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Species-specific effects of biocrust-forming lichens on soil properties under simulated climate change are driven by functional traits

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SUMMARY

(1) Biocrusts are key drivers of ecosystem functioning in drylands, yet our understanding of how climate change will affect the chemistry of biocrust-forming species and their impacts on carbon (C) and nitrogen (N) cycling is still very limited.

(2) Using a manipulative experiment conducted with common biocrust-forming lichens with distinct morphology and chemistry (*Buellia zoharyi, Diploschistes diacapsis*, *Psora decipiens* and *Squamarina lentigera*), we evaluated changes in lichen total and isotopic C and N and several soil C and N variables after 50 months of simulated warming and rainfall reduction.

(3) Climate change treatments reduced $\delta^{13}C$ and C:N ratio in *B. zoharyi*, and increased $\delta^{15}N$ in *S. lentigera*. Lichens had species-specific effects on soil dissolved organic N (DON), NH_4^+, β-glucosidase and acid phosphatase activity regardless of climate change treatments, while these treatments changed how lichens affected several soil properties regardless of biocrust species. Changes in thallus $\delta^{13}C$, N and C:N drove species-specific effects on DON, NH_4^+, β-glucosidase and acid phosphatase activity.

(4) Our findings indicate that warmer and drier conditions will alter the chemistry of biocrust-forming lichens, affecting soil nutrient cycling, and emphasize their key role as modulators of climate change impacts in dryland soils.

Keywords: biological soil crusts, climate change, drylands, lichens, morphology, functional traits, soil fertility

INTRODUCTION

Biological processes are the main determinants of carbon (C) and nitrogen (N) fixation and the subsequent transformation and release of C and N products in the soil (Chapin *et al.*, 2011), which in turn are affected by climate and associated ecosystem-climate feedbacks (Gruber & Galloway, 2008; Heimann & Reichstein, 2008). However, our understanding of how climate change will alter current patterns of C and N cycling in drylands, which occupy ~41% of the terrestrial surface and host ~40% of the global population (Cherlet *et al.*, 2018), is still limited (Maestre *et al.*, 2016). Drylands store about 32% and 40% of global soil organic C and total N, respectively (Plaza *et al.*, 2018), and play a fundamental role in the global terrestrial C sink and its interannual variability (Poulter *et al.*, 2014; Ahlström *et al.*, 2015). Understanding how climate change will impact C and N cycling in drylands is thus fundamental to better forecast its impacts on global biogeochemical cycles and on the capacity of drylands to provide fundamental ecosystem services, such as soil fertility and forage production, for the more than two billion people inhabiting them (Cherlet *et al.*, 2018).

Biocrusts, complex communities composed of lichens, mosses and other soil microorganisms (e.g., cyanobacteria, fungi and algae) living in the soil surface, are a major feature of drylands worldwide (Weber *et al.*, 2016). By fixing atmospheric N, regulating N mineralization and influencing soil respiration and net CO_2 uptake, among other processes (Belnap, 2002; Maestre *et al.*, 2013; Delgado-Baquerizo *et al.*, 2014), biocrusts are major drivers of C and N cycling and storage in these ecosystems (Elbert *et al.*, 2012). The activity and nutrient status of biocrust constituents such as lichens and mosses is highly dependent on environmental conditions due to their poikilohydric nature and their lack of true roots (Nash, 2008; Goffinet & Shaw, 2009). As such, they are in constant equilibrium with the environment due to their limited capacity to regulate their water status, temperature, and nutrient uptake, which makes them highly sensitive to variations in abiotic conditions (Weber *et al.*, 2016). It is thus not surprising to find that forecasted changes in precipitation and temperature have large impacts on biocrust communities and associated ecosystem processes (Reed *et al.*, 2012; Maestre *et al.*, 2013; Ferrenberg *et al.*, 2015). For example, increased temperature and altered precipitation have been found to drastically reduce biocrust cover and diversity (Maestre *et al.*, 2013; Ferrenberg *et al.*, 2015), which in turn can result in shifts in microbial community composition, C and N cycling (Ladrón de Guevara *et al.*, 2014; Delgado-Baquerizo *et al.*, 2014; Darrouzet-Nardi *et al.*, 2015). Similarly, climate change

can affect the role that biocrusts play in modulating key variables such as soil moisture (Lafuente *et al.*, 2018), likely exacerbating the direct impact of climate change on soil microbial communities.

Previous research indicates that the physiological performance and growth of biocrust constituents will be negatively affected by ongoing climate change in drylands (Escolar *et al.*, 2012; Maphangwa *et al.*, 2012; Reed *et al.*, 2012; Ferrenberg *et al.*, 2015). However, this research has mainly considered biocrust communities as a whole, i.e. without exploring specific responses of coexisting species within the same phylum/class (but see Maphangwa *et al.*, 2012; Ladrón de Guevara *et al.*, 2018). Specifically, it has been barely studied how climate change will affect the tissue composition of biocrust constituents and associated soil properties in drylands, despite the potential consequences for ecosystem processes such as nutrient cycling. Moreover, the rare exceptions are mostly dedicated to the desert moss *Syntrichia caninervis* (e.g., Stark *et al.*, 2007; Reed *et al.*, 2012; Young & Reed, 2017), while studies focusing on biocrust-forming lichens, one of the most conspicuous and abundant biocrust constituents in global drylands (Weber *et al.*, 2016), are still lacking. The effects of biocrust-forming lichens on soil nutrients and microbial communities are species-specific and dependent on the chemical and morphological traits of lichen thallus (Miralles *et al.*, 2012; Concostrina-Zubiri *et al.*, 2013; Maier *et al.*, 2014; Delgado-Baquerizo *et al.*, 2015). The nutrient status (i.e., total C and N) of both biocrust-forming lichens and mosses is expected to be reduced with increased temperature and altered rainfall regimes due to reduced physiological performance (Reed *et al.*, 2012), as has been shown along climatic gradients in the field (Concostrina-Zubiri *et al.*, 2018). Thus, lichens are expected to play an important role in modulating climate change effects on soil diversity and functioning (Maestre *et al.*, 2015; Liu *et al.*, 2016; Dacal *et al.*, 2020). Understanding how climate change will differentially impact dominant biocrust-forming lichen species is critical to better forecast how climate change will impact ecosystem functioning in drylands.

To the best of our knowledge, no previous study has experimentally evaluated how climate change drivers like warming and reduced precipitation impact both the chemistry of lichen thalli and soil nutrient cycling. We aimed to do so by conducting a microcosm experiment where we evaluated the impacts of a ~2ºC warming and 35% rainfall reduction on monocultures of four dominant biocrust-forming lichens with diverse morphology and chemical traits (*Buellia zoharyi*, *Diploschistes diacapsis*, *Psora decipiens* and *Squamarina lentigera*). In particular, we evaluated changes in biocrust thallus composition (i.e., total and isotopic C and N composition, and C:N

ratio) and on multiple soil variables related to soil functioning (soil total and isotopic C and N, dissolved organic nitrogen, ammonium, nitrate, β-glucosidase and acid phosphatase activity, pH) after 50 months of warming and rainfall reduction. We tested the following hypotheses: i) warming and rainfall reduction will induce species-specific changes in biocrust C and N composition due to species-specific differences in functional traits. These include, for instance, differences in morphology, which regulate biocrust water relations (Larson, 1981; Mallen-Cooper & Eldridge, 2016; Concostrina-Zubiri *et al.*, 2017) and thus, their photosynthetic capacity (Lange *et al.*, 1988, 1994); ii) warming and rainfall reduction will affect soil properties (Ladrón de Guevara *et al.*, 2014; Delgado-Baquerizo *et al.*, 2014; Darrouzet-Nardi *et al.*, 2015), albeit the magnitude of their effects will be species-specific; and iii) changes in the C and N composition of lichen thalli drive their impacts (Delgado-Baquerizo *et al.*, 2015), and modulate those of simulated climate change on soil properties (Ladrón de Guevara *et al.*, 2014; Delgado-Baquerizo *et al.*, 2014; Darrouzet-Nardi *et al.*, 2015). By simultaneously assessing the effects of climate change drivers and biocrust-forming lichen species on soil functioning, we will be able to better understand the role of biocrusts in mediating climate change impacts on the functioning of drylands soils.

MATERIALS AND METHODS

Species used and biocrust C and N variables studied

We selected four lichen species that coexist and dominate biocrust communities in drylands worldwide (Galun & Garty, 2001; Maestre *et al.*, 2011; Weber *et al.*, 2016). They are also easy to manipulate (e.g., to be collected and used as transplants) and have been successfully used in manipulative experiments before (Maestre *et al.*, 2012a; Castillo-Monroy *et al.*, 2014). These species show marked differences in thallus morphology, colour and chemistry (Fig. S1), and exert species-specific effects on soil chemistry and microbial communities (Concostrina-Zubiri *et al.*, 2013; Delgado-Baquerizo *et al.*, 2015). Their performance and abundance in the field are also affected by simulated warming (Escolar *et al.*, 2012; Ladrón de Guevara *et al.*, 2018).

To evaluate changes in biocrust C and N composition, and their effects on associated soil properties, we focused on total C and N content and ratio (C:N) and C and N isotope ratios ($\delta^{13}C$ and $\delta^{15}N$), which are considered good indicators of changes in climate and soil physico-chemical properties (e.g., Concostrina-Zubiri *et al.*, 2018). Lichen $\delta^{13}C$ reflects the discrimination against ^{13}C in favour of ^{12}C during CO_2 diffusion into the lichen and the CO_2 source (Lakatos *et al.*,

2007). Similarly, lichen $\delta^{15}N$ is very sensitive to N availability and sources (e.g., Pinho *et al.*, 2017). In addition, total C and N and C:N are expected to influence soil nutrient cycling via nutrient leaking and decomposition (Cornelissen *et al.*, 2007).

Experimental design

We carried out a microcosm experiment in the Climate Change Outdoor Laboratory (CCOL), located at the facilities of Rey Juan Carlos University (URJC, Móstoles, Spain: 40°20'37''N, 3°52'00''W, 650 m a.s.l.; Fig. S2a), between March 2013 and May 2017. The climate is semi-arid, with mean annual temperature and precipitation of 16.6°C and 362 mm, respectively. Soil and biocrust-forming lichen species for the experiment were collected from gypsum outcrops present in the surroundings of the Aranjuez Experimental Station, located in the centre of the Iberian Peninsula (40°020N–3°320W; 590 m a.s.l.) and over 50 km south of the CCLOL. All specimens were collected in plant interspaces and lacked visible damage.

Microcosms consisted of plastic pots (depth 8 cm, diameter 20 cm) filled with 4.5 cm of homogenized nutrient-poor field soil, and 3 cm of stones for drainage at the base (Fig. S1). Intact lichen pieces were collected from the field, separated into species, and cut into homogeneous 1.21 cm^2 square fragments (Fig. S1). These fragments were placed on the soil surface to achieve a ~60% coverage of each microcosm unit (excluding a buffer zone; Fig. S1), which is within the range found in the field (39-98%; Maestre *et al.*, 2005). As the spatial pattern of lichen thalli can affect their impact on soil properties in this type of experiments (Maestre *et al.*, 2012a), the same spatial pattern was used in all microcosms (Fig. S1). The microcosms were set up in March 2013. To help the establishment of the lichen fragments, water was sprayed into each microcosm during the first 4 weeks, once per week, before the start of the experiment.

The experiment consisted of two treatments: climate change (three levels: control, ~2.3ºC annual temperature increase and the combination of 35% rainfall reduction and temperature increase), and lichen species (*B. zoharyi*, *D. diacapsis*, *P. decipiens*, *S. lentigera*). Five replicates of each lichen species and four of bare soil (i.e. without lichens) were established for each level of the climate change treatment. One *D. diacapsis* and two *S. lentigera* samples in the control and warming treatments, respectively, were discarded from analyses due to sample contamination, resulting in a total of 69 microcosms.

The warming treatment aimed to simulate climatic predictions for central Spain for the second half of the 21st century (2046-2065), i.e., an increase in annual temperature ranging

between 2.1ºC – 3.2ºC (De Castro *et al.*, 2005; IPCC, 2013). This temperature increase was achieved by using open-top chambers (OTCs; Fig. S2) built with six methacrylate plates open on the top to allow rainfall and elevated 5 cm from the soil surface to achieve adequate airflow and avoid excessive overheating (Figure S2). The OTCs used promoted a 2.3ºC warming on average throughout the study period (see Fig. S3).

The rainfall reduction treatment consisted of passive rainfall shelters based upon the design of Yahdjian & Sala (Yahdjian & Sala, 2002) that reduced the total amount, but not the intensity, of rainfall reaching the soil surface. Each rainfall shelter has a roof composed of six methacrylate grooves (Fig. S2) covering, approximately, 35% of the surface. Rainfall reduction values obtained (35.4% ± 2.2 on average; means ± SE; n = 25 rain events; Valencia et al., 2018) are consistent with predictions from climatic models in central Spain, which forecast reductions between 10% and 33% in the total amount of rainfall received during spring and fall for the second half of the 21st century (De Castro *et al.*, 2005).

Harvest and analyses

All microcosms were harvested in May 2017. Biocrusts were carefully removed with a knife and attached soil particles were discarded before storage at -20ºC until further analysis. At each microcosm, the first 2 cm of the soil were collected and passed through a 2 mm sieve, then air-dried at room temperature for one month. Dry soil samples were kept in sealed plastic bags and stored in the dark until further analysis to avoid gas exchange and sample contamination. Air-drying and storage in the dark is considered an effective method to preserve soil bio-physico-chemical properties in similar soils (Delgado-Baquerizo *et al.*, 2015) and in dryland soils worldwide (Maestre *et al.*, 2012b). Lichen thalli were thoroughly cleaned with a brush to remove soil particles, and then oven-dried (48 h at 60ºC). After this, they were ground in a homogenizer (Precellys® 24, Bertin Technologies, Montigny-le-Bretonneux, France) and analysed for total and isotopic N and C on a Sercon Hydra 20-22 (Sercon, Crewe, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA3000 (EuroVector, Pavia, Italy) elemental analyser. Isotope ratios are given in the notation δ, calculated as $\delta = (R_{sample}/R_{standard} - 1) \times 1000$ (‰). International standards IAEA CH6 (sucrose) and IAEA CH7 (polyethylene) and IAEA N1 (ammonium sulphate) were used to calibrate C and N isotope ratios. We report $\delta^{13}C$ values standardized against Vienna Pee Dee Belemnite and $\delta^{15}N$ relative to $\delta^{15}N$ of atmospheric air.

In each air-dried soil sample, we measured the following variables: total and isotopic C (^{13}C) and N (^{15}N), organic C (SOC), dissolved organic N (DON), ammonium (NH_4^+) and nitrate (NO_3^-) availability. These variables have been extensively used as proxies of ecosystem functioning in many ecosystems (e.g., Austin & Vitousek; Maestre *et al.*, 2012; Singh *et al.*, 2018). To help us elucidating how climate change drivers and biocrusts affect C and N cycles indirectly, e.g., via changes in microbial communities and soil chemistry, we also measured two soil enzymatic activities (β-glucosidase and acid phosphatase) and soil pH. The activity of these enzymes is a good indicator of metabolic and stress status for microbial communities in drylands (e.g., Sardans *et al.*, 2008; Delgado-Baquerizo *et al.*, 2014). Also, it is known that soil pH regulates microbial growth and extracellular enzyme activities (Sinsabaugh *et al.*, 2008), and thus soil nutrient cycling. These soil variables were analysed as described in Maestre *et al*. (Maestre *et al.*, 2012b). Total C and N concentration and isotopic composition were measured in aliquots of 2 g of soil that were processed and analysed following the same methodology as for lichen thalli. Total N concentration in our soils (<0.05% on average) was insufficient to measure N isotopic composition. All lichen and total and isotopic soil C and N analyses were conducted at the Stable Isotopes and Instrumental Analysis Facility – Universidade de Lisboa (Lisbon, Portugal).

Data analyses

To evaluate the effects of climate change drivers on the total C and N, $\delta^{13}C$, $\delta^{15}N$ and C:N ratio of lichen thalli (hypothesis i), we conducted a semiparametric permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001). Climate change treatments were considered as a fixed factor for these analyses, which were conducted for each species separately since they were expected to show contrasting differences in lichen C and N composition (Delgado-Baquerizo *et al.*, 2015). When the main factor (i.e., treatment) had a significant effect on the response variable, we conducted a pairwise comparison between treatments.

To assess whether climate change drivers and biocrust species induced shifts in soil fertility and functioning (hypothesis ii), we calculated the Relative Interaction Intensity (RII) index (Armas *et al.*, 2004) separately for each lichen species and treatment. RII was calculated as $(S_{bc} - S_{bs})/(S_{bc} + S_{bs})$; where S_{bc} and S_{bs} are the values of a given soil variable under each species and treatment in lichen microcosms (n=3-5) and in bare soil microcosms (as the average of the four bare soil replicates for each treatment, n=4), respectively. The RII values range from -1 to +1; a value of zero indicates no effects of a given biocrust species on the variable of interest, while RII

values above and below zero indicate positive and negative effects on such variable, respectively, compared to bare soil. To test whether RII values were significantly different from zero, we computed bootstrapped 95% confidence intervals using the boot R package (Canty & Ripley, 2019). Since RII values did not follow a normal distribution, we evaluated the effects of biocrust species and climate change drivers on RII values for each soil variable using a two-way PERMANOVA. In these models, treatment and species were fixed factors. When main factors (i.e., treatment and species) had a significant effect on response variables, we conducted a pairwise comparison between treatments and/or species.

To evaluate if changes in lichen C and N composition drive biocrust species and climate change impacts on soil properties (hypothesis iii), we conducted a non-metric multidimensional scaling (NMDS) ordination with soil data (from lichen microcosms) and tested for individual correlations with lichen C and N variables using the envfit function from the vegan R package (Oksanen *et al.*, 2019). The best solution for the soil data ordination (i.e., lowest final stress) was found for a 3-dimensional NMDS evaluated with a permutation test (999 permutations). All soil variables were relativized (by maximum) before NMDS analysis due to differences in measurement units. To evaluate if the effects of lichen C and N variables on soil properties were driven by biocrust species or climate change drivers, we conducted a two-way PERMANOVA on soil data with species and treatment as fixed factors. When the main factor (i.e., species or treatment) had a significant effect on soil properties, we conducted a pairwise comparison between levels of each factor. As an aid for the interpretation of our results, we visually examined the relationships between significant biocrust C and N variables and individual soil variables for each level of significant main factors after PERMANOVA.

PERMANOVA and NMDS analyses were conducted using Euclidean distance on unrestricted permutation of raw data (999 permutations) with the vegan R package (functions adonis and metaMDS) (Oksanen *et al.*, 2019). We chose PERMANOVA and NMDS due to small sample size and data heterogeneity. All analyses were performed with R version 3.6.1 (R Core Team, 2019). Data are available from Figshare (Concostrina-Zubiri *et al.*, 2020).

RESULTS

The combination of warming and rainfall reduction decreased $\delta^{13}C$ and C:N ratio in *B. zoharyi* (Fig. 1b,e, Table S1). Both warming and the combination of warming and rainfall reduction

increased $\delta^{15}N$ in *S. lentigera* (Fig. 1d, Table S1). Our climate change treatments did not modify thallus total C and N concentration in any species (Fig. 1a,c, Table S1).

Climate change treatments and biocrust species had significant effects on the RII of several soil variables; however, we only found significant climate change treatment x species interactions for the acid phosphatase activity (Fig. 2, Table S2). Lichens increased total C in the warming treatment (compared to bare soil), but reduced it in the control and the warming x reduced precipitation treatments (Fig. 2a, Table S2). Lichens also increased SOC in the control and warming treatments, but this effect was negative in the warming x rainfall reduction treatment (Fig. 2b, Table S2). Additionally, lichens reduced soil $\delta^{13}C$ in the warming treatment regardless of species identity (Fig. 2c, Table S2). Lichens also decreased total N and NH_4^+ in the warming and the warming x rainfall reduction treatments (Fig. 2d,f, Table S2), and reduced NO_3^- in the warming treatment (Fig. 2g, Table S2). In contrast, lichens increased DON in the control treatment, an effect that was the opposite in the warming treatment (Fig. 2e, Table S2). *Psora decipiens* and *B. zoharyi* increased and reduced, respectively, soil DON (compared to bare soil; Fig. 3e, Table S2). *Buellia zoharyi*, *D. diacapsis* and *S. lentigera* reduced soil NH_4^+ (Fig. 3f, Table S2). All species had a positive effect on β-glucosidase activity, being higher for *P. decipiens* lower for *B. zoharyi* and *D. diacapsis* (Fig. 3h, Table S2). Similarly, *P. decipiens* significantly increased phosphatase activity (Fig. 3i, Table S2). PERMANOVA analysis did not detect any statistically significant effect of climate change treatments or biocrust species on soil pH (Table S2).

The NMDS ordination (final stress = 0.12) showed that soil properties varied with lichen $\delta^{13}C$ (R^2=0.42), N (R^2=0.14) and C:N (R^2=0.22, Fig. 4, Table S3). Higher lichen N was related to changes in soil properties driven by increased DON, NH_4^+, acid phosphatase and β-glucosidase activity, while the opposite pattern was found for lichen $\delta^{13}C$ and C:N (Fig. 4, Table S3). Also, PERMANOVA analyses showed that biocrust species affected soil properties (Table S4). The subsequent pairwise tests indicated differences between *P. decipiens* and the other studied species ($P < 0.01$ in all cases). Such differences were observed in the ordination; i.e., soil microcosms of *P. decipiens* separated from those of other species (Fig. 4). PERMANOVA analyses did not detect any effect of climate change treatments or the combination of species and climate change treatments on soil properties (Fig. 4, Table S4) or between species other than *P. decipiens* ($P > 0.05$ in all cases).

DISCUSSION

Most previous studies about climate change impacts on biocrusts and associated soil properties have generally considered these communities as a whole (e.g., Reed *et al.*, 2012; Maestre *et al.*, 2013). However, we found that lichen identity was a major driver of the nature and extent of these impacts. Our results, obtained with a manipulative experimental approach, also show that important changes can occur in biocrust-forming lichen thallus composition under simulated climate change and that the specific lichen species largely impact soil nutrient cycling and microbial activity. In addition, we found that the composition of lichen thalli and several soil variables were coupled regardless of climate change treatments, although the nature of these relationships was, again, highly species-specific. These findings highlight the need of evaluating the responses and effects of biocrust constituents at the species level to better understand the potential implications of climate change for soil nutrient cycling in biocrust-dominated drylands.

Responses of biocrust C and N composition to simulated climate change are species-specific

We found empirical evidence that the C and N composition of lichen thalli responds to simulated climate change, and that the nature of this response differed among species. For example, $\delta^{13}C$ in *B. zoharyi* decreased under warming and reduced precipitation, while $\delta^{13}C$ in other species showed no differences with climate change treatments. These results suggest that *B. zoharyi* is particularly sensitive to climate change drivers, or that it had an early response compared to the others. Lichen $\delta^{13}C$ values result from multiple processes related to C source, assimilation and use (Lakatos *et al.*, 2007). Moreover, due to their poikilohydric nature, these processes are known to be governed by ambient humidity and temperature in lichens (Nash, 2008), and thus by traits defining their water and temperature relations (e.g., morphology, hydrophobicity and anatomical structure; (Shirtcliffe *et al.*, 2006; Mallen-Cooper & Eldridge, 2016; Concostrina-Zubiri *et al.*, 2017). For example, when lichens are beyond their water saturation point, $\delta^{13}C$ is expected to be higher (i.e., less negative) due to a decrease in the CO_2 diffusion rate into the lichen thallus (Batts *et al.*, 2004). In contrast, at low thallus water content carboxylation is limited and CO_2 internal concentration is high, leading to higher C discrimination and more negative $\delta^{13}C$ values (Lange *et al.*, 1988). The particularly thin and discontinuous thallus of *B. zoharyi* (<0.4 mm; Trinkaus & Mayrhofer, 2000) might have allowed faster evaporation rates after liquid precipitation events and, simultaneously, to hydrate faster from other precipitation forms such as dew (Larson, 1981; Lange *et al.*, 1994), leading to the lower $\delta^{13}C$ under the combination of warming and rainfall reduction. Alternatively, these results could also be explained by an increased uptake of respired CO_2 from the soil, which

is depleted in $\delta^{13}C$ compared to atmospheric CO_2 (i.e., $\delta_{13}C$ in SOM is ~ -26 while in atmospheric CO_2 is ~ -8; (Lakatos *et al.*, 2007). Increased soil respiration has been reported before in biocrust-dominated soils under simulated warming and rainfall reduction during the first years after the experimental setup (Maestre *et al.*, 2013; Escolar *et al.*, 2015; Dacal *et al.*, 2020). However, it would then be expected that *P. decipiens*, characterized by discontinuous darker squamules (small, scale-like thallus units), should have also higher evaporation rates under these experimental conditions and, simultaneously, capture more water from non-liquid precipitation than more continuous species because of its higher surface area (Raggio *et al.*, 2014). Although we did not find a significant decrease in $\delta^{13}C$ of *P. decipiens* in response to climate change treatments, the overall $\delta^{13}C$ values of this species (Fig. 1b) were remarkably lower than those of the other species.

Importantly, the combination of warming and rainfall reduction also caused a decrease in the C:N ratio of *B. zoharyi*. This may be explained by the similar thallus C and the increase in thallus N observed under warming and rainfall reduction (Fig. 1e), likely due to higher N availability (as indicated by an increasing trend in DON; Fig. S4e). These results contrast with those reported for lichen N content along a climatic gradient in the Mediterranean, which showed an increase in this variable under more humid conditions (Concostrina-Zubiri *et al.*, 2018). The increase in lichen $\delta^{15}N$ values with precipitation was attributed to potentially higher N inputs in form of wet deposition (i.e., NH_4^+ to NO_3^-), which typically has higher $\delta^{15}N$ values (Moore, 1974). Lower C:N ratios have been related to increased decomposition rates in terricolous lichens and mosses and to N release in mosses (Limpens & Berendse, 2003; Berdugo *et al.*, 2020). In addition, higher thallus N content can increase lichen palatability for soil fauna (e.g., snails; Asplund & Wardle, 2013). Our results indicate that climate change drivers can also alter, indirectly, nutrient inputs to the soil and overall C and N turnover. More specifically, our results suggest that *B. zoharyi* microsites could increase their contribution to soil fertility (Delgado-Baquerizo *et al.*, 2015) in a more arid scenario (Fig. S4). *Buellia zoharyi* is a widespread species in the Mediterranean and Macaronesian regions (Chiva *et al.*, 2019) and is particularly common in gypsum soils from central Spain (Concostrina-Zubiri *et al.*, 2014; Ladrón de Guevara *et al.*, 2018).

On the other hand, $\delta^{15}N$ in *S. lentigera* showed a marked increase under warming and the combination of warming and rainfall reduction. The transformation of SOM to NH_4^+ or NH_4^+ to NO_3^- can result in $\delta^{15}N$ increases up to 5 and 40‰, respectively (Dawson *et al.*, 2002), which could be later reflected in plant $\delta^{15}N$ via nutrient uptake. Although lichens lack active mechanisms for nutrient uptake, minerals, water and other compounds can enter the lichen body passively due

to physico-chemical processes such as ion exchange and the uptake and retention of exogenous compounds in the lichen intracellular space (Nash, 2008). Our results suggest that the increase in *S. lentigera* $\delta^{15}N$ under warming and rainfall reduction indicated this species assimilated N from the topsoil (Dahlman *et al.*, 2004; Pavlova *et al.*, 2017). This is supported by the higher NO_3^- concentrations values found in bare soil microcosms under warming and the combination of warming and reduced precipitation and by the lower values for this variable observed under *S. lentigera* in these treatments, compared to the control (Fig. S4g). Higher N uptake under warmer conditions may be due to increased affinity to N sources, as observed in other microbial organisms (e.g., marine algae or bacteria; Reay *et al.*, 1999). Further research aiming to elucidate the role of climate change drivers in lichen-soil N cycling should take into account other N forms such as NH_3, NO and N_2O derived from gaseous losses from the soil, which were not considered in this study and may represent important N sources to biocrust-forming lichens.

Simulated climate change and biocrust-forming lichens impact multiple facets of soil fertility and microbial activity

Our results supported our second hypothesis; i.e. climate change drivers and biocrust-forming lichens induce shifts in soil fertility and functioning. Existing evidence of how biocrusts affect soil fertility and functioning have mostly been gathered using observational approaches, i.e. comparing areas with naturally occurring biocrusts vs. areas without them (but see Sedia & Ehrenfeld, 2005; Maestre *et al.*, 2012), which do not allow to estimate the impacts of biocrusts on soil fertility and functioning with certainty. The experimental nature of our study (i.e., all microcosms had the same initial soil and microcosms with and without lichens) allowed us to provide compelling evidence that biocrust-forming lichens modify the soil where they grow over time, and that differences in soil properties between species or treatments were due to the presence of lichens and to the effects of the climate change treatments evaluated. It is interesting to note that species-specific effects of lichens on several soil properties (i.e., DON, NH_4^+ and β-glucosidase activity) were not affected by climate change treatments and *vice versa* as most soil properties (i.e., total C and N, SOC, $\delta^{13}C$, DON, NH_4^+ and NO_3^-) responded to climate change drivers regardless of lichen identity. These results are in line with previous research showing that biocrusts as a whole (i.e., patches where multiple biocrust constituents and species co-exist) are important regulators of climate change impacts on soil C and N cycling (e.g., Delgado-Baquerizo *et al.*, 2013a, 2014; Maestre *et al.*, 2013).

Regardless of species identity, the presence of biocrusts had important effects (as measured with the RII) on several soil properties. Biocrust-forming lichens, in general, protect the soil from direct solar radiation and erosional forces, such as wind erosion and raindrop impact (Eldridge & Rosentreter, 1999), and increase soil stability (Jimenez Aguilar *et al.*, 2009). Therefore, increased nutrient retention is expected under biocrusts compared to bare ground areas (Cantón *et al.*, 2004; Concostrina-Zubiri *et al.*, 2013, 2017). Nevertheless, changes in climate can decrease biocrust contribution to soil fertility, for example, due to reduced photosynthetic capacity (Maphangwa *et al.*, 2012; Reed *et al.*, 2012; Colesie *et al.*, 2018) and shifts in C and N thallus composition, as seen along climatic gradients in the field (e.g., Concostrina-Zubiri *et al.*, 2018).

Our results show that lichen effects on soil fertility were highly responsive to warming and the combination of warming and reduced precipitation. First, the effect of biocrust presence shifted from positive to negative for total C and SOC in the warming and reduced precipitation treatment (Fig. 2b, Table S2). It is known that lichens can contribute to SOC, for instance, via the release of organic acids produced by the fungal partner (Chen *et al.*, 2000), once they have obtained C compounds from the algae via CO_2 fixation. Thus, although chlorolichens can use water from non-liquid sources (e.g., dew) to be active (Lange *et al.*, 1994; Raggio *et al.*, 2014), a reduction in total precipitation may have resulted in an overall decreased metabolic activity, and thus, in lower production of lichen secondary compounds under drier and warmer conditions (BeGora & Fahselt, 2001; Bjerke *et al.*, 2005). Decreases in lichen photosynthetic activity have been observed in field experiments using the same (Ladrón de Guevara *et al.*, 2014) or similar (Colesie *et al.*, 2018) experimental treatment used here.

Biocrusts had a positive effect on DON in the control treatment, an effect not apparent under warmer and drier conditions (Fig. 2e, Table S2). In contrast, warming alone decreased DON and N availability (i.e., NH_4^+ and NO_3^-; Fig. 2e,f,g, Table S2). This is in agreement with previous experimental work showing lower organic and inorganic N under increased temperature in biocrust-dominated environments (Delgado-Baquerizo *et al.*, 2014). On the one hand, it is known that lichens can assimilate important amounts of organic (i.e., amino acids) and inorganic (i.e., NH_4^+ and NO_3^-) N under laboratory conditions (Dahlman *et al.*, 2004). Field experiments have shown contrasting results, indicating that terricolous lichens cannot derive important amounts of these compounds directly from the soil (Ellis *et al.*, 2004). However, these authors evaluated N uptake in fruticose lichens, which are loosely attached to the soil (Ellis *et al.*, 2004). By contrast, most of the lichen species used in our study (i.e., *B. zoharyi*, *P. decipiens* and *S. lentigera*) grow

strongly attached to the soil surface, likely increasing N exchange between the lichens and the soil. On the other hand, a warmer environment can induce important changes in the abundance of soil fungi and bacteria (Castro *et al.*, 2010; Maestre *et al.*, 2015; Delgado-Baquerizo *et al.*, 2020) and enhance heterotrophic activity in the soil (Davidson & Janssens, 2006), and thus, alter nutrient availability (e.g., decreased N mineralization, increased SOC leaching).

All the lichen species included in our study produce secondary compounds with potential antimicrobial effects (Molnár & Farkas, 2010). Since the production of these compounds is determined by the physiological status of lichens (Stocker-Wörgötter, 2002), warmer conditions may have altered the amount of such substances reaching the soil. Lower concentrations of lichen secondary compounds with potential antimicrobial effects, such as usnic acid, have been reported for the terricolous lichen *Cladonia arbuscula* under experimental warming in the field (Nybakken *et al.*, 2011). On the contrary, usnic acid concentration increased in *C. stellaris* with temperature when cultivated in growth chambers, indicating lichen hydration and UV radiation play an important role in the production of this secondary compound (Asplund *et al.*, 2017). Although an earlier study found very low or no concentration of usnic acid in the soil as a result of leaching from the lichen (Stark *et al.*, 2007), little is known about the release of secondary compounds from biocrust-forming lichens.

The effects of the lichens studied on soil properties were species-specific. For example, *P. decipiens* had a positive effect on DON, while *B. zoharyi* decreased its concentration and the other two studied species showed no effect on this variable (Fig, 3e, Table S2). Higher DON under *P. decipiens* may be related to its overall higher N concentration, compared to the other studied species (Fig. 1c; Delgado-Baquerizo *et al.*, 2015), which may be directly released in the form of amino acids by the fungal partner (Pavlova *et al.*, 2017). Although little is known about the contribution of biocrust lichens as soil nutrient sources in drylands via decomposition, it is also expected that these lichens will eventually be incorporated into the soil in the form of litter or after fragmentation and burial, enhancing soil fertility. Recently, a decomposition experiment using biocrust-forming lichens has found that lichen litter can decompose as fast as that from vascular plants and that it loses C and N with time (Berdugo *et al.*, 2020). These findings suggest nutrients present in lichen tissue can be transferred to the soil as a result of decomposition processes. Similarly, all species decreased NH_4^+ concentration except *P. decipiens* (Fig, 3f, Table S2). A plausible explanation for these results is the rather different morphology and colour of *P. decipiens*, characterized by a discontinuous thallus consisting of small, dark orange squamules,

while the other studied species are squamulose-crustose lichens with a more continuous and light coloured thallus. As previously discussed, a homogeneous *P. decipiens* cover may generate a warmer microenvironment compared to the other species (Kershaw, 1975; George *et al.*, 2003; Raggio *et al.*, 2014), because of darker thallus colour. Also, this species may enhance soil moisture (Raggio *et al.*, 2014). First, because in the absence of liquid water precipitation (i.e., the typical condition in our study site) air moisture gets directly to the soil through squamules interspaces, while the contrary is expected in the other species, with a more continuous and hydrophobic thallus (Shirtcliffe *et al.*, 2006). Second, *P. decipiens* may absorb more water not only from liquid precipitation but also from dew and air humidity due to its higher area/volume ratio (Larson, 1981; Raggio *et al.*, 2014). Increased soil moisture and temperature can, in turn, enhance N mineralization under biocrusts, even with reduced liquid precipitation (Delgado-Baquerizo *et al.*, 2013b). This is mainly explained by the more favourable conditions for microbial communities to be active, which is somehow supported by the highest enzymatic activities found for *P. decipiens* (Fig. 3h,i). Indeed, although all of the studied species produce chemical compounds with potential antifungal, antibacterial or antimicrobial effects (Kosanić & Rankovic, 2015; but see Stark *et al.*, 2007), those produced by *P. decipiens* (i.e., anthraquinones) can only be found in the fruiting bodies, which are generally scattered over the lichen thallus and, thus, rarely in direct contact with the soil (and not always present). Conversely, the chemical compounds produced by *B. zoharyi*, *D. diacapsis* and *S. lentigera* may reach the soil in higher concentrations or be particularly effective on the microbial communities present in our soils. Indeed, microbial communities under *P. decipiens* have been reported to be different from other biocrust-forming lichens(Maier *et al.*, 2014). Although lichen compounds show little to moderate water solubility (Iskandar & Syers, 1971; Zagoskina *et al.*, 2013), contrasting results have been reported for their capacity to leak from the lichen into the soil (e.g., Dawson *et al.*, 1984; Stark *et al.*, 2007). These studies have focused on a few fruticose species and cold climates. Thus, future research should test whether chemical compounds from biocrust-forming lichens can reach the soil surface at greater amounts in drylands (e.g., high temperature and soil pH). The typically high thallus N content in *P. decipiens* (Delgado-Baquerizo *et al.*, 2015) may have also contributed to a lower dependence of soil N (i.e., lower N uptake) in this species. Finally, the lower NH_4^+ values found beneath *B. zoharyi*, *D. diacapsis* and *S. lentigera*, compared to the bare soil (Fig. S4, Table S5), suggests that these species may also be capable of deriving nitrogen from the substrate below and at a higher rate than *P. decipiens*, which showed generally higher $\delta^{15}N$ values (Fig. 1d). The low N

content of our soils impeded us to obtain soil $\delta^{15}N$ values to be compared with $\delta^{15}N$ values in the lichens. However, it would be interesting to previously apply isotopically marked N (Ellis *et al.*, 2004) to the soil in future experiments to better understand the role of biocrust-forming lichens in N cycling in drylands.

We did not evaluate the effects of rainfall exclusion alone due to logistic limitations. However, we wouldn't expect the rainfall exclusion treatment to affect soil functioning and fertility under the studied species, as previous research has shown that changes in C and N cycling under simulated climate change are mainly governed by warming, rather than by reduced precipitation, in biocrusts dominated by them (e.g., Maestre *et al.*, 2013; Delgado-Baquerizo *et al.*, 2014; Escolar *et al.*, 2015). Nevertheless, it would be interesting to study the impacts of climate change drivers such as altered rainfall frequency and intensity, which can exert large impacts on the physiological activity of multiple biocrust-forming lichen and moss species (Reed *et al.*, 2012; Liu *et al.*, 2016; Baldauf *et al.*, 2018).

Biocrust thallus composition and soil properties are coupled regardless of climate change drivers

We found support for our third hypothesis, i.e., changes in thalli C and N composition drive the impacts of lichen species and climate change treatments on soil properties. Previous studies have found strong relationships between biocrust lichen nutrient content (e.g., C, N, P) and isotopic ratios and changes in climate and soil properties in the field (Delgado-Baquerizo *et al.*, 2015; Concostrina-Zubiri *et al.*, 2018). Here we show that these relationships are the result of species effects on soil properties through time (i.e., all soils had similar soil properties at the beginning of the experiment). We also found that these effects are maintained across climate change treatments. For example, increases in lichen N were correlated to increases in DON, NH_4^+ and soil β-glucosidase and acid phosphatase activity (Fig. 4), suggesting that lichen N is incorporated into the soil via litter decomposition or through N leaching (Barger *et al.*, 2016), enhancing soil microbial activity. Interestingly, these results were mainly driven by the higher thallus N in *P. decipiens*, compared to the other species (Fig. 5b,e,h,k). Moreover, the opposite pattern was found for lichen C:N, with decreasing soil N and microbial activity rates from *P .decipiens* to *S. lentigera* (Fig. 5c,f,i,l). These results agree with previous work showing that lichen tissue with higher nutrient content and lower secondary compounds promotes lichen decomposition and N release to the soil (Asplund & Wardle, 2013; Berdugo *et al.*, 2020).

CONCLUDING REMARKS

We found that changes in thallus chemistry drove observed species-specific effects of lichens on soil functioning, and modulated soil C and N cycling to simulated climate change. These findings constitute, to the best of our knowledge, the first experimental evidence that the chemistry of biocrust-forming lichen thallus is sensitive to warming and rainfall reduction. Our results also suggest that some species are good indicators of changes in the organic matter pool and microbial activity in the soil (e.g., C and N composition in *P. decipiens*). In addition, some lichen species promote N availability but derive small amounts (i.e., *P. decipiens*), leaving important stocks of organic N in the soil, while others may simultaneously inhibit microbial activity and uptake higher quantities of inorganic N, acting as a sink for soil N (e.g., *S. lentigera*). Overall, our results indicate that biocrust thallus traits can be considered reliable indicators of changes occurring in the biocrust-soil interphase (Cornelissen *et al.*, 2007; Mallen-Cooper & Eldridge, 2016; Deane-Coe & Stanton, 2017). They advance our understanding of nutrient cycling in drylands, where biocrusts dominate plant interspaces and show species-specific vulnerability to climate change drivers (Ladrón de Guevara *et al.*, 2018). Future research should specifically consider species-specific effects on soil properties of biocrust-forming lichens, as this will allow us to hone forecasts of climate change impacts on dryland ecosystems.

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AUTHOR CONTRIBUTIONS

F.T.M. planned and designed the experiment, E.V., V.O., B.G. and B.J.M. set up and maintained the experiment and conducted laboratory analyses, L.C-Z., E.V., V.O. and B.G. processed and analysed data, L.C-Z, E.V. and F.T.M. wrote the manuscript and all authors contributed to the final review.

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Zagoskina NV, Nikolaeva TN, Lapshin PV, Zavarzin AA, Zavarzina AG. **2013**. Water-soluble phenolic compounds in lichens. *Microbiology* **82**: 445–452.

Fig. 1 Changes in lichen carbon (C) and nitrogen (N) composition of the studied species under simulated climate change. (a) Total C content; (b) C isotope ratio ($\delta^{13}C$); (c) total N content; (d) N isotope ratio ($\delta^{15}N$); (e) C:N ratio. Boxes show the median, 25th and 75th percentiles; vertical lines show the minimum and maximum values that fall within 1.5 times the height of the box. Different letters above bars indicate differences between treatments for each species ($P < 0.05$, after PERMANOVA analysis). BuZo, *Buellia zoharyi*; DiDi, *Diploschistes diacapsis*; PsoDe, *Psora decipiens*; SquLe, *Squamarina lentigera*; W, warming and W+RR, warming and rainfall reduction.

Fig. 2 Effects of climate change treatments on soil properties, as measured with the Relative Interaction Intensity (RII) index. Panels show RII indices for soil (a) total carbon (C) content; (b) organic matter content; (c) C isotope ratio ($\delta^{13}C$); (d) total nitrogen (N) concentration; (e) dissolved organic N (DON); (f) ammonium concentration (NH_4^+); (g) nitrate concentration (NO_3^-); (h) β-glucosidase activity; (i) acid phosphatase activity; and (j) pH. RII indices and corresponding CIs above zero indicate a significant ($P < 0.05$) and positive effect of lichens (all species combined) on a given soil property, relative to bare soil, while RII indices and corresponding CIs below zero indicate the opposite. Data are mean ± 95% bootstrap CIs. Different letters above/below bars indicate differences in the RII index between treatments ($P < 0.05$, after PERMANOVA analysis). β-glu., β-glucosidase activity; A. pho., acid phosphatase activity; W, warming and W+RR, warming and rainfall reduction.

Fig. 3 Effects of lichen species on soil properties, as measured with the Relative Interaction Intensity (RII) index. Panels show RII indices for soil (a) total carbon (C) content; (b) organic matter content; (c) C isotope ratio ($\delta^{13}C$); (d) total nitrogen (N) concentration; (e) dissolved organic N (DON); (f) ammonium concentration (NH_4^+); (g) nitrate concentration (NO_3^-); (h) β-glucosidase activity; (i) acid phosphatase activity; and (j) pH RII indices and corresponding CIs above zero indicate a significant ($P < 0.05$) and positive effect of a given lichen species (all treatments combined) on a given soil property, relative to bare soil, while RII indices and corresponding CIs below zero indicate the opposite. Data are mean ± 95% bootstrap CIs. Different letters above/below bars indicate differences in the RII index between species ($P < 0.05$, after PERMANOVA analysis). β-glu., β-glucosidase activity; A. pho., acid phosphatase activity. BuZo, *Buellia zoharyi*; DiDi, *Diploschistes diacapsis*; PsoDe, *Psora decipiens* (PsoDe) and SquLe, *Squamarina lentigera*.

Fig. 4 Non-metric multidimensional scaling (NMDS) ordination plot of soil properties. NMDS plot is based on the two axes of a three-dimensional ordination of soil properties to which significant lichen carbon (C) and nitrogen (N) composition variables ($P < 0.05$), represented as vectors, showed the highest correlations to ordination (Supporting Information Table S4). Note that lichen $\delta_{13}C$ values changed from negative to positive after relativization. β-glu., β-glucosidase activity; A. pho., acid phosphatase activity. Each point represents a microcosm.

Fig. 5 Relationships between lichen carbon (C) and nitrogen (N) composition variables significantly correlated ($P < 0.05$) to changes in the combination of soil properties (NMDS ordination; see Fig. 4) and individual soil properties for each species. Panels show lichen C isotope ratio ($\delta^{13}C$) (a,d,g,j), total N content (b,e,h,k) and C:N ratio (c,f,i,l) versus soil dissolved organic N concentration (DON) (a-c), ammonium concentration (NH_4^+) (d-f), β-glucosidase (g-i) and acid phosphatase activity (j-l). Data are mean ± 95% bootstrap CIs. Only individual soil variables acting as major drivers of differences in the combination of soil properties (NMDS ordination; see Fig. 4) are shown. Relationships between significant biocrust C and N composition variables ($\delta^{13}C$, N, C:N) and all individual soil properties are available in Supporting Information Figs S5–S7. β-glu., β-glucosidase activity; A. pho., acid phosphatase activity.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Lichen species used and experimental work.

Fig. S2 View of the experimental site (a) and details of simulated climate change treatments: warming, consisting of OTC (b), and the combination of warming and reduced precipitation, consisting of OTC + rainfall shelter (c).

Fig. S3 Differences in air temperature between climate change treatments (warming and warming + rainfall reduction) and control plots.

Fig. S4 Changes in each soil property for the studied species under simulated climate change.

Fig. S5. Relationships between lichen C and N composition variables ($\delta^{13}C$, N, C:N) significantly correlated ($P < 0.05$) to changes in the combination of soil properties (NMDS ordination; see Fig. 4) and C-related individual soil properties for each species.

Fig. S6. Relationships between lichen C and N composition variables ($\delta^{13}C$, N, C:N) significantly correlated ($P < 0.05$) to changes in the combination of soil properties (NMDS ordination; see Fig. 4) and N-related individual soil properties for each species.

Fig. S7. Relationships between lichen C and N composition variables ($\delta^{13}C$, N, C:N) significantly correlated ($P < 0.05$) to changes in the combination of soil properties (NMDS ordination; see Fig. 4) and β-glucosidase activity (β-glu.,), acid phosphatase activity (A. pho.), and pH for each species.

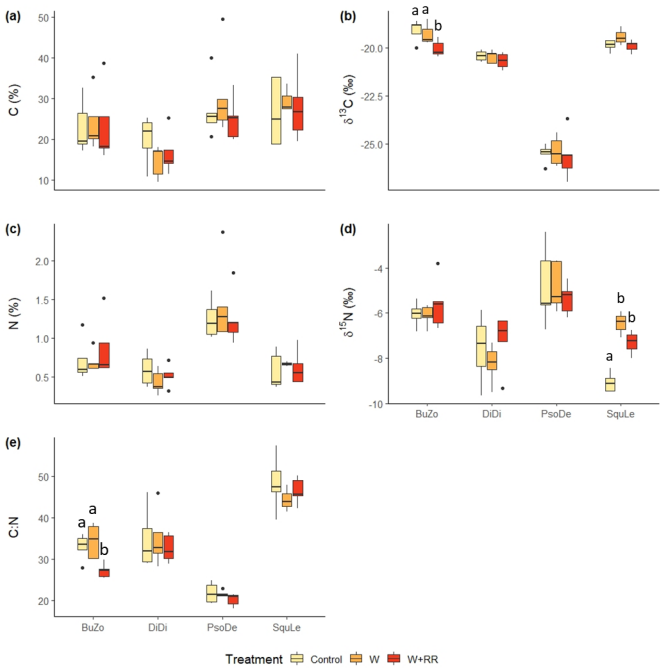
Table S1 Results of the one-way PERMANOVA analysis for lichen C and N composition variables based on species data.

Table S2 Results of the two-way (species and climate change treatments) PERMANOVA analysis for RII values for each soil variable.

Table S3 Correlation coefficients between lichen C and N composition variables and changes in the combination of soil properties (NMDS ordination; see Fig. 4).

Table S4 Results of the two-way (species and climate change treatments) PERMANOVA analysis for soil data.

Table S5 Results of the two-way (species and climate change treatments) PERMANOVA analysis for each soil variable.



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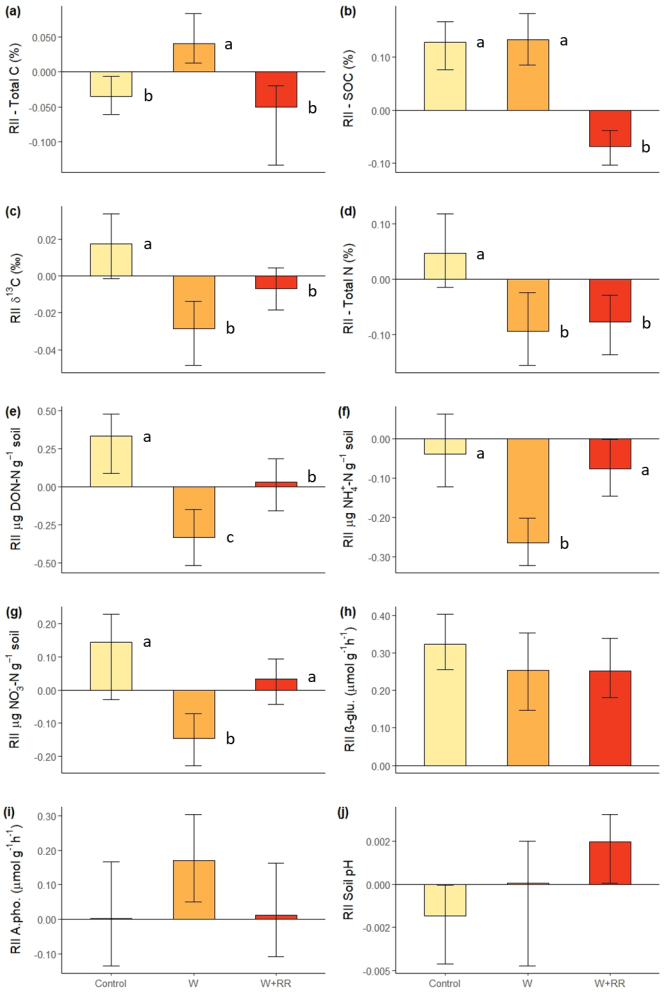

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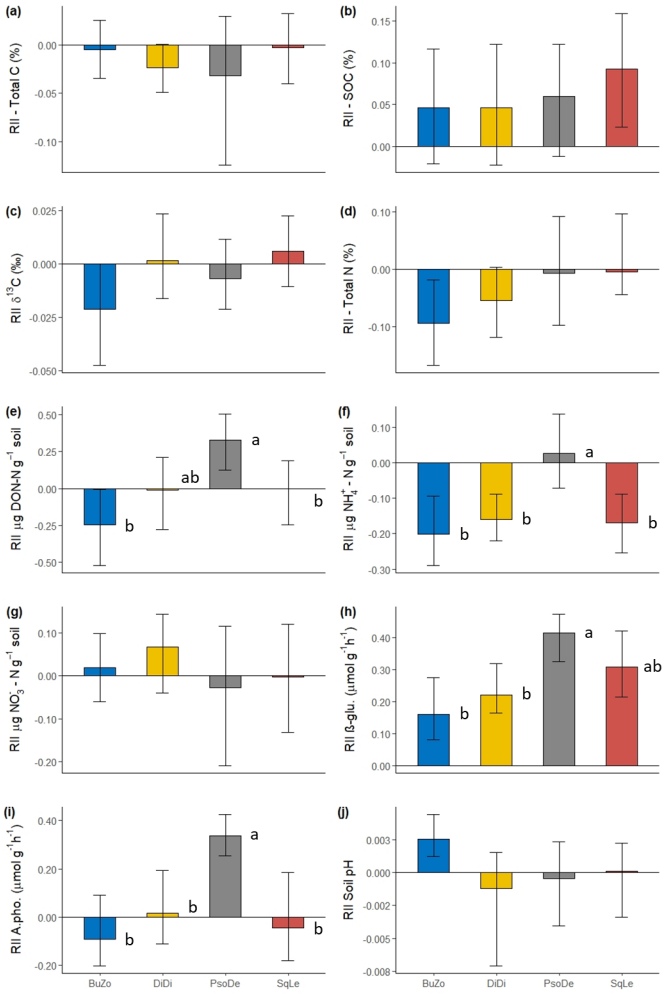

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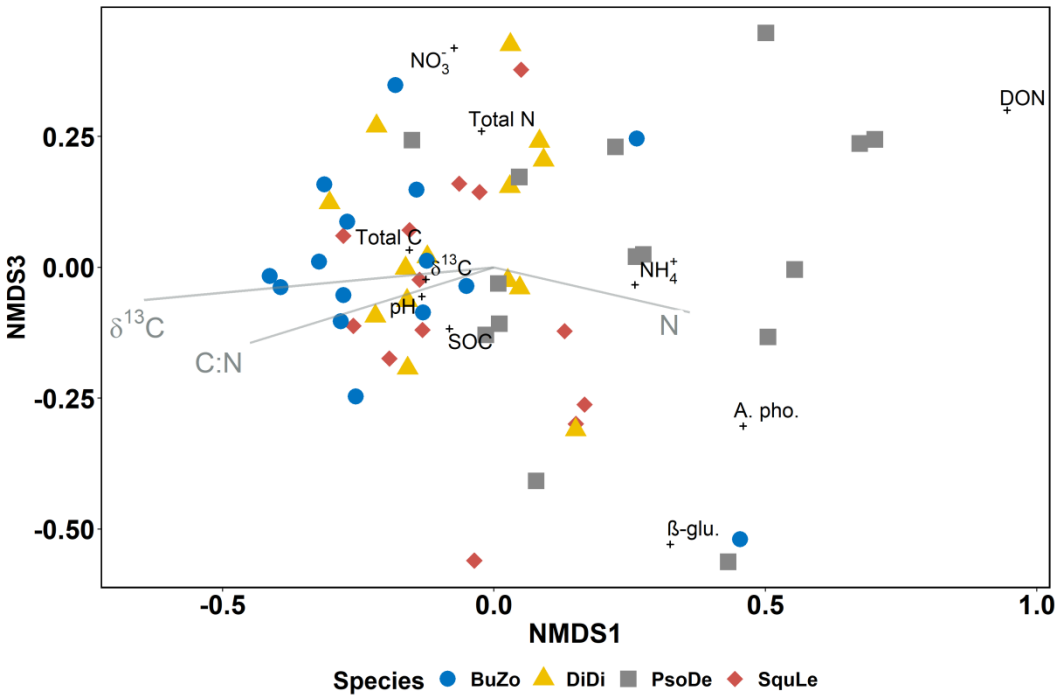

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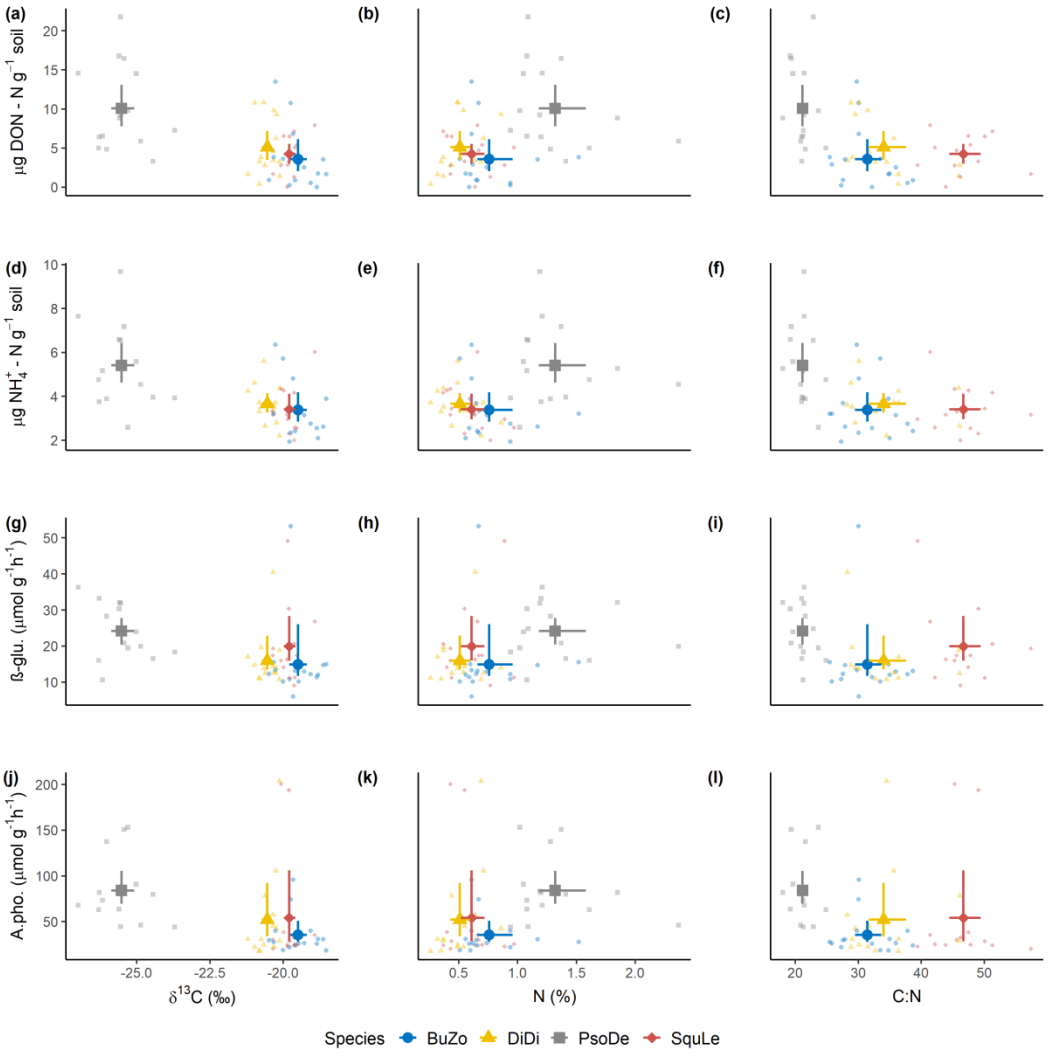

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