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Research article

Environmental change alters nitrogen fixation rates and microbial parameters in a subarctic biological soil crust

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Oikos
2022: e09239
doi: 10.1111/oik.09239
Subject Editor:
Marco A. Molina-Montenegro
Editor-in-Chief:
Gerlinde B. De Deyn
Accepted 15 July 2022

Together, biological soil crust (BSC) and other cryptogamic groundcovers can contribute up to half of the global nitrogen (N) fixation. BSC also stabilizes the soil (reducing erosion and dust emissions), fixes carbon (C), retains moisture and acts as a hotspot of microbial diversity and activity. Much of the knowledge about how climate change is affecting the composition and functioning of BSC comes from hot arid and semiarid regions. The comparatively smaller body of research on BSC from cold and mesic environments has been primarily observational, for example along chronosequences after a glacier retreat. Few studies have experimentally investigated the effects of the environment on BSC from high latitudes. Such experiments allow unraveling of relationships at a resolution that can only be achieved by controlling for confounding factors. We measured short-term (2–4 days) responses of a liverwort-based *Anthelia juratzkana* BSC from the south of Iceland to a range of temperature, moisture and light conditions. Warming increased N fixation rates, especially when moisture was at a saturation level, and only when light was not limiting. A correlation analysis suggests that increases in N fixation rates were linked to cyanobacterial abundance on the BSC surface and to the rates of their metabolic activity. Warming and moisture changes also induced compositional and structural modification of the bacterial community, with consequences at the functional level. In contrast to many observations on BSC from hot drylands, the BSC from our cold and mesic study site is more limited by low temperature and light than by moisture. Our findings show possible ways in which BSC from cold and mesic ecosystems can respond to short-term manifestations of climate change, such as increasingly frequent heat waves.

Keywords: biological soil crust (BSC), climate change, cyanobacterial abundance and composition, nitrogen (N) fixation, subarctic ecosystems

Introduction

Biological soil crust (BSC) is a skin-like system generally dominated by cyanobacteria, fungi, lichens and bryophytes (Belnap 2003, Bowker et al. 2018). In some areas this system can cover over 90% of the soil surface (Williams et al. 2017). BSC-forming

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organisms can colonize soils in harsh environments, such as drylands (Belnap 2003, Elbert et al. 2012, Rodríguez-Caballero et al. 2018) or newly exposed soil after glacier retreat (Breen and Lévesque 2008, Yoshitake et al. 2018). Once they establish, they stabilize the soil (Gao et al. 2017), increase moisture retention (Breen and Lévesque 2008), fix nitrogen (N) (Dickson 2000, Elbert et al. 2012) and carbon (C) (Yan-Gui et al. 2011, Elbert et al. 2012), and become hotspots of microbial abundance (Yoshitake et al. 2018) and diversity (Steven et al. 2013). Under constant high environmental stress, BSC becomes a permanent feature of the undisturbed ecosystem. When high abiotic stress decreases over time, BSC acts like a transient colonizer in primary succession (Bowker 2007).

BSC covers 12% of the Earth's terrestrial surface (Rodríguez-Caballero et al. 2018). It is estimated that before the end of the century climate change and intensification of land use will decrease this area by 25–40%, thus enhancing emissions of soil dust and reducing BSC's contributions to the global C and N cycles (Rodríguez-Caballero et al. 2018).

The rates at which BSC influences N and C cycling depend on environmental factors such as temperature, moisture and light intensity, and on intrinsic properties of its biological components, such as the N fixing activity of cyanobacteria (Belnap 2001) and the differential decomposing activity of bacteria and fungi (Zhao et al. 2020). Together, BSC and other cryptogamic ground covers (i.e. rock crust and bryophyte and lichen carpets) globally account for up to half of the terrestrial N fixation, and 7% of the C fixation (Elbert et al. 2012, Porada et al. 2014). Uncertainty in model projections remains high (global N fixation estimates ranging between 3.5 and 34 Tg yr^{-1}; Porada et al. 2014), in part due to limited knowledge of the extent and structure of crusted communities in many ecosystems (Ferrenberg et al. 2017).

Cold-adapted BSC covers vast areas at high latitudes (Pushkareva et al. 2016), the part of the world experiencing the fastest warming (IPCC 2021, chapter 2, p.316). One of the most visual and consequential manifestations of climate change in these regions and globally is the increase in frequency and intensity of short-term climatic anomalies such as heat waves (Perkins et al. 2012). Some high-latitude systems are more resistant to climatic anomalies than others (Jónsdóttir et al. 2005). Because of its relative simplicity and the fast turnover rates of its biological components, BSC may be particularly sensitive to short-term changes in the environment (Finger-Higgens et al. 2022).

The potential effects of climate change on cold-adapted BSC, and on the ecosystem services it provides, are still largely unknown. Most of the knowledge on BSC responses to the environment comes from hot arid or semiarid ecosystems (Rodríguez-Caballero et al. 2018, Havrilla et al. 2019, Xu et al. 2022). The comparatively smaller body of research on BSC adapted to cold and mesic conditions has been primarily observational, for example along chronosequences after a glacier retreat (Breen and Lévesque 2008, Yoshitake et al. 2010, 2018, Borchhardt et al. 2019), along climatic gradients (Stewart et al. 2011, Blay et al. 2017, Pushkareva et al. 2021, Novakovskaya et al. 2022) or in the context of assisting BSC development for restoration purposes (Aradottir and Halldorsson 2018, Faist et al. 2020). This research has shown, for example, that BSC can act as ecosystem engineers of cold environments, e.g. by increasing physiological performance of vascular plants (Barrera et al. 2022). While observational approaches are useful to study ecosystem processes under natural conditions, experiments allow study of relationships at a resolution that can only be achieved by controlling for confounding factors. Only a handful of studies have used an experimental approach to investigate the effects of the environment on cold-adapted BSC (Colesie et al. 2014, Alatalo et al. 2015, Rousk et al. 2018).

From a methodological perspective, experimental research on BSC offers many advantages (Bowker et al. 2014, Maestre et al. 2016). Because of its size, it is possible to collect entire BSC blocks and study them as closed systems, even under laboratory conditions (Supporting information). Also, because of the small size and relatively fast turnover rates of BSC organisms, it can respond rapidly to external factors. This has advantages for early detection of environmental changes. Finally, since BSC can act as the first link in a chain of ecological succession (Godínez-Alvarez et al. 2011), understanding BSC responses to the environment not only provides information about the BSC itself, but also about the potential ecosystems and ecological dynamics that can evolve from it.

Here we conducted a laboratory experiment to study the compositional and functional responses of a BSC adapted to cold and mesic conditions, BSC to short-term (2–4 days) incubations at a range of temperature, moisture and light intensities. The ranges of temperature varied between average and maximum values during the snow-free season in an Icelandic ecosystem dominated by BSC. Unless limited by other biotic and/or abiotic factors, we expected warming to increase N fixation rates either via growth of N fixers, or via increases in their metabolic activity, or both. Also, we expected environmental treatments to exert a selective pressure, leading to structural and possibly compositional changes within the N fixing communities (e.g. favoring warm adapted taxa); and for this to contribute to explaining N fixation responses to the environment. Given the well known dependence of biological N fixation on moisture (Rousk et al. 2018), and the need of photosynthetically (i.e. light-fueled) synthesized C to run the N fixing machinery (Scherer et al. 1988), we expected the effects of warming on N fixation to be directly dependent on moisture and light intensity. Also, we hypothesized a positive correlation between N fixing rates and chlorophyll *a* (Chl *a*) content (Staal et al. 2001) – an indicator of net photosynthetic rate (Yan-Gui et al. 2011). Overall, we expected subarctic BSC to respond to short-term environmental manipulations and for these responses to indicate possible ways in which this system, and the key ecosystem services it provides, could be altered by climate anomalies like increasingly frequent heat waves (Perkins et al. 2012).

Material and methods

We designed a controlled laboratory experiment to investigate the responses of a subarctic liverwort-based *Anthelia juratzkana* BSC from the south of Iceland to different levels of temperature, moisture and light. We studied how these environmental factors affect the capacity of subarctic BSC to fix N, and whether these responses were linked to changes in the abundance of N fixers and/or to structural changes in the BSC microbial communities.

Sample collection

In September 2018 we collected BSC from a site adjacent to the Climate Research Unit at Subarctic Temperatures (CRUST) experiment (Salazar et al. in progress), near Landmannahellir, Iceland (64°02′N, 19°13′W; 590 m a.s.l.). Mean annual temperature and precipitation at the site are ca 5°C and 1500 mm, respectively. Surface cover in this area is primarily liverwort-based BSC (ca 50%), followed by mosses (ca 30%) and *Salix herbacea* dwarf willow (ca 20%), on an andosol/vitrisol substratum.

We randomly collected eight BSC blocks (i.e. replicates) of 13 × 16 cm^2 and ca 5 cm deep (Supporting information). Blocks were separated by at least 10 m. Since the focus of this study is on BSC, patches of moss or vascular plants were avoided. We transported (approx. 5 h) the BSC blocks in coolers with ice packs and stored them in a dark room at 5°C for 2–5 weeks while we performed the analyses described below. We kept wet paper towels inside the coolers to prevent desiccation. We subsampled BSC disks of 5 cm diameter and 1.5 cm depth out of the 13 × 16 × 5 cm^3 BSC blocks (Supporting information) for N fixation analyses ('N fixation under controlled temperature, moisture and light conditions' section). Then, we subsampled BSC disks of 1.5 cm diameter, 1.5 cm depth from each 5 cm diameter BSC disk, for Chl *a* ('Cyanobacteria and liverwort cover on BSC' section) and cyanobacteria and liverwort cover ('Chlorophyll *a*' section) analyses and for DNA extractions ('DNA extraction and analysis' section).

Experimental design and environmental treatments

We studied the effects of temperature, moisture and light on N fixation and the microbial community structure. For this, we conducted a factorial experiment (4 × 2 × 2) with four levels of temperature: 10, 15, 20 and 25°C; two levels of moisture: ca. 75% (close to moisture at the moment of sampling) and 100% (saturated); and two levels of light ca 2 μmol m^{-2} s^{-1} (low intensity) and ca 90 μmol m^{-2} s^{-1} (high intensity; Supporting information). Light was available all the time (i.e. we did not set day/night cycles), to simulate conditions similar to those in the sampling site during the summer. Temperature and light treatments were set in a growth chamber (Termaks series 8000, Bergen, Norway), and monitored hourly with temperature/light loggers (HOBO Pendant® MX Temperature/Light Data Logger, MX2202, Onset, Bourne, MA, USA). Levels of these environmental variables were selected within ranges commonly experienced by BSC at the sampling site (between ca > 0 and 25°C; 0 and > 100 μmol m^{-2} s^{-1}; and between dryness for short periods of time during the summer, and saturation e.g. after the winter snow is melted; unpublished observations) and comparable ecosystems (e.g. a mesic-dry heath in Greenland; Rousk et al. 2018). We compared ambient versus saturation moisture levels because mean annual precipitation in subarctic and arctic regions is projected to increase in the coming decades (IPCC 2021, chapter 4, p. 638). The maximum temperatures in our experimental design were selected based on peaks of warming (measured at the soil surface) recorded during previous growing seasons (unpubl.). In this sense, our high temperature treatment should simulate BSC responses to heat waves at the study site, under different moisture and light conditions.

Average temperature and light intensity inside the jars were 11.1 ± 0.7, 16.5 ± 0.7, 21.5 ± 0.7 and 26.6 ± 0.9°C (2 loggers × 2 light levels; $n = 4$) and 2.3 ± 0.04 and 88.0 ± 1.6 μmol m^{-2} s^{-1} (2 loggers × 4 temperature levels; $n = 8$) respectively (Supporting information). Temperature levels inside the jars were slightly higher than temperatures set in the growing chamber due to a greenhouse effect.

To create a saturation level in the moisture treatment, we wetted each sample with an excess of deionized water and waited for approximately one minute until it stopped dripping. Moisture was maintained between analyses by placing wet towels in the coolers stored in the cold, dark room. After environmental treatments and N fixation measurements (see following section), we oven dried (60°C, 24 h) BSC disks to estimate the dried weight of the samples, and to prepare them for chlorophyll *a* analysis and DNA extraction. Average moisture content was 75.5 ± 2.4 and 107.2 ± 2.3% (Supporting information).

N fixation under controlled temperature, moisture and light conditions

We estimated N fixation rates with the acetylene reduction assay (ARA; Hardy et al. 1968). We used eight 5-cm subsampled disks (i.e. replicates) per combination of temperature and moisture treatments. Thus, each temperature-specific ARA analysis was composed of a total of 16 samples with two levels of moisture, eight saturated and eight unsaturated, plus controls with acetylene, ethylene and air. The BSC disks were weighed (for further water content analysis) and placed in 350 ml glass jars with rubber septa in the lids (Supporting information). These jars were then placed in an environmental chamber (Termaks series 8000, Bergen, Norway) at fixed temperature and light conditions. We acclimated the samples to each combination of temperature and light for 24 h. We then manually aerated the jars for a few seconds, closed the jars tightly and replaced 10% of the headspace with acetylene (except in jars used as ethylene and air controls). We incubated the jars at the set temperature and light conditions for 24 h. Then, we collected 22 ml of gas from each jar and analyzed it using a Clarus 400 gas chromatograph (PerkinElmer

Ltd., Beaconsfield, UK) equipped with an automatic split/splitless injector and a flame ionization detector (FID), and an Elite-Alumina column (30 m, 0.53 mm; PerkinElmer Ltd., Beaconsfield, UK).

At the end of each 48 h acclimation–incubation period, we manually aerated the samples and started a new acclimation–incubation at a different light (but same temperature) condition. To control for a possible effect of the storing time in the cold room, we randomized the order of the temperatures for the incubations. We incubated first samples (8 replicates at ca 75% and 8 at 100% moisture content) at 20°C, then at 10, 25 and 15°C. Also, to control for a possible cumulative effect between light levels, we switched the order of the light levels for each temperature treatment. For example, for samples incubated at 20°C we measured ethylene production first at low light (48 h) and then at high light (48 h). For the next quarter of the samples, incubated at 10°C, we measured ethylene production first at high light (48 h) and then at low light (48 h), and so on, for the other two temperature treatments. Since ARA is a non-destructive method, we were able to estimate N fixation rates on the same sample at different light treatments. For the rest of the analysis, based on destructive methods (see details below), we measured BSC responses to moisture and temperature.

Cyanobacteria and liverwort cover on BSC

We estimated the cover of cyanobacteria and liverwort (*Anthelia juratzkana*; Supporting information) on the BSC surface by epifluorescence microscopy (Supporting information; similar to Lan et al. 2019). After ARA measurements, BSC samples were stored in a dark room at 5°C for 1–4 days. Plant and cyanobacterial growth was assumed to be minimal under these conditions. From each 5 cm diameter BSC disk (Supporting information), we subsampled a 1.5 cm diameter BSC disk and imaged the plant (liverwort) chlorophyll using a Leica DM6000B fluorescent microscope (Leica, Heerbrugg Switzerland) equipped with an I3 filter cube (Ex 450/90, Di 510, Em 515), and the cyanobacterial phycocyanin with a TX2 filter cube (Ex 560/40, Di 595, Em 630/30). Multiple fields of view were measured using both filter cubes and stitched together to form an image of 1 × 1 cm of BCS surface (Supporting information) using the Leica software. Images were analyzed in ImageJ/Fiji (Collins 2007; Schindelin et al. 2012), and estimates of cyanobacterial and plant covers calculated as percentage of BSC surface cover.

We did not subsample BSC disks between light levels, but rather used samples that were exposed to low light for 48 h (24 h acclimation plus 24 h ARA) and then to high light for another 48 h, or vice versa. Therefore, the treatments in this part of our analysis include temperature and moisture, but not light.

Chlorophyll *a*

We estimated Chl *a* content as an indicator of net photosynthetic rate in BSC (Yan-Gui et al. 2011). Similar to our BSC cover analysis, we subsampled a 1.5 cm diameter, 1.5 cm depth BSC disk from each 5 cm diameter BSC disk (Supporting information) used for ARA analysis. We dried subsamples at 60°C for 24 h, extracted Chl *a* using DMSO (65°C, 90 min) and then estimated Chl *a* content by spectrophotometry (665 and 750 nm; Genesys 20, Thermo Scientific, Waltham, MA), as in Caesar et al. (2018) (Eq. 1, 2):

$$\text{Chl } a\ \mu\text{g} = \left(11.9035 \times \left(\text{A665} - \text{A750}\right)\right) \times S \qquad (1)$$

$$\text{Chl } a\left[\text{mg} \times \text{m}^{-2}\right] = \text{Chl } a\left[\text{mg}\right] / \left(\text{AR} \times 1000\right) \qquad (2)$$

where S is volume of solvent, AR is area (in m^{-2}) and A665 and A750 are absorbances at 665 and 750 nm, respectively.

As for BSC cover, treatments in this part of our analysis included temperature and moisture, but not light.

DNA extraction and analysis

Immediately after the fluorescence microscopy measurements ('Cyanobacteria and liverwort cover on BSC' section), we dried (60°C, 24 h) and ground (1 min, Mini bead beater 16; Biospec products) the 1.5 cm diameter, 1.5 cm depth BSC disks used for the cyanobacteria/liverwort cover analysis and stored them at −80°C for up to four months for DNA extraction. We pooled together replicates in pairs, combining them in equal weight parts (125 mg each for a total of 250 mg). We used the PowerSoil® DNA extraction kit (MOBIO/Qiagen), and shotgun sequencing approaches and analyses via the alignment-free fast taxonomic annotation tool Kraken2 (Wood and Langmead 2019) with the Kraken2 Refseq Standard plus protozoa and fungi database and the web-based pipeline Kaiju (Menzel et al. 2016). We estimated relative abundance of microbial groups using Kraken2 and fungal:bacteria ratios based on Kaiju taxonomic assignments (see sections below). After quality filtering the raw reads using Trim Galore microbial metagenome functional profiling was performed using HUMAnN 3 (Beghiji et al. 2021). For the functional annotation, UniRef50 (Suzek et al. 2015), KEGG (Kanehisa and Goto 2000) and BioCyc databases (Karp et al. 2019) were used. As for BSC cover and Chl *a*, treatments for this part of our analysis included temperature and moisture but not light. We characterized microbial communities only at two temperature levels: 10 and 20°C, which showed significant differences in N fixation and cyanobacterial cover (Results).

Fungal:bacterial ratios

Fungi and bacteria decompose organic matter at different rates, which affects the N and C biogeochemistry of substrates like BSC. To study potential effects of the environment on the biogeochemistry of BSC via differential effects on fungi and bacteria, we estimated fungal:bacterial ratios.

We calculated fungal:bacterial ratios based on numbers of gene copies assigned to each group by Kaiju.

Microbial community and statistical analyses

Microbial community analyses were performed using the microeco package in R ver. 3.5.0. We first investigated the most important Orders for classifying samples into different treatments using a random forest approach. We then conducted an ANOVA test followed by a Tukey's HSD test, $\alpha < 0.05$, as well as Pearson correlations and PERMANOVA analyses between the Bray–Curtis dissimilarity score and moisture content. Finally, we conducted a distance-based redundancy analysis (dbRDA) to assess the effects of the abiotic treatments on the top most abundant bacterial orders. To identify distinctive molecular pathways between treatments, we performed a linear discriminant analysis (LDA) effect size (LEfSe) analysis as implemented in the microeco package, then we selected the functions with a LDA score ≥ 3.5.

We used a mixed model *'lmer'* function in R ver. 3.6.1 to analyze the fixed effects of environmental manipulations on N fixation, while accounting for the random effect of measurements on the same sample at two light levels. For the other response variables, which varied in response to temperature and moisture but not light, we used fixed models *'lm'* function in R ver. 3.6.1. We compared models based on the Bayesian information criterion (BIC; Supporting information).

Results

Nitrogen fixation potential

N fixation (using acetylene to ethylene conversion as a proxy) in BSC increased with light, temperature and moisture (Fig. 1). Responses to environmental manipulations were better explained by the direct and additive effects of light, temperature and moisture than by their interactions (Supporting information). The minimum N fixation (ca 0.5 nmol $\text{cm}^{-2}\ \text{h}^{-1}$) occurred in the BSC at low temperatures, regardless of moisture and light intensity, whereas the maximum (4.4 ± 0.5 nmol $\text{cm}^{-2}\ \text{h}^{-1}$) occurred in moisture saturated BSC at 25°C and high light intensity (Fig. 1).

Cyanobacteria and liverwort cover

Cyanobacterial cover on BSC increased with temperature ($p < 0.05$) and was not affected by moisture (Fig. 2). BSCs incubated at 20°C had ca 5–30% more cyanobacteria on the surface than BSCs incubated at 10°C. There was no difference in cyanobacterial cover between BSCs incubated at 20 and 25°C. Temperature alone was a better predictor of cyanobacterial cover than moisture, or temperature and moisture combined (Supporting information). Liverwort cover was not affected by temperature or moisture (Fig. 2).

N fixation and cyanobacterial cover

Increases in N fixation rates were correlated ($p < 0.05$) to cyanobacterial cover (Fig. 3). Both cyanobacterial cover and N fixation rates increased with temperature between 10 and 20°C. Between 20 and 25°C N fixation rates continued increasing (Fig. 1) but cyanobacterial cover did not change (Fig. 2). Chl *a* did not vary with environmental treatments (Supporting information) and was not related to N fixation rates (Supporting information).

Relative abundance of cyanobacteria

Of the 20 most abundant Orders in the BSC microbial community, six had the highest importance score given by the random forest classification algorithm (Fig. 4a, b and

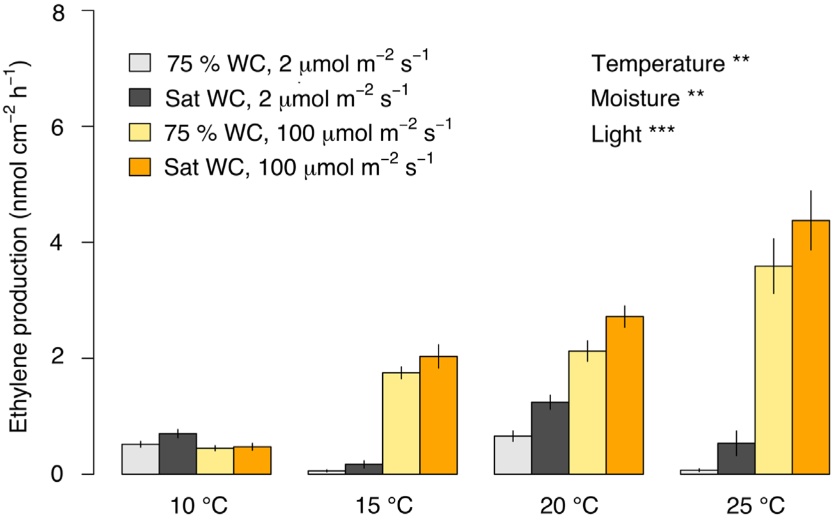

Figure 1. Ethylene production in response to temperature, moisture (light and dark tones) and light intensity (grey and yellow colors). Significance codes (here and elsewhere): $p < 0.001$ '***', $p < 0.01$ '**'. Significance levels shown in the legend are from the statistical model with the lowest BIC in the Supporting information. Values are means ± se. $n = 8$. WC: Water content. Sat: Saturated.

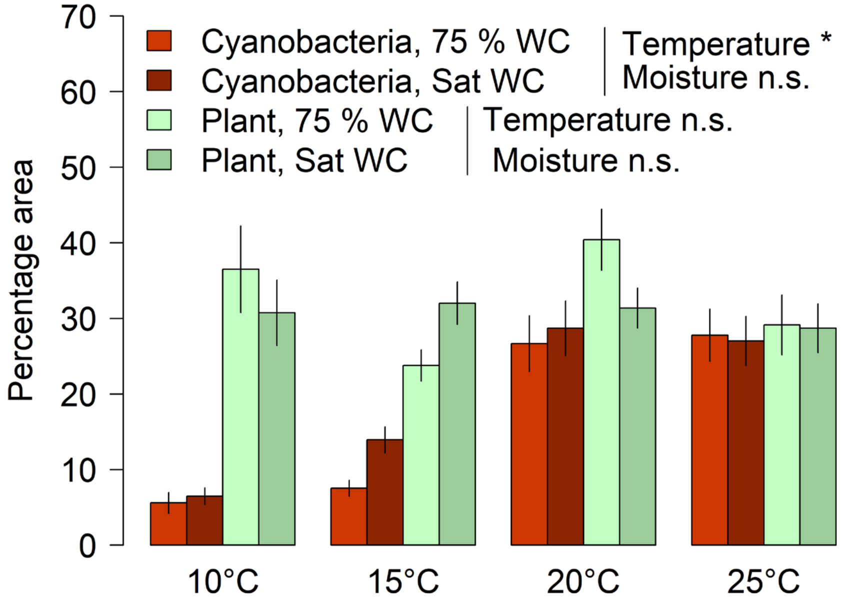

Figure 2. Cover of cyanobacteria and liverwort after 96 h incubation under different conditions. WC: water content (%). Significance codes (here and elsewhere): $p < 0.05$ '*'. Values are means ± SE. n = 8. WC: Water content. Sat: Saturated.

Supporting information). Alphaproteobacteria belonging to the Burkholderiales and Sphingomonadales, as well as Actinobacteria from Corynebacteriales were more abundant at 20°C than 10°C; Burkholderiales and Sphingomonadales being more abundant at 20°C with saturated water content (Fig. 4a and b). Cyanobacteria from the Nostocales are overall more abundant at 10°C than 20°C. Actinobacteria from Pseudonocardiales and Planctomycetes from Isosphaerales are more abundant at 75% water content than in saturated BSC. Pearson correlations and PERMANOVA analyses between the Bray–Curtis dissimilarity score and moisture content showed a significant effect of moisture on the microbial community structure (Fig. 4b, Supporting information). Moisture had a significant effect on the α-diversity, based on the observed richness and Chao1 index of microbial taxa at the phylotype level (Fig. 4c, Supporting information). Significant differences in Chao1 were observed between 75% and saturated BSC at 10°C and at 20°C, with overall higher richness observed in saturated BSC than 75% (Fig. 4c).

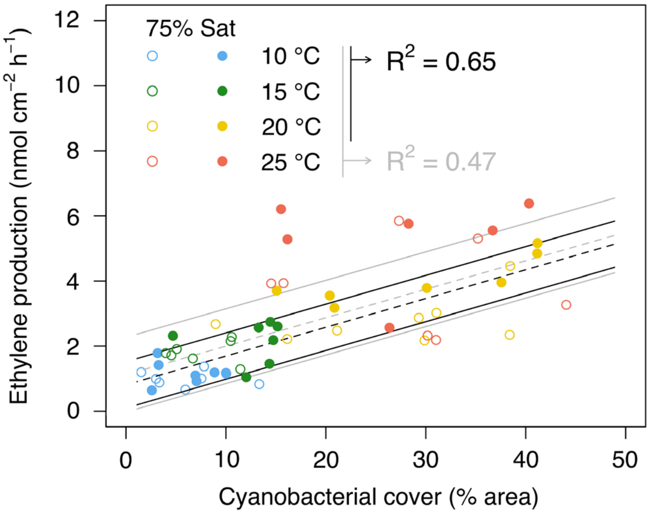

Figure 3. Correlation between cyanobacterial cover and N fixation rates across different temperature, moisture and light levels in a subarctic BSC. Dashed lines show the mean regression line, and solid lines the standard deviation of the mean. Grey lines and text show correlation between cyanobacterial cover and ethylene production for all temperature treatments ($y_i = 1.12 + 0.09 \times x_i + \varepsilon$; $\varepsilon \sim N(0, 1.16^2)$, where ε, here and elsewhere, is the error term describing the random component of the linear relationship). Black lines and text, show results excluding the 25°C level ($y_i = 0.81 + 0.09 \times x_i + \varepsilon$; $\varepsilon \sim N(0, 0.74^2)$ – to illustrate the strong correlation between cyanobacterial cover and ethylene production between 10 and 20°C. $p < 0.05$ '*', with and without including the 25°C level. (n = 8). Sat: Saturated (water content).

Fungal:bacterial ratios were slightly affected by a combination of warming and wetting (Supporting information). Total gene numbers indicate that bacteria grew faster than fungi in the saturated BSC at 20°C, resulting in a decrease in fungal:bacterial ratios (Supporting information). Total DNA did not change significantly across environmental treatments ($p > 0.05$), but it tended to increase with moisture, especially under warming (Supporting information).

BSC microbial functions

The LEfSe results show the effect size of the top 15 differentially abundant MetaCyc metabolic pathways between temperature (Fig. 5a) and moisture (Fig. 5b). At 10°C, pathways linked to autotrophy and anaerobic respiration such as nucleoside and nucleotide biosynthesis/degradation, cofactors, carrier and vitamin biosynthesis, glycolysis and photosynthesis were significantly enriched. At 20°C, central metabolism pathways associated with cellular respiration

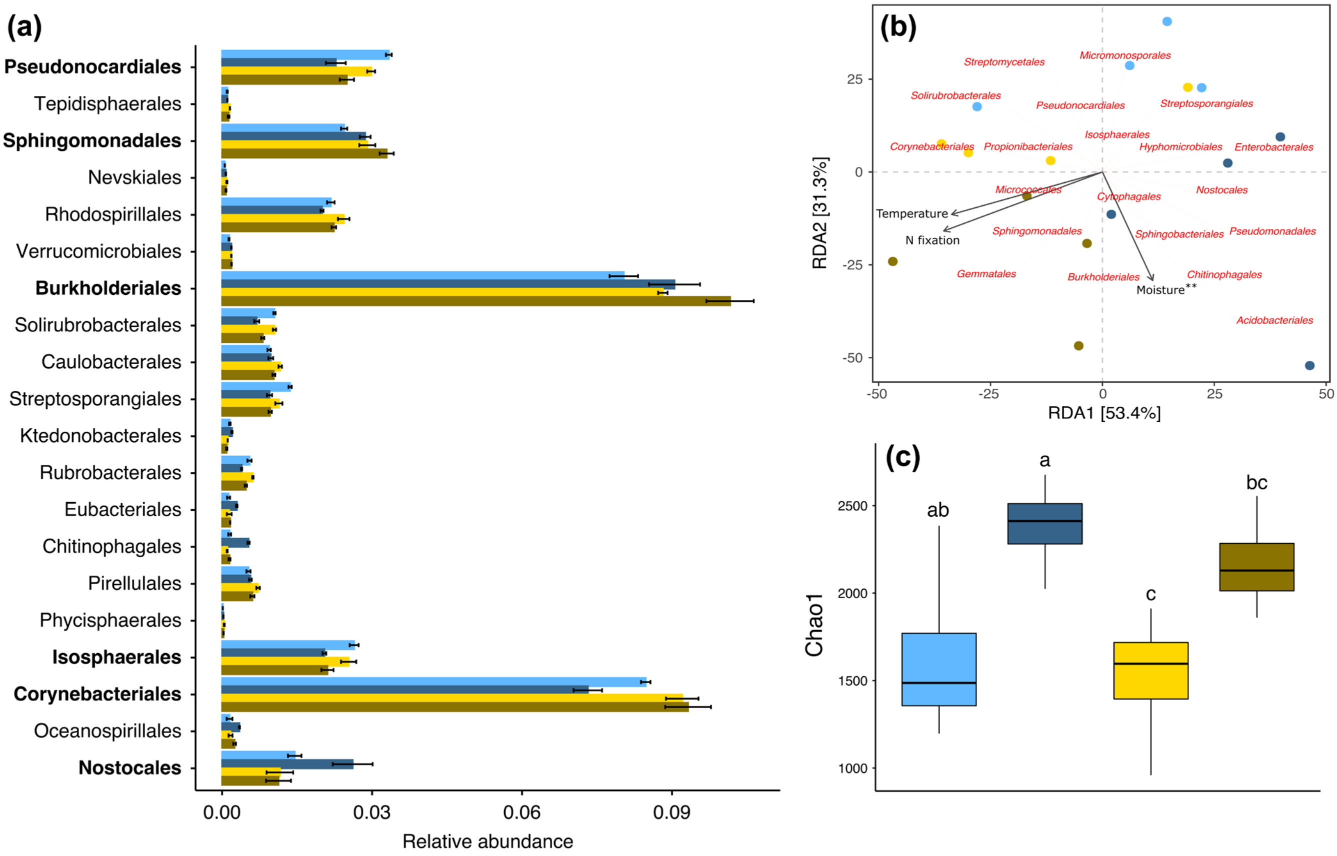

Figure 4. Microbial community composition and richness in a subarctic BSC under different temperature and moisture treatments. Blue and yellow indicate 10 and 20°C, and light and dark tones indicate 70 and 100% moisture content, respectively. (a) Relative read abundances of the 20 most important Orders in our study system, identified by Random Forest classification (Supporting information). Taxa belonging to the 20 most abundant Orders in the BSC are highlighted in bold. (b) dbRDA ordination plots of the community-treatment relationships of microbial communities. The top 20 most abundant Orders are shown in red. The stars ($r = 0.30$, $p < 0.01$) represent significant Pearson correlations between the Bray–Curtis dissimilarity score and moisture content using mantel test. (c) The α-diversity Chao1 richness index across treatments. Different letters above data points indicate statistically significant differences ($p < 0.05$).

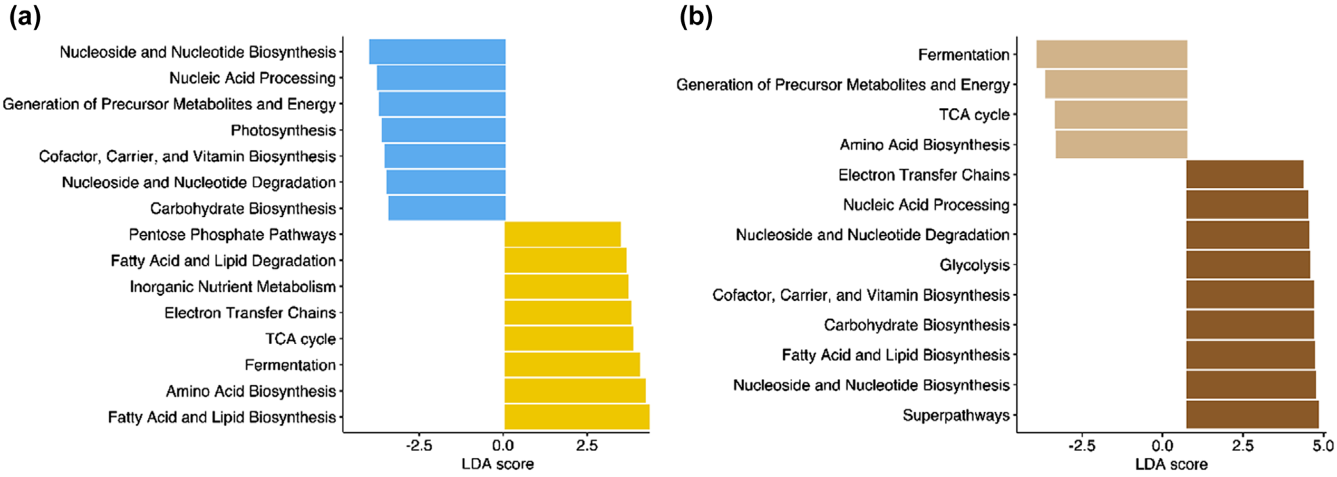

Figure 5. Top 15 MetaCyc pathways to be differentially abundant at (a) temperature (10°C in blue versus 20°C in yellow) and (b) moisture (70% in light brown versus 100% in dark brown). LDA scores were calculated using LEfSe analysis.

and fermentation were more abundant such as the pentose phosphate pathway, TCA cycle and electron transport chain. Pathways associated with fermentation and TCA cycle were found enriched at 75% water content compared to saturated water content which was characterized by an enrichment in cellular respiration related pathways as well as carbohydrate, vitamin, nucleotide, fatty acid and lipid biosynthesis.

Discussion

Climate-driven shifts in the cover, abundance, composition and activity of BSC have the potential to alter the biogeochemistry of terrestrial ecosystems (Elbert et al. 2012, Porada et al. 2014, Weber et al. 2015, Ferrenberg et al. 2017). In hot drylands, warming alone may have little or no effect on the bacterial communities of BSC, wetting alone can increase cyanobacterial abundance, and wetting and warming combined can decrease it (Steven et al. 2015). This shows how bacterial communities from dryland BSC can buffer moderate levels of warming to some extent, but that when combined with altered precipitation the effects of warming can be noticeable (Steven et al. 2015). In our experiment with a BSC adapted to cold and mesic conditions, wetting did not have an effect on the surface cover (a proxy for abundance) of cyanobacteria, and warming increased it. This suggests that the growth of cyanobacteria in our system may be more limited by low temperatures than by lack of moisture. Alternatively, this may reflect the fact that whereas temperatures in our experiment (10–25°C) covered a large part of the range found in nature, both moisture levels (ca 70 and 100%) provided ample biologically-available water. Mean annual temperature and precipitation are projected to increase in the high north in the coming decades (IPCC 2021, chapter 2, p. 316 and chapter 4, p. 638). If the Icelandic BSC used in this study is representative of BSCs from cold and mesic climates in other high latitudes, our results suggest that the abundance of cyanobacteria in this type of BSC could be affected more by warming than by increased precipitation.

The time-scales at which BSC responds to the environment varies with stages of ecological succession (Pushkareva et al. 2017). In the Arctic, temperature and light intensity can more rapidly affect N fixation rates in less-developed BSC dominated by cyanobacteria than in more-developed BSC with dense lichen covers (Pushkareva et al. 2017). The BSC used in this study has the signatures of an early successional stage, including abundant cyanobacteria, being virtually free of lichen patches, having fungal:bacterial ratios closer to those found in deserts than in the tundra (Fierer et al. 2009), and having a mean Chl *a* content closer to those reported for cyanobacterial soil crust than for lichen crusts (Wu et al. 2017). Also, rather than a permanent feature of the ecosystem, the BSC in our study site may be a transient colonizer facilitating the establishment and growth of mosses and vascular plants. Ecosystems dominated by BSC similar to the one used in this study could be particularly sensitive to climate change.

Our correlation analysis suggests that the positive relationship between cyanobacterial cover and N fixation in our study system is in part related to net abundance of cyanobacteria on the soil surface, and in part to the level of their metabolic activity. Between 10 and 20°C, warming increased both cyanobacterial cover and N fixation, suggesting that the increase in N fixation within this temperature range may have been caused by an increase in the number of heterocysts – the N fixing cells of cyanobacteria. If total DNA is assumed to be an indicator of microbial biomass (Semenov et al. 2018, but see Leckie et al. 2004), the previous interpretation is challenged by the lack of difference in total DNA between environmental treatments. However, it is plausible that warming caused a growth of cyanobacteria and a reduction of other microbial groups, resulting in no change in total DNA. A comparison of long-term climatic experiments in drylands in the USA, for example, showed that wetting BSC increased the abundance of cyanobacteria while reducing the abundance of other photosynthetic organisms (Steven et al. 2015).

Alternatively, warming may have altered the microbial composition of the BSC profile, which varies with depth (Maire et al. 2014). Many cyanobacteria commonly found in BSC such as *Microcoleus vaginatus* are motile (Campbell 1980), and can use this motility to reach out for light (Biddanda et al. 2015, Schuergers et al. 2016). In a laboratory experiment with cultured *Oscillatoria*-like cyanobacteria, warming increased the speed at which cells moved towards light (~50 µm min^{-1} at 10°C and ~215 µm min^{-1} at 35°C; Biddanda et al. 2015). An increase in cyanobacterial cover with no change in total DNA could have been caused by a temperature-accelerated migration of cyanobacteria to the BSC surface, where there is more light. There, they could have fixed more N than in deeper and darker BSC layers. Overall, our results suggest that between 10 and 20°C, increases in N fixation rates in our cold- and mesic-adapted BSC were caused by increases in cyanobacterial abundance, migration of cyanobacteria to the BSC surface, or a combination of both.

Between 20 and 25°C, we observed an increase in N fixation rates with no increase in cyanobacterial cover, suggesting that it may have been caused by changes in the metabolic activity of the N fixers. In a semi-arid *Pinus halepensis* plantation, warming did not affect the ratios between major microbial groups in lichen and moss crusts, but it did affect the level of physiological stress of the Gram negative bacterial community, as indicated by phospholipid fatty acid ratios (Maestre et al. 2015). Similarly, in soil from a temperate forest, short-term pulses of microbial respiration were caused by metabolic activation of dormant microbes, with no changes in total microbial biomass (Salazar-Villegas et al. 2016). Our observations suggest that under certain conditions the environment can affect N fixation rates in BSC by altering the metabolic rates of major N fixers, like cyanobacteria, even if there are no changes in their net abundance.

Contrary to previous observations (Staal et al. 2001) and to our hypothesis, we did not find a relationship between N fixation rates and Chl *a*. Moreover, Chl *a* did not vary with the temperature and moisture treatments. As with N fixation at the highest temperature of our experiment, the short-term responses of C fixation to temperature and moisture may be more dependent on the activity than on the abundance of photosynthetic cells and pigments.

Much of the research on the effects of climate change on ecosystem function focuses on temperature and moisture (Zelikova et al. 2012, Hu et al. 2014, Salazar-Villegas et al. 2016, Rousk et al. 2018). The comparatively lower number of studies that have included light intensity have found, as in our own study, clear interactions between light, temperature and moisture on the cycling of elements through BSC (Lange et al. 1998, Grote et al. 2010). Although our experiment only captures two levels of light intensity, it shows a quantitative relationship that, if assumed or proved linear, could be used to estimate N fixation at a range of light (and temperature and moisture) conditions representative of natural daily and seasonal variations. Our findings suggest that the projected decreases in downward solar radiation in the high north by 2100 (as much as -10 W m^{-2} relative to the reference period of 1986–2005, under the RCP4.5 scenario; KNMI 2021), could reduce N fixation rates in cold-adapted BSCs.

The ways in which BSC interacts with the environment largely depends on its community composition (Belnap 2002a, Bowker et al. 2021). On one hand, the rates at which BSC assimilates C and N depend on the abundance, type and activity of species in the system. BSCs dominated by *Collema* soil lichens, for example, has higher levels of nitrogenase activity and therefore can fix more N than BSCs dominated by *Microcoleus vaginatus* (Belnap 2002a). On the other hand, the capacity of a BSC to resist environmental change depends on its community composition (Bowker et al. 2021). The presence of the lichens *Enchylium* and *Peltula* can increase the capacity of BSC to resist stress caused by wetting pulses at supra-optimal temperatures (Bowker et al. 2021).

Although the BSC in our study has commonalities in structure, composition and function to BSC from analog cold environments in the Arctic (Pushkareva et al. 2016) and in other locations in Iceland (Pushkareva et al. 2021), such as a high presence of cyanobacteria (e.g. Nostocales sp.); it also has distinctive compositional features, such as being dominated by *Anthelia juratzkana*, at the macroscopic scale, and by *Burkholderiales* and *Corynebacteriales*, at the microscopic scale. The main microbial phototrophs found in our study are well known N and C fixers in BSC, and liverworts like *Anthelia* are known for surviving in harsh environments, in part because of the protection of leaf surface waxes (Heinrichs et al. 2000). However, the functional roles of the diversity of taxonomic groups in cold-adapted BSC are yet to be studied in detail.

It is well known that warming and moisture can induce significant compositional and structural changes in the bacterial communities of biocrust (Garcia-Pichel et al. 2013, Steven et al. 2015, Delgado-Baquerizo et al. 2018), with associated effects on bacterial functions (Steven et al. 2015). This can happen, for example, by environmental change differentially altering the growth rate and survival of different taxonomic groups (Lürling et al. 2013, Colesie et al. 2014). Our pCoA analysis (Fig. 4) indicates that the treatments drastically influenced the community composition in our study system, and samples that underwent the same treatment tend to have a more similar microbial community composition. This means that before the incubations, samples had either a very similar community or a dissimilar community that was then shaped to a similar one due to treatment. The most parsimonious hypothesis is that the microbial communities before treatment were very similar. Overall, our results support the idea that warming can exert a selective pressure on warm-adapted microbial groups over those adapted to colder regimes (Muñoz-Martín et al. 2018). Moreover, our findings show that this selective pressure can manifest within a few days after environmental change. This was observed in our metagenomic investigations where photosynthesis-related genes are enriched at 10°C but not at 20°C, which corresponds to a decreased relative abundance of Nostocales. In addition, pathways related to a heterotrophic lifestyle are enriched at higher temperature and moisture, which correspond to a shift in the community composition with Alphaproteobacteria (e.g. Burkholderiales and Sphingomonadales) becoming more dominant in those conditions. In a broader sense, this suggests that climate change could lead to a replacement of the photosynthetic and N fixing Nostocales by non-photosynthetic N fixing bacteria. Such alteration could affect photosynthetic activity supported by cold adapted biocrust, and thus C cycling, as well as N cycling. It remains to be studied if and how an environment-driven exchange of species in cold-adapted BSC could result in an acclimation of N fixation rates or other biogeochemical processes (Colesie et al. 2014). A deeper understanding of the contribution of microbial community composition to N fixation activity appears to be critical to predicting the potential effects of projected climates (IPCC 2021, chapter 2, p. 316 and chapter 4, p. 638) on the productivity of cold biocrusts and the ways it interacts with its natural surroundings.

Short-term ecosystem responses to the environment may not necessarily reflect long-term trends (Alatalo et al. 2015). More multi-year, in situ experiments are needed at high latitudes (Rousk et al. 2018, Salazar et al. in progress) to better understand the effects of climate change on cold- and mesic-adapted BSC over large spatial and temporal scales. However, our short-term laboratory experiment 1) highlights direct and interacting effects of environmental factors on BSC composition and functioning, which could be useful to inform the structure and/or parameterizarion of ecosystem models that explicitly take into account the inherent dynamics of BSC (Rodríguez-Caballero et al. 2015); 2) provides data that can help with BSC characterization (Pietrasiak et al. 2013), which could further serve as reference when assessing the impacts of human disturbances on BSC (Szyja et al. 2018) and/or with the design and implementation of BSC-based restoration practices on degraded land (Bowker 2007, Velasco-Ayuso et al. 2017, Tucker et al. 2020); and 3) provides quantitative data on the N fixation responses of BSC to short-term environmental variations, such as diurnal cycles or short-term climatic anomalies like the increasingly frequent and intense heat waves.

Speculations

BSCs around the globe are highly diverse in their physical structure, function and taxonomy – so much that even the definition of what BSC is, and what it is not, can be tricky. However, a refined definition of BSC has been developed over the years (Weber et al. 2022). This has allowed, for example, grouping BSC types depending on their habitat. For understanding how the different types of BSC respond to climate change, we speculate that perhaps the most important distinction to make is between BSCs adapted to warm drylands, and those adapted to cold, mesic environments. The two types of BSC are being affected by climate change in fundamentally different ways. Rising temperatures are pushing warm-adapted BSCs out of their fundamental niche; while it may be affecting cold-adapted BSCs both positively via increases in metabolic rates, and negatively via out-competition by plants, especially in mesic environments. Cold-adapted BSCs seem to cover areas much larger than generally considered in the scientific literature (Pushkareva et al. 2016). Studying this overlooked system, with emphasis on its responses to the environment, could modify our global view on how BSCs are responding to climate change.

Alternative views

Although we discuss how increases in N-fixation appear to be correlated with changes in cyanobacterial abundance on the BSC surface, Andrésson believes that increases in N-fixation might also have been largely due to increases in activity of major N-fixing taxa, e.g. the Rhizobiales. Warshan thinks that this calls for the use of methods that investigate the physiological response of microbes at lower scale, such as NanoSIMS (nanoscale secondary ion mass spectrometry), otherwise we will remain with correlations. Studying the role of abiotic factors (temperature, light, etc.) together with biotic interactions (microbe–microbe and microbe–eukaryote interactions) is of crucial importance to be able to understand BSC functioning. Further studies are called for to clarify this issue.

Acknowledgements – We thank Katrín Halldórsdóttir for her help with soil DNA extractions.
Funding – Most AS work, including data collection and analysis, was done when working as a postdoc at the University of Iceland, under the Icelandic Research Fund 2016 grant no. 163336.

Author contributions

Alejandro Salazar: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Denis Warshan**: Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Visualization (supporting); Writing – review and editing (supporting). **Clara Vásquez**: Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Visualization (supporting); Writing – review and editing (supporting). **Ólafur S. Andrésson**: Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Writing – review and editing (supporting).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.x95x69pmw> (Salazar et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Aguilar, A. J. et al. 2009. Biological soil crusts exhibit a dynamic response to seasonal rain and release from grazing with implications for soil stability. – J. Arid Environ. 73: 1158–1169.

Alatalo, J. M. et al. 2015. Testing reliability of short-term responses to predict longer-term responses of bryophytes and lichens to environmental change. – Ecol. Indic. 58: 77–85.

Aradottir, A. L. and Halldorsson, G. 2018. Colonization of woodland species during restoration: seed or safe site limitation? – Restor. Ecol. 26: S73–S83.

Barrera, A. et al. 2022. Biological soil crusts as ecosystem engineers in antarctic ecosystem. – Front. Microbiol. 13: 755014.

Beghini, F. et al. 2021. Integrating taxonomic, functional and strain-level profiling of diverse microbial communities with bioBakery 3. – eLife 10: e65088.

Belnap, J. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. – In: Belnap, J. and Lange, O. (eds), Biological soil crusts: structure, function and management. Springer, pp. 241–261.

Belnap, J. 2002a. Nitrogen fixation in biological soil crusts from southeast Utah, USA. – Biol. Fertil. Soils 35: 128–135.

Belnap, J. 2002b. Impacts of off-road vehicles on nitrogen cycles in biological soil crusts: resistance in different US deserts. – J. Arid Environ. 52: 155–165.

Belnap, J. 2003. The world at your feet: desert biological soil crusts. – Front. Ecol. Environ. 1: 181–189.

Biddanda, B. A. et al. 2015. Seeking sunlight: rapid phototactic motility of filamentous mat-forming cyanobacteria optimize photosynthesis and enhance carbon burial in Lake Huron's submerged sinkholes. – Front. Microbiol. 6: 930.

Blay, E. S. et al. 2017. Variation in biological soil crust bacterial abundance and diversity as a function of climate in cold steppe ecosystems in the Intermountain West, USA. – Microb. Ecol. 74: 691–700.

Borchhardt, N. et al. 2019. Soil microbial phosphorus turnover and identity of algae and fungi in biological soil crusts along a transect in a glacier foreland. – Eur. J. Soil Biol. 91: 9–17.

Bowker, M. A. 2007. Biological soil crust rehabilitation in theory and practice: an underexploited opportunity. – Restor. Ecol. 15: 13–23.

Bowker, M. A. et al. 2014. Biological soil crusts (biocrusts) as a model system in community, landscape and ecosystem ecology. – Biodivers. Conserv. 23: 1619–1637.

Bowker, M. A. et al. 2018. Biocrusts: the living skin of the earth. – Plant Soil 429: 1–7.

Bowker, M. A. et al. 2021. Community composition influences ecosystem resistance and production more than species richness or intraspecific diversity. – Oikos 130: 1399–1410.

Breen, K. and Lévesque, E. 2008. The influence of biological soil crusts on soil characteristics along a High Arctic glacier foreland, Nunavut, Canada. – Arctic Antarctic Alpine Res. 40: 287–297.

Caesar, J. et al. 2018. Revisiting chlorophyll extraction methods in biological soil crusts–methodology for determination of chlorophyll a and chlorophyll a+ b as compared to previous methods. – Biogeosciences 15: 1415–1424.

Campbell, S. 1980. Soil stabilization by a prokaryotic desert crust: implications for Precambrian land biota. – In: Ponnamperuma, C. and Margulis, L. (eds), Limits of life. Springer, pp. 85–98.

Colesie, C. et al. 2014. Habitat stress initiates changes in composition, CO_2 gas exchange and C-allocation as life traits in biological soil crusts. – ISME J. 8: 2104–2115.

Collins, T. J. 2007. ImageJ for microscopy. – Biotechniques 43 Supplement: S25–S30.

Delgado-Baquerizo, M. et al. 2018. Biocrust-forming mosses mitigate the impact of aridity on soil microbial communities in drylands: observational evidence from three continents. – New Phytol. 220: 824–835.

Dickson, L. G. 2000. Constraints to nitrogen fixation by cryptogamic crusts in a polar desert ecosystem, Devon Island, Nwt, Canada. – Arctic Antarctic Alpine Res. 32: 40–45.

Elbert, W. et al. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. – Nat. Geosci. 5: 459.

Faist, A. M. et al. 2020. Inoculation and habitat amelioration efforts in biological soil crust recovery vary by desert and soil texture. – Restor. Ecol. 28: S96–S105.

Ferrenberg, S. et al. 2015. Climate change and physical disturbance cause similar community shifts in biological soil crusts. – Proc. Natl Acad. Sci. USA 112: 12116–12121.

Ferrenberg, S. et al. 2017. Biological soil crusts: diminutive communities of potential global importance. – Front. Ecol. Environ. 15: 160–167.

Finger-Higgens, R. et al. 2022. Decline in biological soil crust N-fixing lichens linked to increasing summertime temperatures. – Proc. Natl Acad. Sci. USA 119: e2120975119.

Gao, L. et al. 2017. Biological soil crusts decrease erodibility by modifying inherent soil properties on the Loess Plateau, China. – Soil Biol. Biochem. 105: 49–58.

Garcia-Pichel, F. et al. 2013. Temperature drives the continental-scale distribution of key microbes in topsoil communities. – Science 340: 1574–1577.

Godínez-Alvarez, H. et al. 2012. Germination, survival and growth of three vascular plants on biological soil crusts from a Mexican tropical desert. – Plant Biol. 14: 157–162.

Grote, E. E. et al. 2010. Carbon exchange in biological soil crust communities under differential temperatures and soil water contents: implications for global change. – Global Change Biol. 16: 2763–2774.

Guo, J. et al. 2015. Microbial community analysis with ribosomal gene fragments from shotgun metagenomes. – Appl. Environ. Microbiol. 82: 157–166.

Hardy, R. W. et al. 1968. The acetylene–ethylene assay for N_2 fixation: laboratory and field evaluation. – Plant Physiol. 43: 1185–1207.

Havrilla, C. A. et al. 2019. Towards a predictive framework for biocrust mediation of plant performance: a meta-analysis. – J. Ecol. 107: 2789–2807.

Heinrichs, J. et al. 2000. Surface wax, a new taxonomic feature in Plagiochilaceae. – Plant Syst. Evol. 225: 225–233.

Hu, R. et al. 2014. The response mechanisms of soil N mineralization under biological soil crusts to temperature and moisture in temperate desert regions. – Eur. J. Soil Biol. 62: 66–73.

IPCC 2021. Climate Change 2021: the physical science basis. – In: Masson-Delmotte, V. et al. (eds), Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. Cambridge Univ. Press.

Jónsdóttir, I. S. et al. 2005. Variable sensitivity of plant communities in Iceland to experimental warming. – Global Change Biol. 11: 553–563.

Kanehisa, M. and Goto, S. 2000. KEGG: kyoto encyclopedia of genes and genomes. – Nucleic Acids Res. 28: 27–30.

Karp, P. D. et al. 2019. The BioCyc collection of microbial genomes and metabolic pathways. – Brief. Bioinform. 20: 1085–1093.

KNMI 2021. KNMI Climate Change Atlas. – <https://climexp.knmi.nl/plot_atlas_form.py>, retrieved on 11 October 2021.

Lan, S. et al. 2019. Small-scale spatial heterogeneity of photosynthetic fluorescence associated with biological soil crust succession in the Tengger Desert, China. – Microb. Ecol. 78: 936–948.

Lange, O. L. et al. 1998. Photosynthesis of the cyanobacterial soil-crust lichen Collema tenax from arid lands in southern Utah, USA: role of water content on light and temperature responses of CO_2 exchange. – Funct. Ecol. 12: 195–202.

Leckie, S. E. et al. 2004. Comparison of chloroform fumigation–extraction, phospholipid fatty acid and DNA methods to determine microbial biomass in forest humus. – Soil Biol. Biochem. 36: 529–532.

Maestre, F. T. et al. 2016. Biological soil crusts as a model system in ecology. – In: Weber, B. et al. (eds), Biological soil crusts: an organizing principle in drylands. Springer, pp. 407–425.

Menzel, P. et al. 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. – Nat. Commun. 7: 11257.

Novakovskaya, I. V. et al. 2022. Distribution of algae and cyanobacteria of biological soil crusts along the elevation gradient in mountain plant communities at the Northern Urals (Russian European Northeast). – J. Mount. Sci. 19: 637–646.

Perkins, S. E. et al. 2012. Increasing frequency, intensity and duration of observed global heatwaves and warm spells. – Geophys. Res. Lett. 39: 1–5.

Pietrasiak, N. et al. 2013. Biological soil crust community types differ in key ecological functions. – Soil Biol. Biochem. 65: 168–171.

Porada, P. et al. 2014. Estimating impacts of lichens and bryophytes on global biogeochemical cycles. – Global Biogeochem. Cycles 28: 71–85.

Pushkareva, E. et al. 2016. A review of the ecology, ecophysiology and biodiversity of microalgae in Arctic soil crusts. – Polar Biol. 39: 2227–2240.

Pushkareva, E. et al. 2017. Nitrogen fixation and diurnal changes of photosynthetic activity in Arctic soil crusts at different development stage. – Eur. J. Soil Biol. 79: 21–30.

Pushkareva, E. et al. 2021. Diversity of microbial phototrophs and heterotrophs in Icelandic biocrusts and their role in phosphorus-rich Andosols. – Geoderma 386: 114905.

Rodríguez-Caballero, E. et al. 2015. Biological soil crust effects must be included to accurately model infiltration and erosion in drylands: an example from Tabernas Badlands. – Geomorphology 241: 331–342.

Rodriguez-Caballero, E. et al. 2018. Dryland photoautotrophic soil surface communities endangered by global change. – Nat. Geosci. 11: 185.

Rousk, K. et al. 2018. What drives biological nitrogen fixation in high arctic tundra: moisture or temperature? – Ecosphere 9: e02117.

Salazar, A. et al. 2022. Data from: Environmental change alters nitrogen fixation rates and microbial parameters in a subarctic biological soil crust. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.x95x69pmw>.

Salazar-Villegas, A. et al. 2016. Changes in the size of the active microbial pool explain short-term soil respiratory responses to temperature and moisture. – Front. Microbiol. 7: 524.

Scherer, S. et al. 1988. Interaction of photosynthesis, respiration and nitrogen fixation in cyanobacteria. – Photosynth. Res. 15: 95–114.

Schindelin, J. et al. 2012. Fiji: an open-source platform for biological-image analysis. – Nat. Methods 9: 676.

Schuergers, N. et al. 2016. Cyanobacteria use micro-optics to sense light direction. – eLife 5: e12620.

Semenov, M. et al. 2018. DNA-based determination of soil microbial biomass in alkaline and carbonaceous soils of semi-arid climate. – J. Arid Environ. 150: 54–61.

Staal, M. et al. 2001. Nitrogenase activity in cyanobacteria measured by the acetylene reduction assay: a comparison between batch incubation and on-line monitoring. – Environ. Microbiol. 3: 343–351.

Steven, B. et al. 2012. Dryland biological soil crust cyanobacteria show unexpected decreases in abundance under long-term elevated CO_2. – Environ. Microbiol. 14: 3247–3258.

Steven, B. et al. 2013. High bacterial diversity of biological soil crusts in water tracks over permafrost in the high arctic polar desert. – PLoS One 8: e71489.

Steven, B. et al. 2015. Climate change and physical disturbance manipulations result in distinct biological soil crust communities. – Appl. Environ. Microbiol. 81: 7448–7459.

Stewart, K. J. et al. 2011. Bryophyte-cyanobacterial associations as a key factor in N_2-fixation across the Canadian Arctic. – Plant Soil 344: 335–346.

Suzek, B. E. et al. 2015. UniRef clusters: a comprehensive and scalable alternative for improving sequence similarity searches. – Bioinformatics 31: 926–932.

Tucker, C. et al. 2020. Biological soil crust salvage for dryland restoration: an opportunity for natural resource restoration. – Restor. Ecol. 28: S9–S16.

Williams, L. et al. 2017. Biological soil crusts of Arctic Svalbard and of Livingston Island, Antarctica. – Polar Biol. 40: 399–411.

Wood, D. E. et al. 2019. Improved metagenomic analysis with Kraken 2. – Gen. Biol. 20: 1–13.

Xu, H. et al. 2022. Soil nitrogen and climate drive the positive effect of biological soil crusts on soil organic carbon sequestration in drylands: a meta-analysis. – Sci. Total Environ. 803: 150030.

Yan-Gui, S. et al. 2011. Carbon fixation of cyanobacterial–algal crusts after desert fixation and its implication to soil organic carbon accumulation in desert. – Land Degrad. Devel. 24: 342–349.

Yoshitake, S. et al. 2018. Soil microbial succession along a chronosequence on a High Arctic glacier foreland, Ny-Ålesund, Svalbard: 10 years' change. – Polar Sci. 16: 59–67.

Zelikova, T. J. et al. 2012. Warming and increased precipitation frequency on the Colorado Plateau: implications for biological soil crusts and soil processes. – Plant Soil 355: 265–282.

Zhao, L. et al. 2020. Bacteria and fungi differentially contribute to carbon and nitrogen cycles during biological soil crust succession in arid ecosystems. – Plant Soil 447: 379–392.