factors. The concentrations of carbohydrates at the beginning of the experiment were introduced in the analysis as covariates, but removed when not significant.

Lastly, we tested the effects of warming and initial biocrust cover on the SOC accumulation rate (calculated from the beginning to eight years after the set-up of the experiment) using a two-way ANOVA. This is an adequate metric to assess the capacity of soils to store C over time, as it takes into account the initial variation in soil conditions between plots (Gattinger *et al.*, 2012). This analysis allowed us to evaluate whether the warming effects on R_S agree with changes in the rate at which SOC accumulates in soil. Across all analyses, the distance of each PVC collar from the closest tree individual and its diameter at breast height were introduced as covariates, but removed when not significant. *P* values were not adjusted for multiple testing because this approach is considered overly conservative (Gotelli & Ellison, 2004). All the analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

Results

Warming effects on R_S and Q_{10}

Warming significantly decreased R_S regardless of the initial biocrust cover (Fig. 1, P = 0.004, Table S1). On average, R_S was 0.30 µmol CO_2 m^{-2} s^{-1} (95 % CI = −0.24 to 0.84) lower in the warming than in the control plots across the years. Nevertheless, such difference increased to 0.49 µmol CO_2 m^{-2} s^{-1} (95 % CI =−0.12 to 1.10) from 2012 to

2015, after three years of warming. Accordingly, whereas the negative warming effects on R_S were significant across the experimental duration at high biocrust cover (Fig. 1b), this was only found after three years of warming at low biocrust cover (Fig. 1a). Similar results were found when analyzing the temperature sensitivity of R_S (Fig. 2, Table S2). Warming significantly reduced β and Q_{10} (P_W = 0.046 and 0.040, respectively), although such negative effect was especially marked in high biocrust cover plots for both β (0.01 points, 95 % CI = −0.021 to 0.001) and Q_{10} (0.1 points, 95 % CI = −0.01 to 0.21).

Pathways regulating the warming effects on R_S

We first analyzed the warming effects on the total cover of biocrust four years after the set-up of the experiment, and found a significant warming × initial biocrust cover interaction ($P_{W \times biocrust}$ = 0.031, Table S3, Fig. 3). Warming reduced total biocrust cover by 23% (95 % CI = 4.9 to 41.4) in the plots with high initial biocrust cover respect to the control plots, but had no effect in low biocrust cover plots. Such response was mediated by a significant ($P_{W \times biocrust}$ = 0.008) reduction of 23% (95 % CI = 11.9 to 42.7) in lichen cover in the warmed plots at high biocrust level. Bryophytes experienced a significant (P_W = 0.025) cover gain of 3.5% (95 % CI = 0.9 to 8) in the warmed plots.

During the first two years of the experiment, the activity of β-glucosidase was higher with warming in the low biocrust cover plots ($P_{W \times biocrust}$ < 0.05 in both years, Table S4), with increases of 0.30 µmol PnP g^{-1} soil h^{-1} (95 % CI = −0.47 to 1.07) in 2010 and 0.41 µmol PnP g^{-1} soil h^{-1} (95 % CI =−0.23 to 1.06) in 2011 (Fig. 4a). The opposite pattern was found in the plots with high biocrust cover, where the values of this enzymatic activity were 0.65 µmol PnP g^{-1} soil h^{-1} (95 % CI =−0.12 to 1.42) and 0.42 µmol PnP g^{-1} soil h^{-1} (95 % CI = −0.23 to 1.06) lower in the warmed plots in 2010 and 2011, respectively. In the fourth year of warming (2013), the activity of β-glucosidase was 0.54 µmol PnP g^{-1} soil h^{-1} (95 % CI =−0.15 to 1.23) lower in the warmed plots across both biocrust levels (P_W = 0.006). After eight years of elevated temperatures, the activity of β-glucosidase was still lower with warming in the high biocrust cover plots, although this difference disappeared in the low biocrust cover plots ($P_{W \times biocrust}$ = 0.072). Soil mass-specific respiration was 1.86 µg C g microbial biomass^{-1} day^{-1} (95 % CI = 0.31 to 3.41) lower in the warming than in control plots four years after the set-up of the experiment (Fig. 4b), but this response was only observed at low biocrust cover ($P_{W \times biocrust}$ < 0.041, Table S5). The effect size (response ratio) of warming on soil moisture and R_S were not related at both low ($r = -0.09$, $P = 0.708$, $n = 18$) and high ($r = -0.16$, $P = 0.529$, $n = 18$) biocrust cover levels. After one and two years of warming, the concentration of soil carbohydrates was 0.30 mg C Kg^{-1} soil (95 % CI =−0.47 to 1.07) and 0.41 mg C Kg $^{-1}$ soil (95 % CI =−0.23 to 1.06) higher in warmed than in control plots, respectively (Figs. 5a and 5b). However, this effect was only found at low biocrust cover ($P_{W \times biocrust} < 0.05$ in both years, Table S6). This significant interaction vanished after four and eight years of experimental warming (Figs. 5c and 5d).

Warming effects on soil organic carbon accumulation rates

The presence of a well-develop biocrust community significantly increased SOC accumulation rates throughout the eight years of the experiment ($P = 0.044$, Fig. 6), with a mean difference of 0.12 Mg C ha^{-1} yr^{-1} (95 % CI =−0.10 to 0.34) from low to high biocrust cover sites. Warming increased SOC accumulation rates by 0.11 Mg C ha^{-1} yr^{-1} (95 % CI =−0.11 to 0.33) , albeit these differences were non-significant (P = 0.056, Table S7).

Discussion

The observed responses of R_S to eight years of experimental warming in a biocrust-dominated dryland provide empirical support to recent global extrapolations on soil C losses with warming (Crowther *et al.*, 2016). On average, warming reduced R_S by 13% compared with control plots across the years, although these differences were only significant at low levels of biocrust cover after three years of elevated temperatures. We found support for several pathways regulating the warming-induced reduction in R_S at low (microbial thermal acclimation) vs. high (microbial thermal acclimation jointly with a reduction in autotrophic respiration from biocrust) levels of initial biocrust cover.

Warming decreases soil respiration and its temperature sensitivity

Previous studies in drylands have found different warming effects on R_S, ranging from positive (Shen *et al.*, 2009; Maestre *et al.*; 2013; Darrouzet-Nardi *et al.*, 2015) to negative (Xu *et al.*, 2016; Wertin *et al.*, 2017). Our results from eight-year experimental warming indicated that whereas elevated temperatures decreased R_S over the study duration at high biocrust cover, this reduction was significant at low biocrust cover only after three years of warming. According to this, the two metrics addressing the temperature sensitivity of R_S over the study duration, β (the slope of the relationship between R_S and soil temperature) and Q_{10} (the respiration quotient), were significantly lower in the warmed plots at high but not at low biocrust cover. These results are in disagreement with those from Maestre *et al.* (2013) and Darrouzet-Nardi *et al.* (2015), which were also performed in biocrust-dominated hot drylands. Both studies found a positive warming effect on R_S, and linked such pattern with an increased in soil CO_2 efflux from the heterotrophic component, as lower soil CO_2 net uptake was unlikely to be driven by biocrust photosynthesis. Therefore, the apparent mismatch with our results may be driven by a different response of heterotrophic respiration to warming. To better understand the role played by biocrusts mediating the effects of elevated temperatures on R_S, potential autotrophic (decreased biocrust cover) and heterotrophic (substrate depletion, warming-induced reduction in soil moisture and microbial thermal acclimation) pathways should be investigated.

Warming effects on soil respiration via autotrophic pathways

In biocrust-dominated drylands, soil respiration has both autotrophic (e.g., bryophytes and lichens) and heterotrophic (microbial biomass) components (Darrouzet-Nardi *et al.*, 2015). Thus, beyond the microbial mechanisms typically addressed in more mesic biomes, the respiration contribution from autotrophic biocrust organisms may be a particularly important driver of warming effects on R_S in these areas. In our study, the total cover of biocrust was considerably reduced after four years of warming, mainly due to lichen mortality, although this was only found in plots with high initial biocrust cover. These results are in agreement with previous findings in other biocrust-dominated ecosystems (Belnap *et al.*, 2006; Maphangwa *et al.*, 2012; Maestre *et al.*, 2013). Elucidating the physiological drivers of such dramatic reduction in lichen cover is beyond the scope of our study. However, we hypothesize that it is is driven by a decrease in C fixation as a consequence of abiotic stress linked with warming-induced reduction in the duration of dew events (Ladrón de Guevara *et al*., 2014), which is a key driver of lichen metabolic activity in sites where summer precipitations are absent (del Prado & Sancho, 2007). If we define dew events as periods with relative air humidity of 100% (Maphangwa *et al*., 2012; Ladrón de Guevara *et al*., 2014), the length of these periods was reduced by 44% in the warming plots (Maestre *et al*., 2015). The decrease in lichen cover, and therefore in CO_2 efflux from lichen respiration, may explain the low R_S found during the first four years of warming in plots with high initial levels of biocrust cover, supporting our first hypothesis. Biocrust-forming lichens are adapted to the extreme climatic conditions found in drylands (Green *et al*., 2011), but our results suggest that a ~ 2ºC warming can lead to major mortality events due to reduced water inputs from dew, with cascading effects on soil C dynamics. This is quite a disturbing result considering that current climate change models suggest that a < 2 °C warming by 2100 is unlikely to be met (Raftery *et al*., 2017).

Warming effects on soil respiration via heterotrophic pathways

Overall, our results point to microbial thermal acclimation as a pathway contributing to decrease R_S and its temperature sensitivity with warming, which agrees with our second hypothesis. Here, we addressed the role of soil microbial thermal acclimation as a pathway driving the effects of warming on R_S, by investigating extracellular enzymatic activities (β-glucosidase) and mass-specific respiration rates (R_{mass}). This indirect approach is adequate to identify potential microbial acclimation to the thermal regime (Rinnan *et al*., 2009; Allison *et al*., 2010; Blagodatskaya *et al.*, 2016), but cannot be used to establish a causal link with the reduced R_S observed under warming. Acclimation to the thermal regime in plants and animals can occur within short-time spans (from days to weeks) when affecting enzymes of respiratory pathways (Hochachka & Somero, 2002; Atkin & Tjoelker, 2003). Such biochemical adaptations are also likely to occur in soil heterotrophic organisms (Bradford, 2013; Crowther & Bradford, 2013). During the first two years of the experiment, we found higher β-glucosidase activity in warmed plots with low biocrust cover, but this response was reversed after four years, matching the R_S results. Enzymatic and R_S responses to warming also matched in plots with high biocrust cover, as both variables decreased with elevated temperatures across the eight years of the study. We also investigated the 'aggregate' respiratory activity of soil microbial communities under controlled biomass, measuring the R_{mass} after four years of warming (Bradford *et al*., 2008, 2010). Our results from the low biocrust cover plots are consistent with those found when measuring the activity of β-glucosidase, but R_{mass} was similar in warming and control plots at high biocrust levels. Importantly, previous results from the same experiment showed that warming did not affect the soil fungal: bacterial ratio, as measured with phospholipid fatty acids (Maestre *et al*., 2015), which indicates that changes in microbial structure are unlikely to drive the β-glucosidase and R_{mass} responses observed (Ziegler *et al.*, 2013). However, we cannot disentangle whether biochemical/physiological adaptation is behind these patterns (Hochachka & Somero, 2002).

The decrease in R_S with warming observed throughout the experiment was independent of changes in soil moisture, which disagrees with our third hypothesis. This pattern suggests that the negative warming effects on R_S are not the product of soil drying reducing microbial activity, as found in other dryland studies with similar climatic conditions (Pendall et al., 2013). However, the average 1.3% soil moisture decrease found in the warming compared with the control plots (Maestre *et al*., 2015) might not have been enough to drive the R_S responses to warming. Alternatively, in Pendall *et al.* (2013), R_S responses to warming were mediated by a 15% decrease in soil moisture over the four years study period. The response of R_S to elevated temperatures may be also limited by the depletion of labile C pools by increased microbial activity with warming (Eliasson *et al*., 2005; Hartley *et al*., 2007). Instead, substrate limitation was unlikely to drive the reduced R_S found with elevated temperatures, which is contrary to our fourth hypothesis. The concentration of soil carbohydrates was not lower in warming than in control plots across the eight years of study duration at both biocrust levels.

Consequences for soil organic carbon accumulation

Soil organic carbon stocks represent the net result of long-term changes in SOC gains and losses (Crowther *et al*., 2016). Thus, the assessment of SOC changes over time (i.e. SOC accumulation rates along eight years) allowed us to evaluate whether warming-induced effects in soil C losses via R_S ultimately impacted the capacity of soils to store C. Soil organic carbon accumulation was higher in high than in low biocrust cover plots, which may be due to the increased transfer of C from biocrust photosynthesis to soil typically observed in high biocrust cover microsites (Maestre et al., 2013; Ladrón de Guevara et al, 2014). At both levels of initial biocrust cover, the SOC accumulation rates were higher in warming than in control plots, although such differences were not statistically significant (P = 0.056). This result agrees with the lower R_S found in warming plots over the study period. Soil microbes may start to acclimate to rising temperatures at low biocrust cover since 2012, as the β-glucosidase and R_{mass} results suggest, likely contributing to explain the significantly lower R_S found in warmed plots from 2012 to 2015. It is important to note that in these areas, biocrust cover was below 15% in both control and warming plots, preventing any potential effect of decreasing biocrust cover with warming on R_S and SOC. Conversely, at high biocrust cover, the decreased soil C losses from R_S found across the study agrees with the higher SOC accumulation rates found.

We describe two potential mechanisms to explain the increase in SOC accumulation with warming. First, the mortality and subsequent decomposition of biocrust-forming lichens with warming may increase soil C gains from recalcitrant C compounds (Stark *et al.,* 2007), but also decrease soil C losses from autotrophic respiration. Both effects may enhance the accumulation of SOC over time. Nevertheless, our results do not support the decomposition pathway suggested by Maestre *et al.* (2013), as the biocrust cover assessed four years after the set-up of the warming experiment was not significantly related with the SOC accumulation rate calculated over that period (r = 0.314, P = 0.178). Secondly, microbial thermal acclimation to elevated temperatures may also play an important role decreasing soil C losses via reduced $R_{S,}$ as the β-glucosidase results suggest (Bradford, 2013), which may have contributed to increase SOC accumulation rates. If biocrust-forming lichens disappear, soil microbial acclimation to warming conditions, in addition to an eventual depletion of labile C sources feeding microbial activity, may actually contribute to reduce R_S under warming conditions. Such an effect may likely depend on the initial biocrust cover levels, substrate and microbial community composition, which might explain the differences for soil respiration found here vs. somewhere else (Maestre *et al.*, 2013; Darrouzet-Nardi *et al.,* 2015). Put simply, the initial conditions from different locations (studies) and the different temporal stage evaluated in current warming experiments in drylands (2-4 years vs. eight years in our study) might lead to different patterns in R_S. For example, R_S in different locations might have a different initial resistance to warming, taking different time to respond to this climate change driver. However, we hypothesize that, in the mid- to long-term (> 8-10 years), as the cover of biocrust-forming lichens in drylands decreases and soil microbes acclimate to elevated temperatures, warming will lead to a generalized decline in soil respiration across different studies.

Methodological caveats and future directions

Open top chambers (OTCs) are a common method to simulate climate warming (Bokhorst *et al*., 2013; Reed *et al*., 2016), but the methacrylate sheets used to build the them can intercept fog circulation, increasing the duration of dew events and thereby confounding the effects of elevated temperatures. Importantly, our OTCs were elevated five cm from the ground, to allow air circulation. In fact, the length of these periods was reduced by 44% in the warming plots (Maestre *et al.*, 2015), neglecting the importance of such methodological caveat in our study. The vicinity of *Pinus halepensis* individuals (average distance of 4 m) from the PVC collars may have influenced our R_S measurements. This is indeed a problem in most studies teasing apart the autotrophic from the heterotrophic component of R_S (Hartley *et al.*, 2007), as root respiration represents a large proportion of R_S (Hanson *et al*., 2000), and it can respond differently than heterotrophic respiration to elevated temperatures (Wang *et al*., 2014). However, since the *P. halepensis* trees were not directly warmed-up with the OTC chambers (40 × 50 × 32 cm), we believe that the responses of R_S to warming may have not been influenced by nearby tree individuals. In fact, to further account for this point, we evaluated the indirect contribution of *P. halepensis* root respiration by including the distance from the closest tree individual and the size of such individual in the models, but found no significant effect on any of the variables measured. We also acknowledge that the frequency of R_S measurements is not enough to estimate annual R_S. However, our goal was to compare R_S rates in warmed vs. control plots, particularly during the dry season, which represents the natural state of drylands during most part of the year, but also avoid the drastic changes in R_S caused by occasional rain pulses (Escolar *et al*., 2015).

Our results indicate that warming has a negative effect on R_S in drylands, and adds to the still scarce number of experimental studies evaluating warming impacts on this important soil C flux. Although our results suggest that contrasting pathways may explain such reduced R_S in microsites with low vs. high biocrust cover, the direct mechanistic linkages remain to be explored. In this context, measuring the respiration of soils from control and warming plots after short-term incubation at increasing temperatures represents an explicit test of microbial thermal acclimation (Bradford *et al*., 2008; Crowther & Bradford 2013; Karhu *et al.*, 2014). Additionally, to quantify the fate of soil C inputs from biocrust-forming lichens, decomposition studies using ^{13}C-labelled lichen material may allow to trace lichen-derived C in soil organic matter and R_S (Cotrufo *et al*., 2015), and constitutes a promising research line.

Our eight-year experimental study indicates that warming decreases R_S and its temperature sensitivity in a biocrust-dominated dryland. We found support for different pathways regulating such pattern, with soil microbial thermal acclimation affecting at both biocrust cover levels, but reduced autotrophic respiration mainly operating in plots with high biocrust cover. These results provide empirical support to previous global extrapolations showing that, in drylands, soil C losses to the atmosphere due to rising temperatures may be lower compared with other biomes (Crowther *et al*., 2016).

However, previous short-term studies have found the opposite pattern (Maestre *et al.*, (2013; Darrouzet-Nardi *et al.*, 2015), which calls for the need of longer-term studies addressing soil carbon losses in drylands, as performed in more mesic biomes (Melillo *et al.*, 2017). Biocrust communities can drive the warming effects on R_S in drylands, and therefore should be explicitly included in modelling efforts aimed to quantify the soil C−climate feedback in this biome.

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References

Ananyeva ND, Susyan EA, Gavrilenko EG (2011) Determination of the soil microbial biomass carbon using the method of substrate induced respiration. *Eurasian Soil Science*, **11**, 1215–1221.

Anderson M, Ingramm JSI (1993) *Tropical Soil Biology and Fertility: A Handbook of Methods*. CAB International, Wallington.

Atkin, OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science*, **8**, 343–351.

Allison SD, Wallenstein MD, and Bradford MA (2010) Soil–carbon response to warming dependent on microbial physiology. *Nature Geoscience*, **3**, 336–340.

Blagodatskaya E., Blagodatsky S, Khomyakov N, Myachina O, Kuzyakov Y (2016). Temperature sensitivity and enzymatic mechanisms of soil organic matter decomposition along an altitudinal gradient on Mount Kilimanjaro. *Scientific Reports*, **6**, 22240.

Blagodatsky SA, Heinemeyer O & Richter J (2000) Estimating the active and total soil microbial biomass by kinetic respiration analysis. *Biology and Fertility of Soils,* **32,** 73–81**.**

Belnap J, Phillips SL, Troxler T (2006) Soil lichen and moss cover and species richness can be highly dynamic: the effects of invasion by the annual exotic grass Bromus tectorum, precipitation, and temperature on biological soil crusts in SE Utah. *Applied Soil Ecology*, **32**, 63–76.

Bokhorst S, Huiskes A, Aerts R *et al.* (2013). Variable temperature effects of Open Top Chambers at polar and alpine sites explained by irradiance and snow depth. *Global Change Biology*, ***19***, 64–74.

Bradford MA (2013) Thermal adaptation of decomposer communities in warming soils. *Frontiers in Microbiology*, **4**, 333.

Bradford MA, Davies CA, Frey SD *et al.* (2008) Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Lett*ers, **11**, 1316–1327.

Bradford MA, Watts BW, Davies CA (2010) Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Global Change Biology,* **16**, 1576–1588.

Bradford MA, Wieder WR, Bonan GB, Fierer N, Raymond PA, Crowther TW (2016) Managing uncertainty in soil carbon feedbacks to climate change. *Nature Climate Change*, **6**, 751–758.

Carey JC, Tang J, Templer PH *et al.* (2016) Temperature response of soil respiration largely unaltered with experimental warming. *Proceedings of the National Academy of Sciences*, **113**, 13797–13802.

Castillo–Monroy AP, Maestre FT, Rey A, Soliveres S, García–Palacios P (2011) Biological soil crust microsites are the main contributor to soil respiration in a semiarid ecosystem. *Ecosystems*, 14, 835**–**847.

Chantigny MH, Angers DA, Kaiser K, Kalbitz K (2006) *Soil Sampling and Methods of Analysis* (eds Carter MR, Gregorich EG), pp. 617–635. CRC Press, Boca Raton.

Cotrufo MF, Soong JL, Horton AJ, Campbell EE, Haddix MH, Wall DL, Parton WJ (2015) Soil organic matter formation from biochemical and physical pathways of litter mass loss. *Nature Geosciences*, **8**, 776–779.

Crowther TM, Bradford MA (2013) Thermal acclimation in widespread heterotrophic soil microbes. *Ecology Letters*, **4**, 469–477.

Crowther T, Todd-Brown KEO, Rowe CW *et al.* (2016) Quantifying global soil C losses in response to warming. *Nature*, **540**, 104–108.

Darrouzet–Nardi A, Reed SC, Grote EE, Belnap J (2015) Observations of net soil exchange of CO_2 in a dryland show experimental warming increases carbon losses in biocrust soils. *Biogeochemistry,* **126,** 363–378**.**

Del Prado R, Sancho, LG (2007) Dew as a key factor for the distribution pattern of the lichen species *Teloschistes lacunosus* in the Tabernas Desert (Spain). *Flora,* **202**, 417–428.

Dorrepaal E, Toet S, van Logtestijn RSP, Swart E, van de Weg MJ, Callaghan TV, Aerts R (2009) Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. *Nature*, **460**, 616–619.

Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, **20**, 601–606.

Elbert W, Weber B, Burrows S, Steinkamp J, Büdel B, Andreae MO, Poschl U (2012) Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. *Nature Geoscience,* **5**, 459–462.

Eliasson PE, McMurtrie RE, Pepper DA, Strömgren M, Linder S, Ågren GI (2005) The response of heterotrophic CO_2 flux to soil warming. *Global Change Biology*, **11**, 167–181.

Escolar C, Maestre FT, Rey A (2015) Biocrusts modulate warming and rainfall exclusion effects on soil respiration in a semi–arid grassland. *Soil Biology & Biochemistry*, **80**, 9–17.

Frey SD, Lee J, Melillo JM, Six J (2013) The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change*, **3**, 395–398.

García-Palacios P, Prieto I, Ourcival JM, Hättenschwiler S (2016) Disentangling the litter quality and soil microbial contribution to leaf and fine root litter decomposition responses to reduced rainfall. *Ecosystems,* **19**, 490–503.

García-Palacios, P. (2018). Data from: Pathways regulating decreased soil respiration with warming in a biocrust-dominated dryland. *Figshare Digital Repository*, https://figshare.com/s/007f6a567ce4111ef3b7.

García-Palacios P, Gattinger A, Bracht-Jørgensen H *et al.* (2018) Crop traits drive soil carbon sequestration under organic farming. *Journal of Applied Ecology*, in press, doi: 10.1111/1365-2664.13113.

Gattinger A, Muller A, Haeni M *et al.* (2012) Enhanced top soil carbon stocks under organic farming. *Proceedings of the National Academy of Sciences,* **109**, 18226–18231.

Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. *Global Planeteray Change,* **63**, 90–104.

Green TGA, Sancho LG, Pintado A (2011) Ecophysiology of Desiccation/Rehydration Cycles in Mosses and Lichens. In: *Plant Desiccation Tolerance* (eds Lüttge U, Beck E, Bartels D), pp. 89–120. Springer, Berlin Heidelberg.

Grote EE, Belnap J, Housman DC, Sparks JP (2010) Carbon exchange in biological soil crust communities under differential temperatures and soil water contents: implications for global change. *Global Change Biology*, **16**, 2763–2774.

Gotelli NJ, Ellison AM (2004) *A Primer of Ecological Statistics*. Sinauer Associates. Sunderland.

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry,* **48**, 115–146.

Hartley IP, Heinemeyer A, Ineson P (2007) Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Global Change Biol*ogy, **13**, 1761–1770.

Hochachka PW, Somero GN (2002) *Biochemical adaptation: Mechanism and process in physiological evolution*. Oxford University Press, Oxford.

IUSS Working Group WRB (2006) *World Reference Base for Soil Resources* 2006. *World Soil Resources Reports No. 103*. FAO, Rome.

Karhu K, Auffret AD, Dungait JAJ, *et al.* (2014) Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature,* **513**, 81–84.

Ladrón de Guevara M, Lázaro, R, Quero, JL, Ochoa V, Gozalo B, Berdugo M, Uclés O, Escolar C, Maestre FT (2014) Simulated climate change reduced the capacity of lichen–dominated biocrusts to act as carbon sinks in two semi–arid Mediterranean ecosystems. *Biodiversity and Conservation,* **23**: 1787–1807.

Ladrón de Guevara M, Gozalo B, Raggio J, Lafuente A, Prieto I, Maestre FT (2018) Warming reduces the cover, richness and evenness of lichen–dominated biocrusts but promotes moss growth: Insights from an eight–year experiment. *New Phytologist*, in press, doi: 10.1111/nph.15000.

Luo Y, Wan S, Hui D, Wallace LL (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature,* **413**, 622–625.

Maestre FT, Quero JL, Gotelli NJ *et al.* (2012) Plant species richness and ecosystem multifunctionality in global drylands. *Science,* **335**, 214–218.

Maestre FT, Escolar C, de Guevara ML *et al.* (2013) Changes in biocrust cover drive carbon cycle responses to climate change in drylands. *Global Change Biol*ogy, **19**, 3835–3847.

Maestre FT, Escolar C, Bardgett R, Dungait JAD, Gozalo B, Ochoa V. (2015) Warming reduces the cover and diversity of biocrust–forming mosses and lichens, and increases the physiological stress of soil microbial communities in a semi–arid *Pinus halepensis* plantation. *Frontiers in Microbiology* **6**: 865.

Maphangwa KW, Musil CF, Raitt L, Zedda L (2012) Experimental climate warming decreases photosynthetic efficiency of lichens in an arid South African ecosystem. *Oecologia*, **169**, 257–268.

Melillo, JM, Butler S, Johnson J *et al.* (2011) Soil warming, carbon –nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences*, **108**, 9508–9512.

Melillo JM, Frey SD, DeAngelis KM, Werner W, Bernard MJ, Bowles FP, Pold G, Knorr MA, Grandy AS (2017) Long–term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science*, **358**, 101–105.

Pachauri RK, Meyer LA (2014) *IPCC Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Geneva.

Pendall E, Heisler-White JL, Williams DG, Dijkstra FA, Carrillo Y, Morgan JA, LeCain DR (2013) Warming reduces carbon losses from grassland exposed to elevated atmospheric carbon dioxide. *Plos One*, **8**, e71921.

Pointing SB, Belnap J (2012) Microbial colonization and controls un dryland systems. *Nature Reviews Microbiology*, **10**, 551–562.

Prăvălie R (2016) Drylands extent and environmental issues. A global approach. Earth *Sciences Review*, **161**, 259–278.

Quinn GP, Keough MJ (2002) *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge.

Raftery AE, Zimmer A, Frierseon DMW, Startz R, Lie P (2017) Less than 2 °C warming by 2100 unlikely. *Nature Climate Change*, **7**, 637–641.

Reed SC, Maestre FT, Ochoa-Hueso R. *et al.* (2016). Biocrusts in the context of global change. In *Biological Soil Crusts: An Organizing Principle in Drylands* (pp. 451-476). Springer International Publishing, Cham.

Rinnan R, Rousk J, Yergeauz E, Kowalchuk GA, Bååth E (2009) Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: predicting responses to climate warming. *Global Change Biology*, **15**, 2615–2625.

Rodríguez-Caballero E, Belnap J, Büdel B, Crutzen PJ, Andreae MO, Pöschl U, Weber B (2018) Dryland photoautotrophic soil surface communities endangered by global change. *Nature Geosciences*, **11**, 185–189.

Safriel U, Adeel Z. (2005) *Ecosystems and Human Well–Being: Current State and Trends*. Island Press, Washington.

Schlesinger WH, Bernhardt E (2013) *Biogeochemistry, An Analysis of Global Change*. Elsevier, Waltham.

Shen W, Reynolds JF, Hui D (2009) Responses of dryland soil respiration and soil carbon pool size to abrupt vs. gradual and individual vs. combined changes in soil temperature, precipitation, and atmospheric CO2: a simulation analysis. *Global Change Biology*, **15**, 2274–2294.

Stark S, Kytöviita M, Neumann AB (2007) The phenolic compounds in Cladonia lichens are not antimicrobial in soils. *Oecologia*, **152**, 299–306.

Thomas AD (2012) Impact of grazing intensity on seasonal variations of soil organic carbon and soil CO_2 efflux in two semi–arid grasslands in southern Botswana. *Philosophical Transactions of the Royal Society B,* 367, 3076–3086.

Van Gestel N, Shi Z, van Groenigen KJ *et al.* (2018) Predicting soil carbon loss with warming. *Nature*, **554**, 4–5.

Wang X, Liu L, Piao S, Janssens IA, Tang J, Liu W, Chi Y, Wang J, Xu S (2014) Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. *Global Change Biology*, **20**, 3229–3237.

Wertin TM, Belnap J, Reed SC (2017) Experimental warming in a dryland community reduced plant photosynthesis and soil CO_2 efflux although the relationship between the fluxes remained unchanged. *Functional Ecology*, **31**, 297–305.

Xu Z, Hou Y, Zhang L, Liu T, Zhou G (2016) Ecosystem responses to warming and watering in typical and desert steppes. *Scientific Reports*, **6,** 34801.

Ziegler SE, Billings SA, Lane CS, Li J, Fogel ML (2013) Warming alters routing of labile and slower–turnover carbon through distinct microbial groups in boreal forest organic soils. *Soil Biology and Biochemistry,* **60**, 23–32.

Figure captions

Figure 1. Soil respiration (R_S, measured as soil CO_2 efflux) along six years of warming at low (a) and high (b) levels of initial biocrust cover (< 15% and > 50%, respectively). Data are means ± SE (n = 10). F = February, M = May, A = August, N = November.

Figure 2. Temperature sensitivity of soil respiration at low (< 15%) and high (> 50%) levels of initial biocrust cover: (a) β, the slope of the linear regression between volumetric soil water content and R_S, and (b) Q_{10}, the respiration quotient. Both variables are unitless. Box plots represent medians, 25th and 75th percentiles (n = 10). Error bars represent 10th and 90th percentiles. Asterisks denote significant differences at $P < 0.05$.

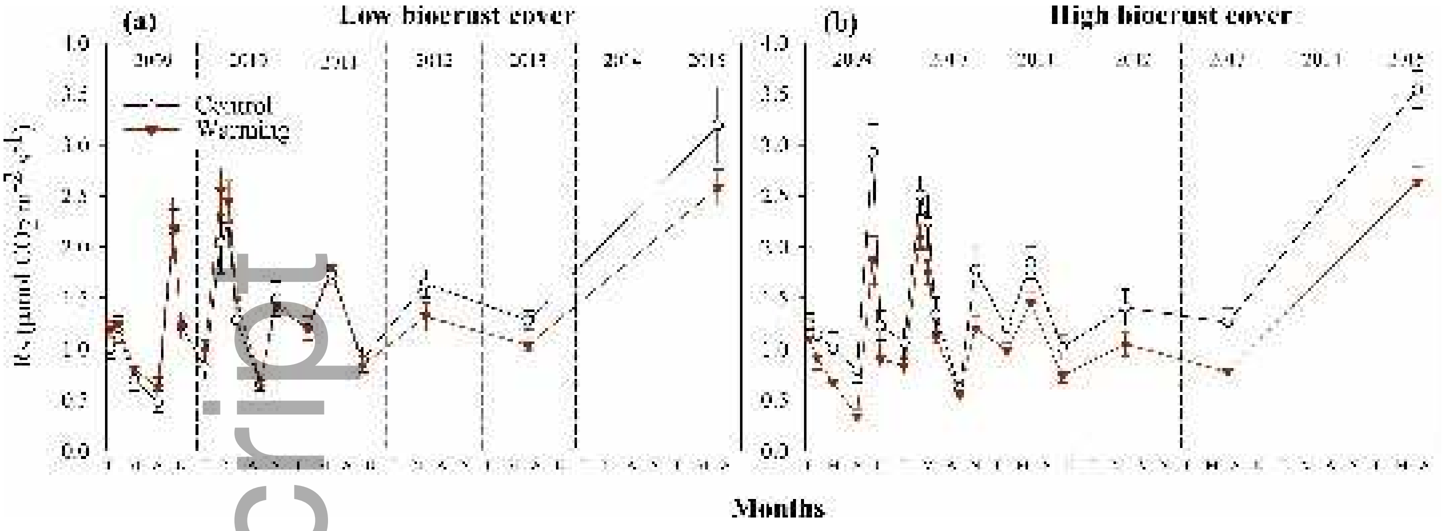
Figure 3. Total cover of biocrust and its major visible components (bryophytes and lichens) after four years (2013) of warming at low (< 15%) and high (> 50%) levels of initial biocrust cover. Box plots represent medians, 25th and 75th percentiles (n = 10). Error bars represent 10th and 90th percentiles. Asterisks denote significant differences at $P < 0.05$.

Figure 4. Soil β-glucosidase activity after one (2010), two (2011), four (2013) and eight (2017) years of warming (a), and soil mass-specific respiration (basal respiration / microbial biomass) after four (2013) years of warming (b). Data are split into low (< 15%) and high (> 50%) levels of initial biocrust cover. Box plots represent medians, 25th and 75th percentiles (n = 5). Error bars represent 10th and 90th percentiles. Asterisks denote significant differences at $P < 0.05$.

Figure 5. Soil carbohydrates concentration after one (2010, a), two (2011, b), four (2013, c) and eight (2017) years of warming at low (< 15%) and high (> 50%) levels of initial biocrust cover. Box plots represent medians, 25th and 75th percentiles (n = 5).

Error bars represent 10th and 90th percentiles. Asterisks denote significant differences at $P < 0.05$.

Figure 6. Soil organic carbon (SOC) accumulation rates after eight years of warming at low (< 15%) and high (> 50%) levels of initial biocrust cover. Box plots represent medians, 25th and 75th percentiles (n = 5). Error bars represent 10th and 90th percentiles. Asterisks denote significant differences at $P < 0.05$.



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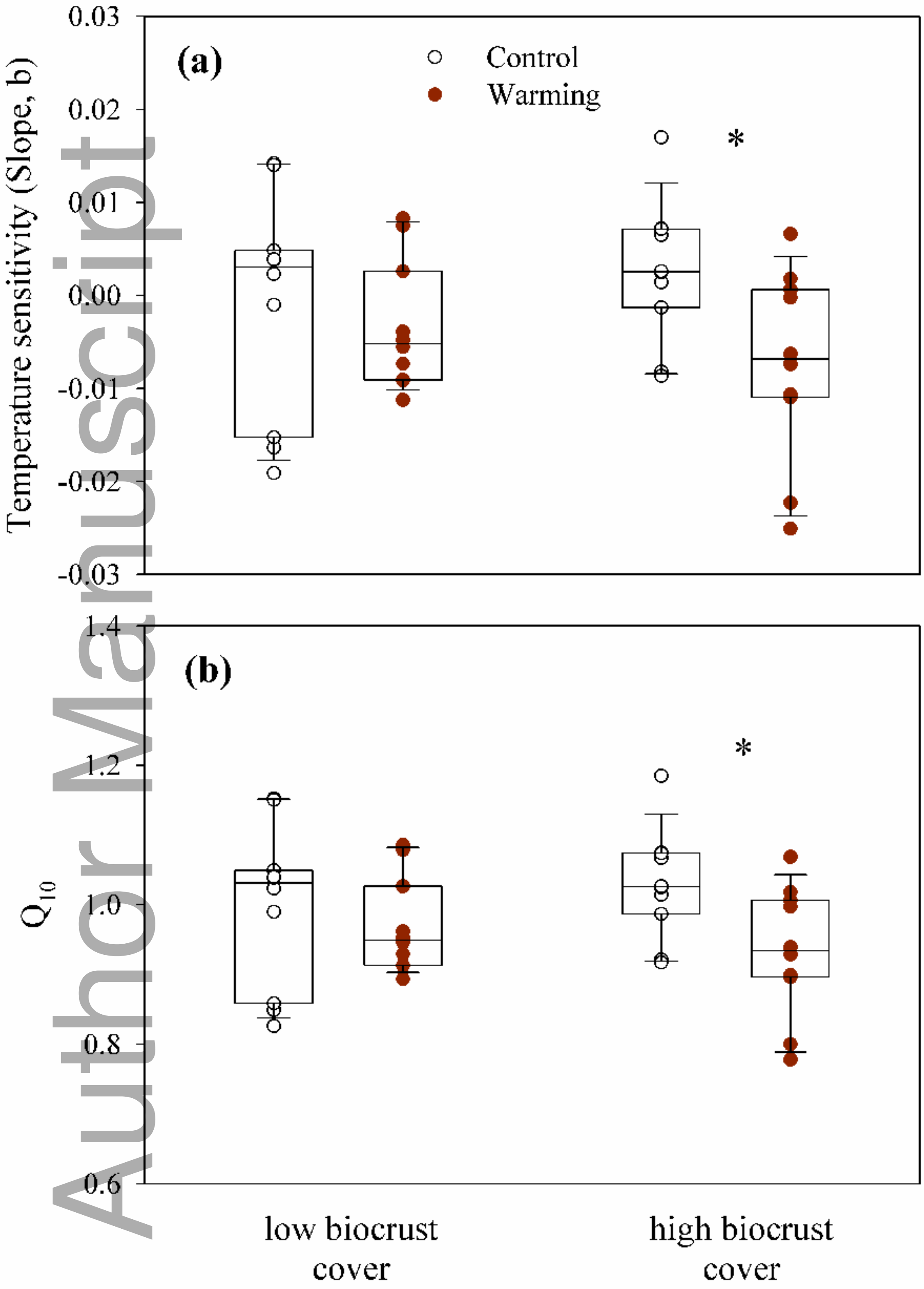

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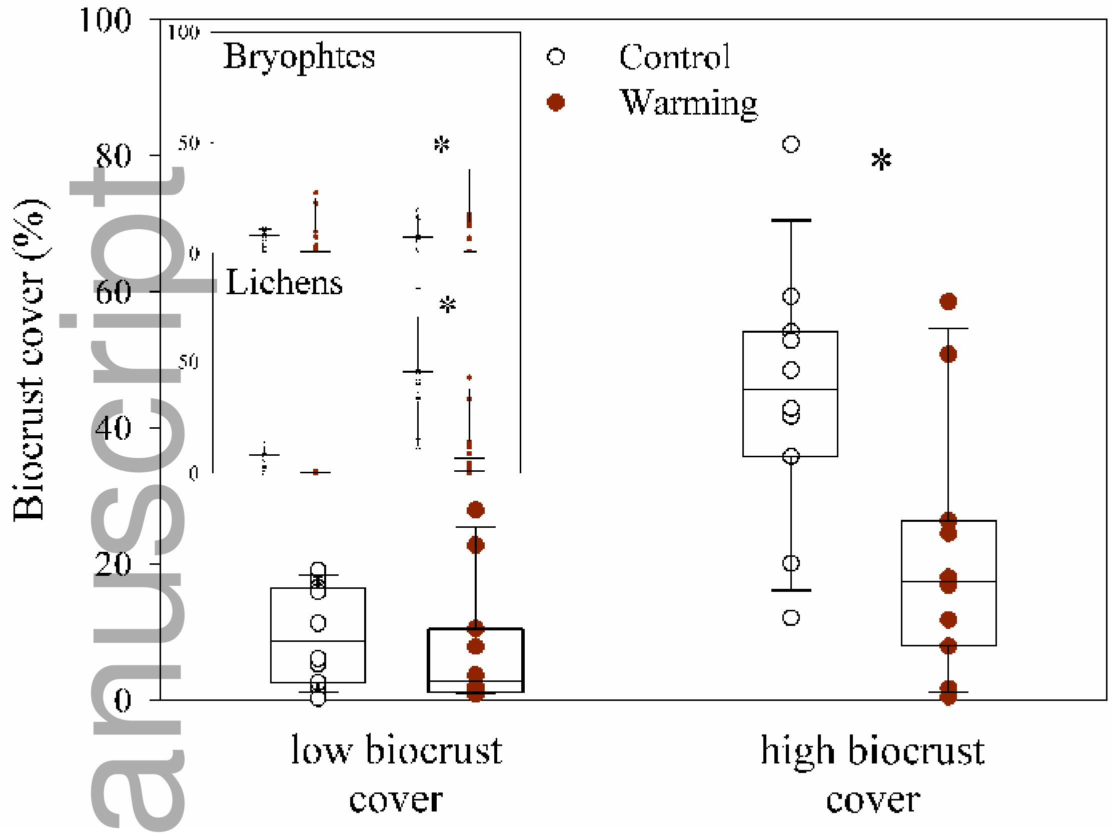

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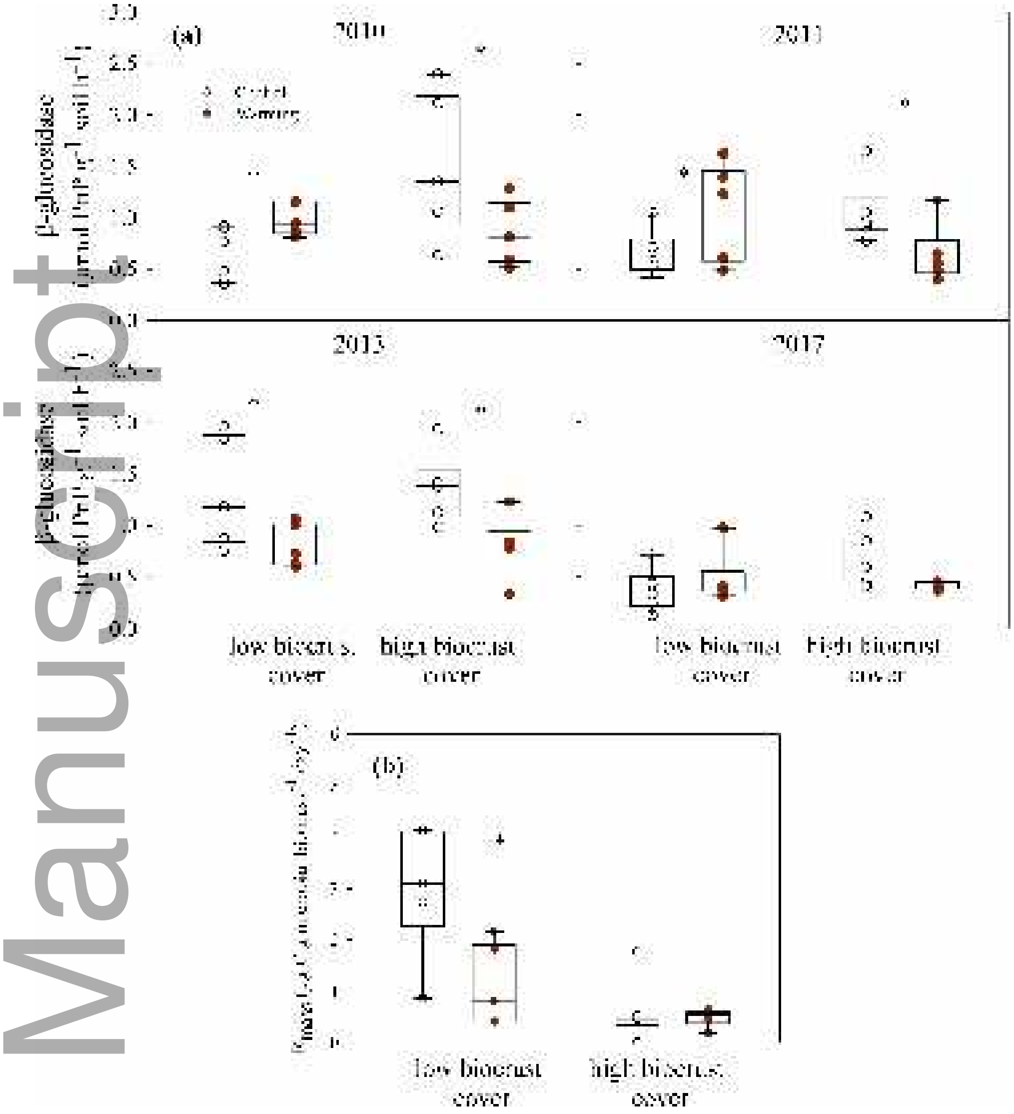

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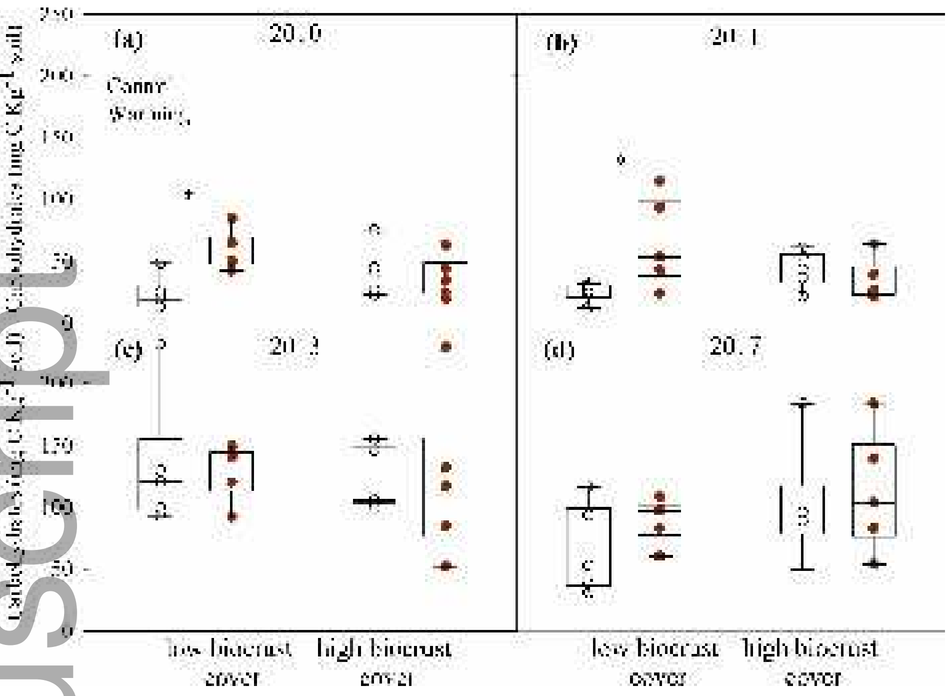

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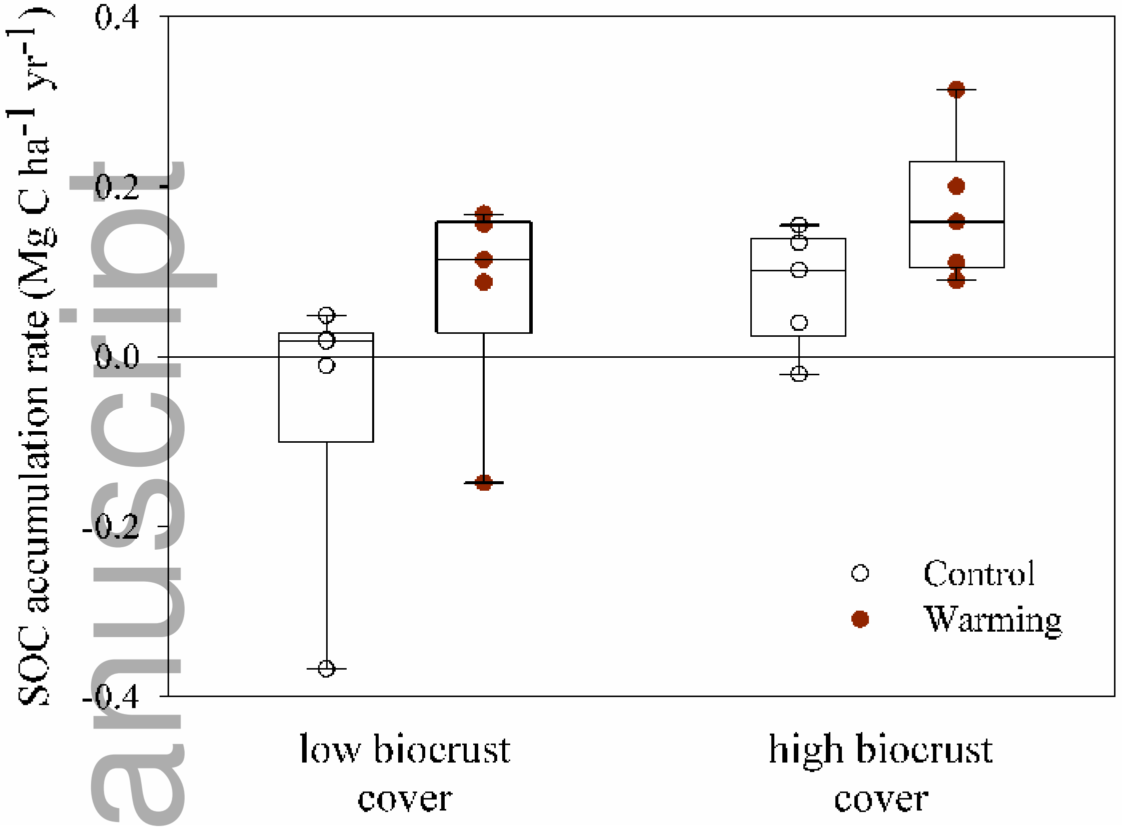

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