Morphological and chemical traits drive how biocrust-forming lichens affect soil properties under simulated climate change

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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 4 years of simulated warming and rainfall reduction.

(3) Climate change treatments reduced δ^{13}C and C:N ratio in *B. zoharyi*, and increased δ^{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 climate change treatments changed how lichens affected several soil properties regardless of biocrust identity. Changes in thallus C and N concentrations drove variations in soil organic C, DON, NH_4^+, NO_3^-, 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

PLAIN LANGUAGE SUMMARY

Biocrust-forming lichens are key drivers of ecosystem functioning in drylands. We show that simulated climate change alters lichen thallus chemistry and their effects on soil nutrient cycling.

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 carbon 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 how climate change will impact global biogeochemical cycles and their capacity to provide fundamental ecosystem services, such as soil fertility and forage production, for the more than 2 billion people inhabiting them.

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 proper 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 performance, cover and diversity (Maphangwa *et al.*, 2012; 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 in drylands will be negatively affected by ongoing climate change. 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), and has barely studied how climate change will affect the thallus composition of biocrust constituents. The nutrient status (i.e., total C and N) of biocrust-forming lichens and mosses is expected to change with increased temperature and altered rainfall regimes due to reduced physiological performance (Reed *et al.*, 2012), as was shown along climatic gradients in the field (Concostrina-Zubiri *et al.*, 2018). 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. This is so because lichens are one of the most conspicuous and abundant biocrust constituents in global drylands (Weber *et al.*, 2016), and because the effects of biocrust-forming lichens on soil nutrients and microbial communities are both 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).

To the best of our knowledge, no previous study has experimentally evaluated how climate change drivers like warming and reduced precipitation impact the chemistry of lichen thallus 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 several 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 morphological and chemical traits, 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 functioning (Ladrón de Guevara *et al.*, 2014; Delgado-Baquerizo *et al.*, 2014; Darrouzet-Nardi *et al.*, 2015), albeit the magnitude of their effects will be modulated by lichen species; and iii) changes in lichen C and N composition drive climate change impacts on soil functioning.

MATERIALS AND METHODS

Species used

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 (Escolar *et al.*, 2012). 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).

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 located over 50 km south of the CCLOL.

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 onto 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.*, 2012), 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, resulting in a total of 72 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; Rhein *et al.*, 2013). This temperature increase was achieved by using open top chambers (OTCs) built with six methacrylate plates that followed a hexagonal design with sloping sides of 65-52-42 cm (Fig. S2). The methacrylate material used to build them transmits ~92% of visible spectrum, has a 4% of emission of the infrared wavelength and pass on 85% of incoming energy (data provided by the manufacturer, Decorplax Metacrilatos S.L., Madrid, Spain). The OTCs were open on the top to allow rainfall, and were elevated 5 cm from the soil surface to achieve adequate air flow 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 on passive rainfall shelters based upon the design of Yahdjian & Sala (Yahdjian & Sala, 2002); these did not modify the frequency of rainfall events but reduced the total amount of rainfall reaching the soil surface. Each rainfall shelter has an area of 1.68 m^2 (1.4 m × 1.2 m), and a mean height of 1 m, and a roof composed of six methacrylate grooves (Fig. S2), which covers 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 plastic bags until further analysis.

Biocrust material (the whole lichen thallus) was thoroughly cleaned with a brush and oven-dried (48 h at 60ºC). It was then 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 has been shown to be 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.*, 2012). 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. Also, one soil sample was discarded from data analyses for potential contamination, as indicated by its high N values relative to the whole sample set. 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 thallus (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). We chose PERMANOVA over ANOVA because biocrust thallus composition variables did not follow a normal distribution nor showed homogeneity of variances. When the main factor (i.e., treatment) had a significant effect on the response variable, we conducted a pairwise comparison for PERMANOVA between treatments. PERMANOVA was run using Euclidean distance and based on unrestricted permutation of raw data (9999 permutations) due to small sample size (n=5).

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 (Sbc - Sbs)/(Sbc + Sbs); where Sbc and Sbs are the values of a given soil variable under each species and treatment (n=5) and in bare soil (as the average of the four replicates for each species and treatment), 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 positive and negative values indicate positive and negative effects on such variable. 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 by means of a two-way PERMANOVA, using Euclidean distance and based on unrestricted permutation of raw data (9999 permutations) due to small sample size (n=5). 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 species.

To evaluate the species-specific effects of lichen thallus C and N composition on soil fertility and functioning (hypothesis iii), we calculated Spearman's rank correlation coefficients for each species separately. We used Spearman correlation coefficients (Delgado-Baquerizo *et al.*, 2015) because preliminary analyses showed that relationships between biocrust and soil variables were monotonic in most of the cases for all the studied species.

PERMANOVA analyses and pairwise comparisons were conducted using the vegan R package (Oksanen *et al.*, 2019). To test whether RII values were significantly different from zero, we computed their bootstrapped 95% confidence intervals using the boot R package (Canty & Ripley, 2019). All analyses were performed with R version 3.6.1 (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, 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

δ^{13}C (compared to microcosms without lichens) 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. 2e, g, 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 microcosms without lichens; Fig. 3e, g, 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 total C and soil pH (Table S2).

The C and N content of lichen thallus were strongly related to several soil variables in all studied species except in *P. decipiens*, which showed no significant correlation between its tissue composition and soil variables (Table S3). For *B. zoharyi*, thallus C and N were positively related to SOC, while thallus C also showed a positive relation to β-glucosidase activity (Table S3). Additionally, *B. zoharyi* δ^{15}N and C:N were negatively related to total C and DON and NO_3^-, respectively (Table S3). Similarly, thallus C and N and δ^{13}C in *D. diacapsis* showed a strong, positive relation to SOC and phosphatase activity, respectively (Table S3). In this species, thallus N and C:N were positively and negatively related to DON, respectively (Table S3). Similarly, thallus C:N in *P. decipiens* showed a negative relation to DON and NH_4^+ (Table S3). For *S. lentigera*, thallus C and N were negatively related to soil NH_4^+ (Table S3). Also, *S.*

lentigera C and N content and ratio had strong positive and negative effects, respectively, on soil pH (Table S3).

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 biocrust thallus composition and several soil variables were coupled regardless of climate change treatments, although the nature of these relationships was, again, highly dependent on the species identity. 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 C and N composition in biocrust lichens 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 (i.e., CO_2 diffusion into lichen thallus) and use (Lakatos *et al.*, 2007). Moreover, due to their poikilohydric nature, in lichens these processes are known to be governed by ambient humidity and temperature (Nash, 2008)(Nash 1999), 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* (<0.4mm; (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 $\delta^{13}C$ values observed 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 experimental setup (Castillo-Monroy *et al.*, 2011; Maestre *et al.*, 2013; Escolar *et al.*, 2015). However, it would then be expected that *P. decipiens*, characterized by discontinuous darker squamules, 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 for this species (Fig. 1b) were remarkably lower.

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 a 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 terricoulous 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 as small “fertility islands” for plants, microbes and other soil fauna in a more arid scenario.

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 DON and NO_3^- concentrations values found in bare soil microcosms under warming and the combination of warming and reduced precipitation, while *S. lentigera* soils showed similar or lower values for these variables, respectively, compared to the control treatment (Fig. S4e, g). Further research 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 species 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 the effects of climate change drivers. 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*; most soil properties responded to climate change drivers (i.e., total C and N, SOC, $\delta^{13}C$, DON, NH_4^+ and NO_3^-) regardless of lichen identity. Previous research has shown 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 the bare ground soil (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.*, 2005b). 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 terricoulous lichens cannot derive important amounts of these compounds directly from the soil (Ellis *et al.*, 2004). However, this study evaluated N uptake in fruticose lichens which are loosely attached to the soil (Ellis *et al.*, 2004). By contrast, the lichen species in our study are 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 broad microbial functional groups (i.e., fungi and bacteria; Castro *et al.*, 2010; Maestre *et al.*, 2015) 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 studied species produce secondary compounds with potential antimicrobial effects (Molnár & Farkas, 2010). Since the production of these compounds are determined by the lichen physiological status (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 two species of boreal terricoulous lichens (i.e., *Cladonia islandica* and *C. arbuscula*) under experimental warming in the field, while other species were unaffected (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, and they had no effect on microbial activity, measured as respiration rates (Stark *et al.*, 2007), little is known for other biocrust lichens and associated secondary compounds. Although the lack of response of lichen effects on soil enzymatic activities to climate change treatments suggests that our idea that lichens are taking N from the soil is more plausible, future research should investigate the capacity of biocrust-forming lichens to uptake organic and inorganic N from the soil (for example using ^{15}N labelling techniques) and whether climate change drivers affect the production and release of lichen secondary compounds.

Lichens also decreased soil $\delta^{13}C$ under warmer conditions (Fig. 2c, Table S2), supporting the idea that biocrusts promote soil respiration under increased temperature at the short-term (Escolar *et al.*, 2015; Darrouzet-Nardi *et al.*, 2015; Dacal *et al.*, 2020). However, the decreasing effect of biocrusts on soil $\delta^{13}C$ was particularly low (~2% increase, Table S2), and thus, our results should be taken cautiously.

Biocrust-forming lichens had strong species-specific effects on soil properties. 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, it has been shown that lichen litter lose C and N with time (Berdugo *et al.*, 2020), suggesting that these nutrients are being transferred to the soil below as a result of decomposition processes. Similarly, *P. decipiens* had a positive effect, on average, on NH_4^+ concentration, while the other species decreased it (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, *P. decipiens* may generate a warmer, because of darker thallus colour, and more humid soil microenvironment compared to the other species, 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. 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; the apothecia, 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 (e.g., highly group-specific). Indeed, microbial communities under *P. decipiens* have been reported to be different from other biocrust-forming lichens previously (Maier *et al.*, 2014). 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 overall lower NH_4^+ observed values found beneath *B. zoharyi*, *D. diacapsis* and *S. lentigera*, compared to the bare soil (Fig. f, Table S4), 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.

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

We found support for our third hypothesis, i.e. changes in biocrust C and N composition and soil fertility and functioning are coupled. 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 some of these effects are maintained across climate change treatments, while others may have changed. For example, thallus C and N and C:N ratio of *B. zoharyi* and *D. diacapsis* were positively and negatively correlated, respectively, to SOC and DON (Table S4), indicating that these lichens may be more easily incorporated into the soil via litter decomposition or through C and N leaching (Barger *et al.*, 2016). This idea is somehow supported by the positive correlation between the C and N content of these species and the soil enzymatic activities beneath them (Table S4). Moreover, our results suggest that, for the studied species, the effects of lichen nutrient content dominate over the potentially detrimental effects of lichen secondary compounds on microbial communities and decomposition rates (Asplund & Wardle, 2013; Kosanić & Rankovic, 2015).

Values of $\delta^{15}N$ appeared to be a good indicator of organic N in the soil in the case of *B. zoharyi* (Table S4). In addition, *S. lentigera* showed a strong negative effect on soil NH_4^+ (Fig. 3f, Table S4). This supports the idea that this lichen uptakes important amounts of inorganic N from the soil. The strong and positive relationship between *S. lentigera* nutrient content and soil pH might be explained by increased soil nutrient availability at higher pH values (Table S4). The lack of effects of *S. lentigera* on soil pH (Fig. 3j, Table S2) suggests that the rates or concentration at which usnic acid is released are highly variable at the individual level (Bjerke *et al.*, 2005a), so are its effect on soil pH and soil nutrient availability. Then, at low acid release rates or concentrations, soil pH would be higher, enhancing soil microbial activity, and, thus, soil nutrient availability, which would be then reflected as a higher nutrient content in *S. lentigera*. Surprisingly, we found no significant correlations between P. decipiens tissue composition and any soil variables. This suggests that the effects found for this species are mainly due to its morphological traits, which drive the response of soil properties to climate change treatments, rather than to its physiological activity, which may have resulted in stronger relationships between tissue composition and soil properties at the individual level. Nevertheless, 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), particularly concerning soil nutrient cycling and microbial activity.

CONCLUDING REMARKS

Our study is the first to provide experimental evidence that the chemistry of biocrust-forming lichen thallus is sensitive to warming and rainfall reduction. Our findings show how changes in thallus chemistry drive observed species-specific effects of lichens on soil functioning, and modulated soil C and N cycling to simulated climate change. This will have important implications to nutrient cycling in drylands, where these communities dominate plant interspaces and show species-specific vulnerability to climate change drivers (Ladrón de Guevara *et al.*, 2018). Our results also suggest that some species are indicators of environmental changes and potential feedbacks occurring in the atmosphere-soil interphase (i.e., isotopic composition in *B. zoharyi* and *S.*

lentigera), while others are good indicators of changes in the organic matter pool and microbial activity in the soil (e.g., C and N composition in *D. diacapsis*). 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 (i.e., *S. lentigera*). 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

FTM planned and designed the experiment, E.V., V.O., B.G. and B.J.M. 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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Fig. 1. Changes in lichen thallus composition of the studied species under simulated climate change. 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. C, Carbon; N, nitrogen. BuZo, *Buellia zoharyi*; DiDi, *Diploschistes diacapsis*; PsoDe, *Psora decipiens*; SquLe, *Squamarina lentigera*. W and W+RR, warming and rainfall reduction.

Figure 2. Effects of climate change treatments on soil properties, as measured with the Relative Interaction Intensity (RII) index. Data are mean ± 95% bootstrap CIs. Different letters above/below bars indicate differences between treatments ($P < 0.05$, after PERMANOVA analysis). β-glu., β-glucosidase activity; A. phos., acid phosphatase activity. W and W+RR, warming and rainfall reduction.

Figure 3. Effects of lichen species on soil properties, as measured with the Relative Interaction Intensity (RII) index. Data are mean ± 95% bootstrap CIs. Different letters above/below bars indicate differences between treatments ($P < 0.05$, after PERMANOVA analysis). β-glu., β-glucosidase activity; A. phos., acid phosphatase activity. BuZo, *Buellia zoharyi*; DiDi, *Diploschistes diacapsis*; PsoDe, *Psora decipiens* (PsoDe); SquLe, *Squamarina lentigera*.

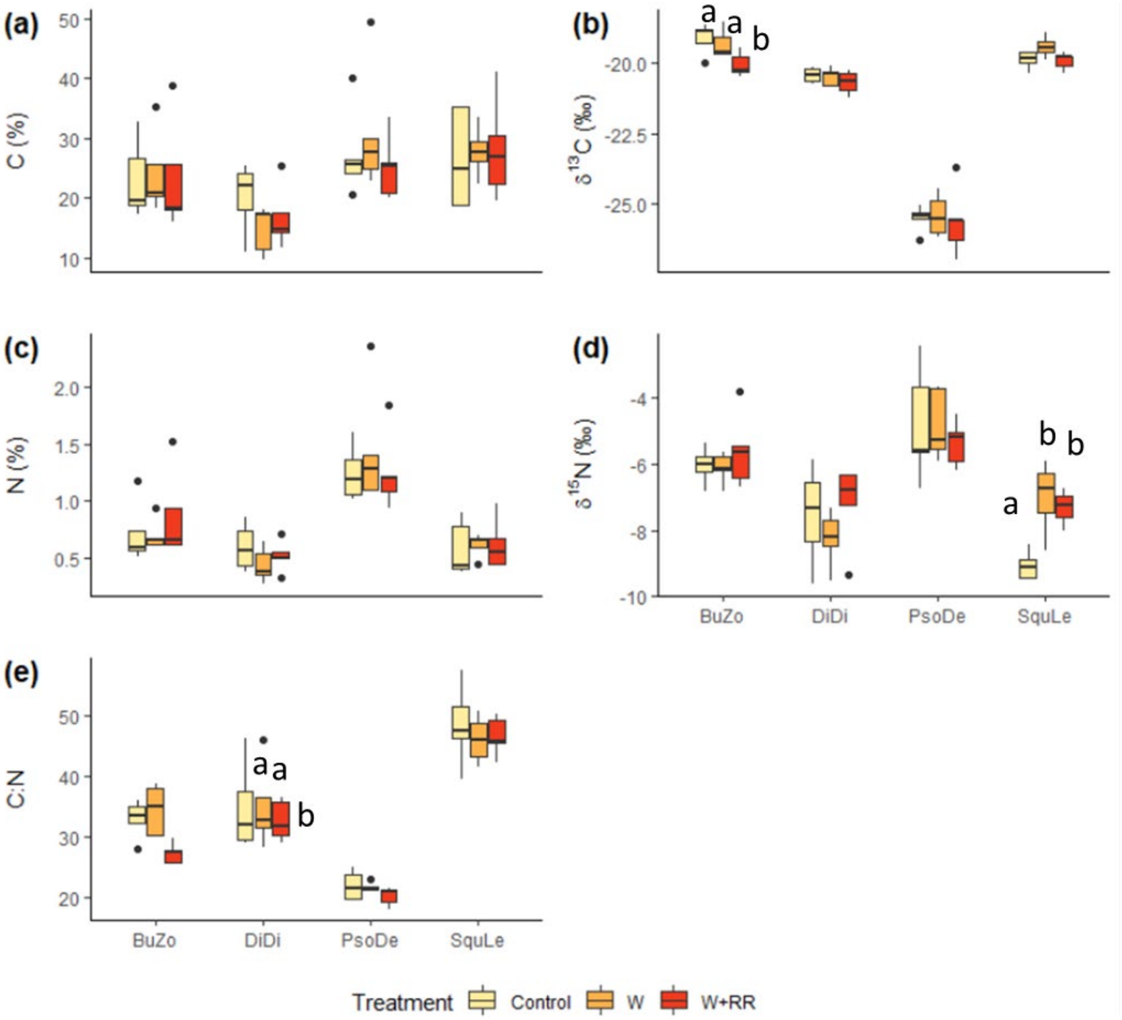
(a)
C (%)
50
40
30
20
10
(b)
δ ^{13}C (‰)
-20.0
-22.5
-25.0
a
a
b
(c)
N (%)
2.0
1.5
1.0
0.5
(d)
δ ^{15}N (‰)
-4
-6
-8
-10
b
b
a
BuZo
DiDi
PsoDe
SquLe
(e)
C:N
50
40
30
20
a
a
b
BuZo
DiDi
PsoDe
SquLe
Treatment
Control
W
W+RR

Figure 1

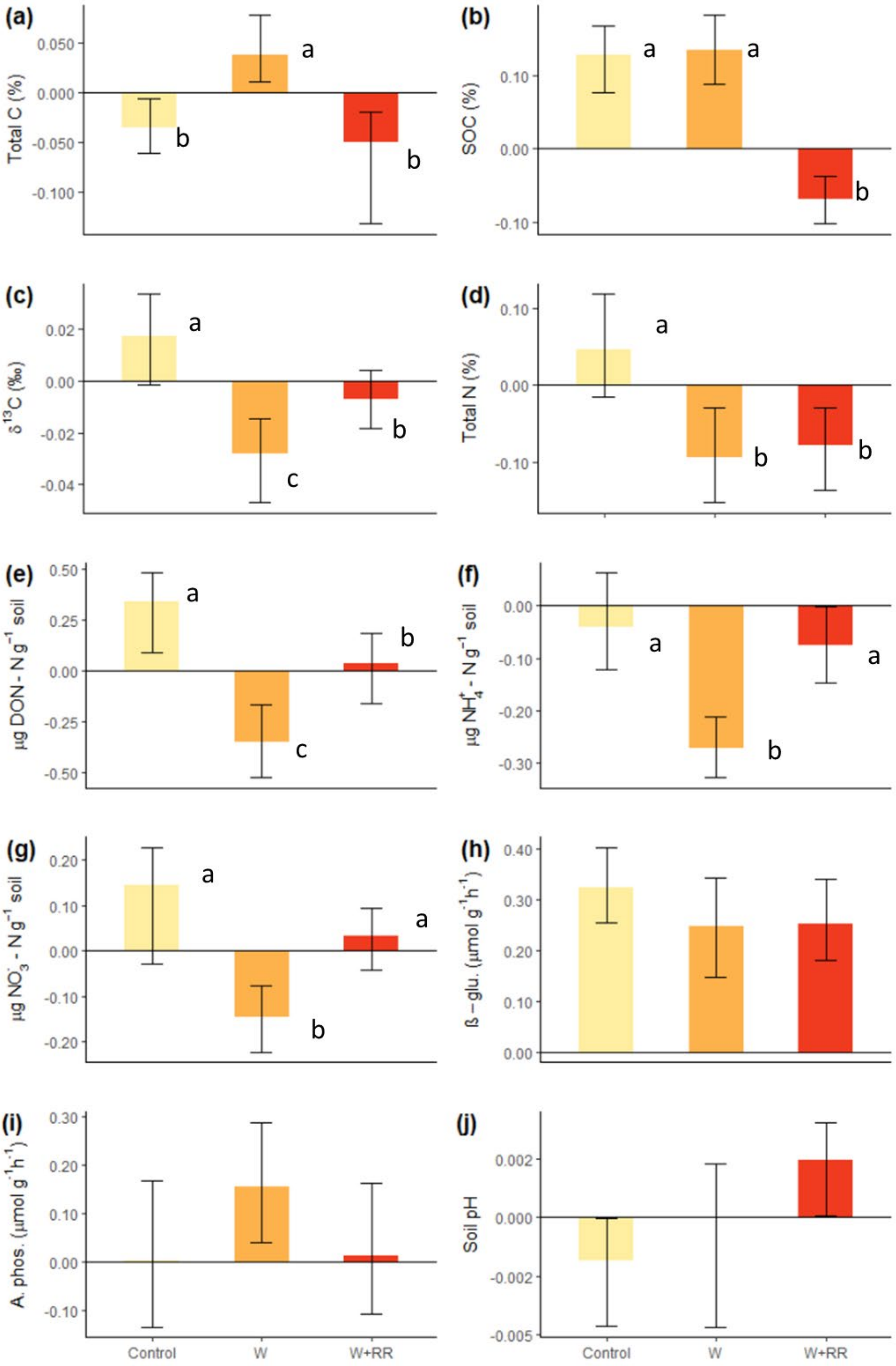
(a)
Total C (%)
0.050
0.000
-0.050
-0.100
b
a
b
(b)
SOC (%)
0.10
0.00
-0.10
a
a
b
(c)
$\delta^{13}C$ (‰)
0.02
0.00
-0.02
-0.04
a
c
b
(d)
Total N (%)
0.10
0.00
-0.10
a
b
b
(e)
µg DON - N g^{-1} soil
0.50
0.25
0.00
-0.25
-0.50
a
c
b
(f)
µg NH_4^+ - N g^{-1} soil
0.00
-0.10
-0.20
-0.30
a
b
a
(g)
µg NO_3^- - N g^{-1} soil
0.20
0.10
0.00
-0.10
-0.20
a
b
a
(h)
ß −glu. (µmol g^{-1} h^{-1})
0.40
0.30
0.20
0.10
0.00
(i)
A. phos. (µmol g^{-1} h^{-1})
0.30
0.20
0.10
0.00
-0.10
(j)
Soil pH
0.002
0.000
-0.002
-0.005
Control
W
W+RR
Control
W
W+RR

Figure 2

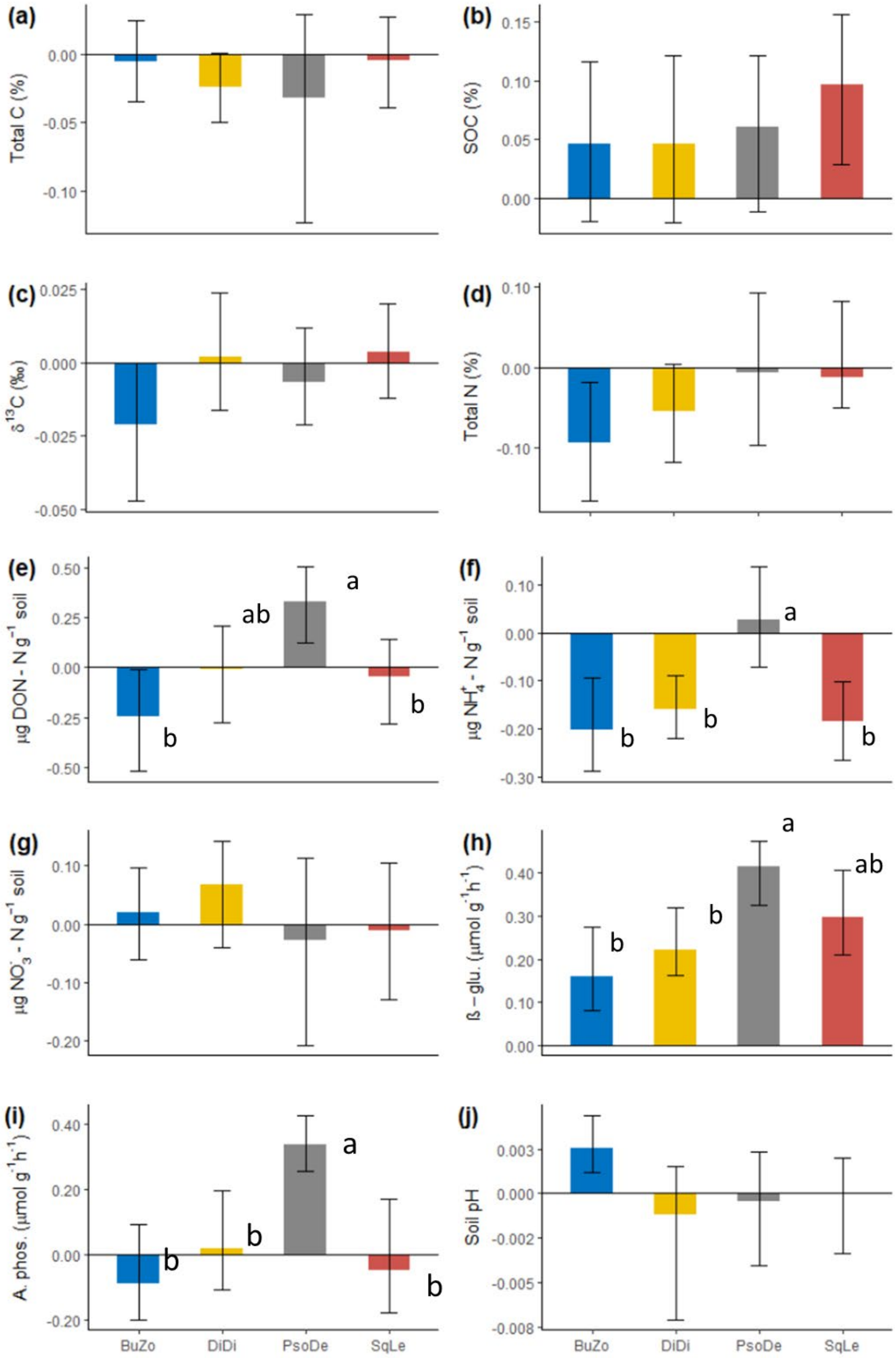
(a)
Total C (%)
0.00
-0.05
-0.10
(b)
SOC (%)
0.15
0.10
0.05
0.00
(c)
$\delta^{13}C$ (‰)
0.025
0.000
-0.025
-0.050
(d)
Total N (%)
0.10
0.00
-0.10
(e)
µg DON - N g^{-1} soil
0.50
0.25
0.00
-0.25
-0.50
a
ab
b
b
(f)
µg NH$_4^+$ - N g^{-1} soil
0.10
0.00
-0.10
-0.20
-0.30
a
b
b
b
(g)
µg NO$_3^-$ - N g^{-1} soil
0.10
0.00
-0.10
-0.20
(h)
ß−glu. (µmol g^{-1}h^{-1})
0.40
0.30
0.20
0.10
0.00
a
ab
b
b
(i)
A. phos. (µmol g^{-1}h^{-1})
0.40
0.20
0.00
-0.20
a
b
b
b
(j)
Soil pH
0.003
0.000
-0.002
-0.005
-0.008
BuZo
DiDi
PsoDe
SqLe
BuZo
DiDi
PsoDe
SqLe

Figure 3