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

3

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9

10 **Abstract**

11

12 Together, Biological Soil Crust (BSC) and other cryptogamic groundcovers can contribute
13 up to half of the global nitrogen (N) fixation. BSC also stabilizes the soil (reducing erosion
14 and dust emissions), fixes carbon (C), retains moisture, and acts as a hotspot of microbial
15 diversity and activity. Much of the knowledge about how climate change is affecting the
16 composition and functioning of BSC comes from hot arid and semiarid regions. The
17 comparatively smaller body of research on BSC from cold and mesic environments has
18 been primarily observational, for example along chronosequences after a glacier retreat.
19 Few studies have experimentally investigated the effects of the environment on BSC from
20 high latitudes. Such experiments allow unraveling of relationships at a resolution that can
21 only be achieved by controlling for confounding factors. We measured short-term (2-4
22 days) responses of a liverwort-based (*Anthelia juratzkana*) BSC from the south of Iceland
23 to a range of temperature, moisture and light conditions. Warming increased N fixation

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24 rates, especially when moisture was at a saturation level, and only when light was not
25 limiting. A correlation analysis suggests that increases in N fixation rates were linked to
26 cyanobacterial abundance on the BSC surface and to the rates of their metabolic activity.
27 Warming and moisture changes also induced compositional and structural modification
28 of the bacterial community, with consequences at the functional level. In contrast to many
29 observations on BSC from hot drylands, the BSC from our cold and mesic study site is
30 more limited by low temperature and light than by moisture. Our findings show possible
31 ways in which BSC from cold and mesic ecosystems can respond to short-term
32 manifestations of climate change, such as increasingly frequent heat waves.

33

34

35 **Keywords:** Biological Soil Crust (BSC), cyanobacteria, nitrogen (N) fixation, climate
36 change, subarctic ecosystems, cyanobacterial abundance and composition.

37

38 ## 1. Introduction

39

40 Biological Soil Crust (BSC) is a skin-like system generally dominated by
41 cyanobacteria, fungi, lichens and bryophytes (Belnap, 2003; Bowker et al., 2018). In some
42 areas this system can cover over 90% of the soil surface (Williams et al., 2017). BSC-
43 forming organisms can colonize soils in harsh environments, such as drylands (Belnap,
44 2003; Elbert et al., 2012; Rodríguez-Caballero et al., 2018) or newly exposed soil after a
45 glacier retreat (Breen and Lévesque, 2008; Yoshitake et al., 2018). Once they establish,
46 they stabilize the soil (Gao et al., 2017), increase moisture retention (Breen and

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47 Lévesque, 2008), fix nitrogen (N) (Dickson 2000; Elbert et al., 2012) and carbon (C) (Yan-
48 Gui et al., 2011; Elbert et al., 2012), and become hotspots of microbial abundance
49 (Yoshitake et al., 2018) and diversity (Steven et al., 2013). Under constant high
50 environmental stress, BSC becomes a permanent feature of the undisturbed ecosystem.
51 When high abiotic stress decreases over time, BSC acts like a transient colonizer in
52 primary succession (Bowker, 2007).

53 BSC covers 12% of the Earth's terrestrial surface (Rodríguez-Caballero et al.,
54 2018). It is estimated that before the end of the century climate change and intensification
55 of land use will decrease this area by 25-40%, thus enhancing emissions of soil dust and
56 reducing BSC's contributions to the global C and N cycles (Rodríguez-Caballero et al.,
57 2018).

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

68 Cold-adapted BSC covers vast areas at high latitudes (Pushkareva et al., 2016),
69 the part of the world experiencing the fastest warming (IPCC, 2021). One of the most

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70 visual and consequential manifestations of climate change in these regions and globally
71 is the increase in frequency and intensity of short-term climatic anomalies such as heat
72 waves (Perkins et al., 2012). Some high-latitude systems are more resistant to climatic
73 anomalies than others (Jónsdóttir et al., 2005). Because of its relative simplicity and the
74 fast turnover rates of its biological componentes, BSC may be particularly sensitive to
75 short-term changes in the environment.

76 The potential effects of climate change on cold-adapted BSC, and on the
77 ecosystem services it provides, are still largely unknown. Most of the knowledge on BSC
78 responses to the environment comes from hot arid or semiarid ecosystems (e.g. Eldridge
79 et al., 2006; Bowker et al., 2008; Büdel et al., 2009; Delgado-Baquerizo et al., 2013; Zhao
80 et al., 2020). The comparatively smaller body of research on BSC adapted to cold and
81 mesic conditions has been primarily observational, for example along chronosequences
82 after a glacier retreat (Breen and Lévesque, 2008; Yoshitake et al., 2010, 2018;
83 Borchhardt et al., 2019) or along climatic gradients (Stewart et al., 2011; Blay et al., 2017;
84 Pushkareva et al. 2021). While observational approaches are useful to study ecosystem
85 processes under natural conditions, experiments allow study of relationships at a
86 resolution that can only be achieved by controlling for confounding factors. Only a handful
87 of studies have used an experimental approach to investigate the effects of the
88 environment on cold-adapted BSC (e.g. Colesie et al., 2014; Alatalo et al., 2015; Rousk
89 et al., 2018).

90 From a methodological perspective, experimental research on BSC offers many
91 advantages (Bowker et al., 2014; Maestre et al., 2016). Because of its size, it is possible
92 to collect entire BSC blocks and study them as closed systems, even under laboratory

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93 conditions (Figure S1). Also, because of the small size and relatively fast turnover rates
94 of BSC organisms, it can respond rapidly to external factors. This has advantages for
95 early detection of environmental changes. Finally, since BSC can act as the first link in a
96 chain of ecological succession (Godínez- Alvarez et al., 2011), understanding BSC
97 responses to the environment not only provides information about the BSC itself, but also
98 about the potential ecosystems and ecological dynamics that can evolve from it.

99 Here we conducted a laboratory experiment to study the compositional and
100 functional responses of a cold- and mesic-adapted BSC to short-term (2-4 days)
101 incubations at a range of temperature, moisture and light intensities. The ranges of
102 temperature varied between average and maximum values during the snow-free season
103 in an Icelandic ecosystem dominated by BSC. Unless limited by other biotic and/or abiotic
104 factors, we expected warming to increase N fixation rates either via growth of N fixers, or
105 via increases in their metabolic activity, or both. Also, we expected environmental
106 treatments to exert a selective pressure, leading to structural and possibly compositional
107 changes within the N fixing communities (e.g. favoring warm adapted taxa); and for this
108 to contribute to explaining N fixation responses to the environment. Given the well known
109 dependence of biological N fixation on moisture (Rousk et al., 2018), and the need of
110 photosynthetically synthesized C to run the N fixing machinery (Scherer et al., 1988), we
111 expected the effects of warming on N fixation to be directly dependent on moisture and
112 light intensity. Also, we hypothesized a positive correlation between N fixing rates and
113 chlorophyll *a* (Chl *a*) content - an indicator of net photosynthetic rate (Yan-Gui et al.,
114 2011). Overall, we expected subarctic BSC to respond to short-term environmental
115 manipulations and for these responses to indicate possible ways in which this system,

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116 and the key ecosystem services it provides, could be altered by climate anomalies like
117 increasingly frequent heat waves (Perkins et al., 2012).
118

119 2. Materials and methods

120 We designed a controlled laboratory experiment to investigate the responses of
121 subarctic BSC from the south of Iceland to different levels of temperature, moisture and
122 light. We studied how these environmental factors affect the capacity of subarctic BSC to
123 fix N, and whether these responses were linked to changes in the abundance of N fixers
124 and/or to structural changes in the BSC microbial communities.
125

126 2.1. Sample collection

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

133 We randomly collected eight BSC blocks (i.e. replicates) of 13x16 cm^2 and ca. 5
134 cm deep (Figure S1a). Blocks were separated by at least 10 meters. Since the focus of
135 this study is on BSC, patches of moss or vascular plants were avoided. We transported
136 (approx. 5 h) the BSC blocks in coolers with ice packs and stored them in a dark room at
137 5 °C for 2 to 5 weeks while we performed the analyses described below. We kept wet
138 paper towels inside the coolers to prevent desiccation.

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139

140 **2.2. Experimental design and environmental treatments**

141 We studied the effects of temperature, moisture and light on N fixation and the
142 microbial community structure. For this, we conducted a factorial experiment (4 x 2 x 2)
143 with four levels of temperature: 10, 15, 20 and 25 °C; two levels of moisture: ca. 75%
144 (close to moisture at the moment of sampling) and 100% (saturated); and two levels of
145 light ca. 2 µmol m^{-2} s^{-1} (low intensity) and ca. 90 µmol m^{-2} s^{-1} (high intensity; Figure S2).
146 Light was available all the time (i.e. we did not set day/night cycles), to simulate conditions
147 in the sampling site during the summer. Temperature and light treatments were set in a
148 growth chamber (Termaks series 8000, Bergen, Norway), and monitored hourly with
149 temperature/light loggers (HOBO Pendant® MX Temperature/Light Data Logger,
150 MX2202, Onset, Bourne, MA, USA). Levels of these environmental variables were
151 selected within ranges commonly experienced by BSC at the sampling site (unpublished
152 observations) and comparable ecosystems (Rousk et al., 2018).

153 Average temperature and light intensity inside the jars were 11.1 ± 0.7, 16.5 ± 0.7,
154 21.5 ± 0.7 and 26.6 ± 0.9 °C (2 loggers x 2 light levels; n = 4) and 2.3 ± 0.04 and 88.0 ±
155 1.6 µmol m^{-2} s^{-1} (2 loggers x 4 temperature levels; n = 8) respectively (Figure S2a and b).
156 Temperature levels inside the jars were slightly higher than temperatures set in the
157 growing chamber due to a greenhouse effect.

158 To create a saturation level in the moisture treatment, we wetted each sample with
159 an excess of deionized water and waited for approximately one minute until it stopped
160 dripping. Moisture was maintained between analyses by placing wet towels in the coolers
161 stored in the cold, dark room. After environmental treatments and N fixation

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162 measurements (see following section), we oven dried (60 °C, 24 h) BSC disks to estimate
163 the dried weight of the samples, and to prepare them for chlorophyll *a* analysis and DNA
164 extraction. Average moisture content was 75.5 ± 2.4 and 107.2 ± 2.3 % (Figure S3c).
165

166 **2.3. N fixation under controlled temperature, moisture and light conditions**

167 We estimated N fixation rates with the Acetylene Reduction Assay (ARA; Hardy
168 et al., 1968). We subsampled disks of 5 cm diameter and 1.5 cm depth (Figure S1c) out
169 of the 13x16x5 cm^3 BSC blocks (Figure S1a). We used eight 5 cm-diameter disks (i.e.
170 replicates) per combination of temperature and moisture treatments. Thus, each
171 temperature-specific ARA analysis was composed of a total of 16 samples with two levels
172 of moisture, eight saturated and eight unsaturated, plus controls with acetylene, ethylene
173 and air. The BSC disks were weighed (for further water content analysis) and placed in
174 350 mL glass jars with rubber septa in the lids (Figure S1c). These jars were then placed
175 in an environmental chamber (Termaks series 8000, Bergen, Norway) at fixed
176 temperature and light conditions. We acclimated the samples to each combination of
177 temperature and light for 24 h. We then manually aerated the jars for a few seconds,
178 closed the jars tightly and replaced 10% of the headspace with acetylene (except in jars
179 used as ethylene and air controls). We incubated the jars at the set temperature and light
180 conditions for 24 h. Then, we collected 22 mL of gas from each jar and analyzed it using
181 a Clarus 400 gas chromatograph (PerkinElmer Ltd., Beaconsfield, UK) equipped with an
182 automatic split/splitless injector and a flame ionization detector (FID), and an Elite-
183 Alumina column (30 m, 0.53 mm; PerkinElmer Ltd., Beaconsfield, UK).

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184 At the end of each 48 h acclimation-incubation period, we manually aerated the
185 samples and started a new acclimation-incubation at a different light (but same
186 temperature) condition. To control for a possible cumulative effect between light levels,
187 we switched the order of the light levels for each temperature treatment i.e. half of the
188 times starting with low light, and the other half starting with bright light.
189

190 **2.4. Cyanobacteria and liverwort cover on BSC**

191 We estimated the cover of cyanobacteria and liverwort (*Anthelia juratzkana*; Figure
192 S1b) on the BSC surface by epifluorescence microscopy (Figure S3). After ARA
193 measurements, BSC samples were stored in a dark room at 5 °C for 1 to 4 days. Plant
194 and cyanobacterial growth was assumed to be minimal under these conditions. From
195 each 5 cm diameter BSC disk (Figure S1c), we subsampled a 1.5 cm diameter BSC disk
196 and imaged the plant (liverwort) chlorophyll using a Leica DM6000B fluorescent
197 microscope (Leica, Heerbrugg Switzerland) equipped with an I3 filter cube (Ex 450/90, Di
198 510, Em 515), and the cyanobacterial phycocyanin with a TX2 filter cube (Ex 560/40, Di
199 595, Em 630/30). Multiple fields of view were measured using both filter cubes and
200 stitched together to form an image of 1x1 cm of BCS surface (Figure S3) using the Leica
201 software. Images were analyzed in ImageJ/Fiji (Collins, 2007; Schindelin et al., 2012),
202 and estimates of cyanobacterial and plant covers calculated as percentage of BSC
203 surface cover.

204 We did not subsample BSC disks between light levels, but rather used samples
205 that were exposed to low light for 48 h (24 h acclimation plus 24 h ARA) and then to high

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206 light for another 48 h, or vice versa. Therefore, the treatments in this part of our analysis
207 include temperature and moisture, but not light.
208
209 **2.5. Chlorophyll *a***
210 We estimated Chl *a* content as an indicator of net photosynthetic rate in BSC (Yan-
211 Gui et al., 2011). Similar to our BSC cover analysis, we subsampled a 1.5 cm diameter
212 BSC disk from each 5 cm diameter BSC disk (Figure S1c) used for ARA analysis. We
213 dried subsamples at 60 °C for 24 h, extracted Chl *a* using DMSO (65 °C, 90 min) and
214 then estimated Chl *a* content by spectrophotometry (665 and 750 nm; Genesys 20,
215 Thermo Scientific, Waltham, MA), as in Caesar et al. (2018):
216
217 $$\text{Chl } a \text{ µg} = (11.9035 \times (A665 - A750)) \times S \quad (1)$$
218 $$\text{Chl } a\ [\text{mg} \times \text{m}^{-2}] = \text{Chl } a\ [\text{µg}] / (AR \times 1000) \quad (2)$$
219
220 Where S is volume of solvent, AR is area (in m^{-2}) and A665 and A750 are
221 absorbances at 665 and 750 nm, respectively.
222 As for BSC cover, treatments in this part of our analysis included temperature and
223 moisture, but not light.
224
225 **2.6. DNA extraction and analysis**
226 Immediately after the fluorescence microscopy measurements (section 2.4), we
227 dried (60 °C, 24 h) and ground (1 min, Mini bead beater 16; Biospec products) the 1.5
228 BSC disks used for the cyanobacteria/liverwort cover analysis and stored them at -80 °C

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229 for up to four months for DNA extraction. We pooled together replicates in pairs,
230 combining them in equal weight parts (125 mg each for a total of 250 mg). We used the
231 PowerSoil® DNA extraction kit (MOBIO/Qiagen), and shotgun sequencing approaches
232 and analyses via the alignment-free fast taxonomic annotation tool Kraken2 (Wood and
233 Langmead, 2019) with the Kraken2 Refseq Standard plus protozoa and fungi database
234 and the web-based pipeline Kaiju (Menzel et al., 2016). We estimated relative abundance
235 of microbial groups using Kraken2 and fungal:bacteria ratios based on Kaiju taxonomic
236 assignments (see sections below). After quality filtering the raw reads using Trim Galore
237 microbial metagenome functional profiling was performed using HUMAnN 3 (Beghiji et
238 al., 2021). For the functional annotation, UniRef50 (Suzek et al., 2015), KEGG (Kanehisa
239 and Goto, 2000), and BioCyc databases (Karp et al., 2019) were used. As for BSC cover
240 and Chl *a*, treatments for this part of our analysis included temperature and moisture but
241 not light. We characterized microbial communities only at two temperature levels: 10 and
242 20 °C, which showed significant differences in N fixation and cyanobacterial cover (see
243 Results).
244
245 **2.7. Fungal:bacterial ratios**
246 Fungi and bacteria decompose organic matter at different rates, which affects the
247 N and C biogeochemistry of substrates like BSC. To study potential effects of the
248 environment on the biogeochemistry of BSC via differential effects on fungi and bacteria,
249 we estimated fungal:bacterial ratios. We calculated fungal:bacterial ratios based on
250 numbers of gene copies assigned to each group by Kaiju.
251

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252 **2.8. Microbial community and statistical analyses**

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

263 We used a mixed model (*lmer* function in R, version 3.6.1) to analyze the fixed
264 effects of environmental manipulations on N fixation, while accounting for the random
265 effect of measurements on the same sample at two light levels. For the other response
266 variables, which varied in response to temperature and moisture but not light, we used
267 fixed models (*lm* function in R, version 3.6.1). We compared models based on the
268 Bayesian Information Criterion (BIC; Figure S4).

269

270

271 **3. Results**

272

273 **3.1. Nitrogen fixation potential**

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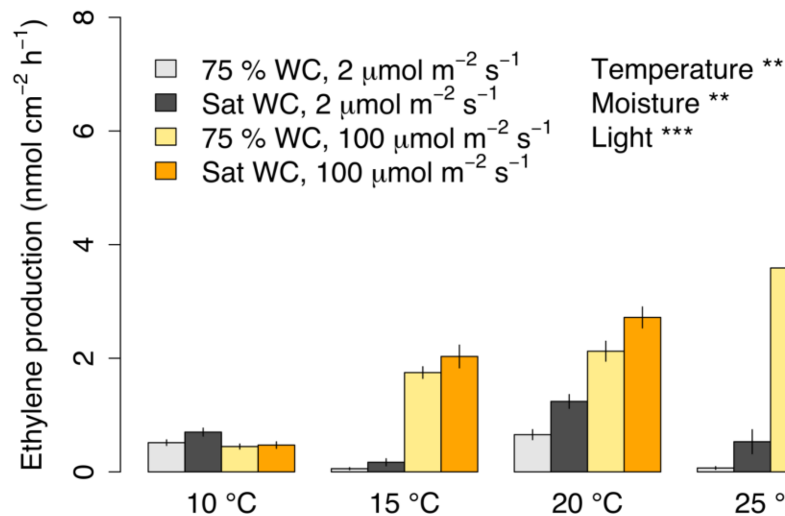
274 N fixation (using acetylene to ethylene conversion as a proxy) in BSC increased
275 with light, temperature and moisture (Figure 1). Responses to environmental
276 manipulations were better explained by the direct and additive effects of light, temperature
277 and moisture than by their interactions (FigS4a). The minimum N fixation (ca. 0.5 nmol
278 cm^{-2} h^{-1}) occurred in the BSC at low temperatures, regardless of moisture and light
279 intensity, whereas the maximum (4.4 ± 0.5 nmol cm^{-2} h^{-1}) occurred in moisture saturated
280 BSC at 25 °C and high light intensity (Figure 1).



281

282 **Figure 1**. Ethylene production in response to temperature, moisture (light and dark
283 tones), and light intensity (grey and yellow colors). Significance codes (here and
284 elsewhere): P < 0.001 '***', P < 0.01 '**'. Significance levels shown in the legend are from
285 the statistical model with the lowest BIC in Figure S4a. Values are means ± se. n = 8.
286 WC: Water content. Sat: Saturated.

287

288 **3.2. Cyanobacteria and liverwort cover**

289 Cyanobacterial cover on BSC increased with temperature (p<0.05) and was not
290 affected by moisture (Figure 2). BSC incubated at 20 °C had ca. 5 to 30% more

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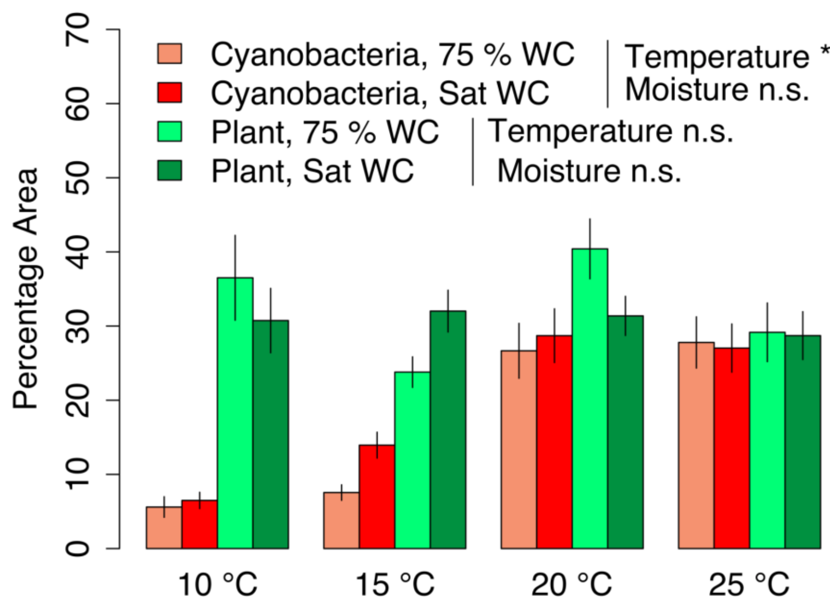
291 cyanobacteria on the surface than BSC incubated at 10 °C. There was no difference in
292 cyanobacterial cover between BSCs incubated at 20 and 25 °C Liverwort cover was not
293 affected by temperature or moisture (Figure 2). Temperature alone was a better predictor
294 of cyanobacterial cover than moisture, or temperature and moisture combined (Figure
295 S4).



296

297 **Figure 2.** Cover of cyanobacteria and liverwort after 96 hours incubation under different
298 conditions. WC: water content (%). Significance codes (here and elsewhere): $P < 0.05$
299 ‘*’. Values are means ± se. n = 8. WC: Water content. Sat: Saturated.

300

301 **3.3. N fixation and cyanobacterial cover**

302 Increases in N fixation rates were correlated ($p < 0.05$) to cyanobacterial cover
303 (Figure 3). Both cyanobacterial cover and N fixation rates increased with temperature
304 between 10 and 20 °C. Between 20 and 25 °C N fixation rates continued increasing

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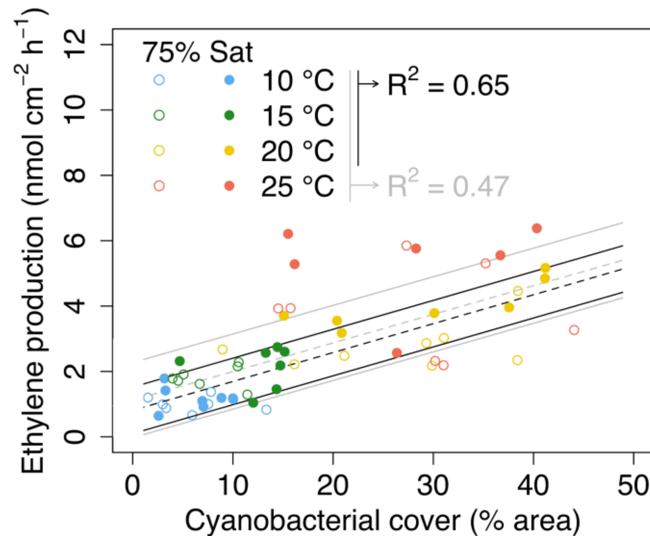
305 (Figure 1) but cyanobacterial cover did not change (Figure 2). Chl *a* did not vary with
306 environmental treatments (Figure S5) and was not related to N fixation rates (Figure S6).



307

308 **Figure 3.** Correlation between cyanobacterial cover and N fixation rates across different
309 temperature, moisture and light levels in a subarctic BSC. Dashed lines show the mean
310 regression line, and solid lines the standard deviation of the mean. Grey lines and text
311 show correlation between cyanobacterial cover and ethylene production for all
312 temperature treatments ($y_i = 1.12 + 0.09*x_i$ + □; □~*N(0, 1.16*^{2}), where □*, here and*
313 *elsewhere, is the error term describing the random component of the linear relationship*).
314 Black lines and text, show results excluding the 25 °C level ($y_i = 0.81 + 0.09*x_i$ + □;
315 □~*N(0, 0.74*^{2})—to illustrate the strong correlation between cyanobacterial cover and
316 ethylene production between 10 and 20 °C. $P < 0.05$ '*', with and without including the 25
317 °C level. (n=8). Sat: Saturated (water content).

318

319 ### 3.4. Relative abundance of cyanobacteria

320 Of the 20 most abundant Orders in the BSC microbial community, six had the
321 highest importance score given by the random forest classification algorithm (Figures 4a

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322 and b and S7). Alphaproteobacteria belonging to the Burkholderiales and
323 Sphingomonadales, as well as Actinobacteria from Corynebacteriales were more
324 abundant at 20 °C than 10 °C; Burkholderiales and Sphingomonadales being more
325 abundant at 20 °C with saturated water content (Figures 4a and b). Cyanobacteria from
326 the Nostocales are overall more abundant at 10 °C than 20 °C. Actinobacteria from
327 Pseudonocardiales and Planctomycetes from Isosphaerales are more abundant at 75%
328 water content than in saturated BSC. Pearson correlations and PERMANOVA analyses
329 between the Bray–Curtis dissimilarity score and moisture content showed a significant
330 effect of moisture on the microbial community structure (Figure 4b, Table S1). Moisture
331 had a significant effect on the α-diversity, based on the observed richness and Chao1
332 index of microbial taxa at the phylotype level (Figure 4c, Table S2 and S3). Significant
333 differences in Chao1 were observed between 75% and saturated BSC at 10 °C and at

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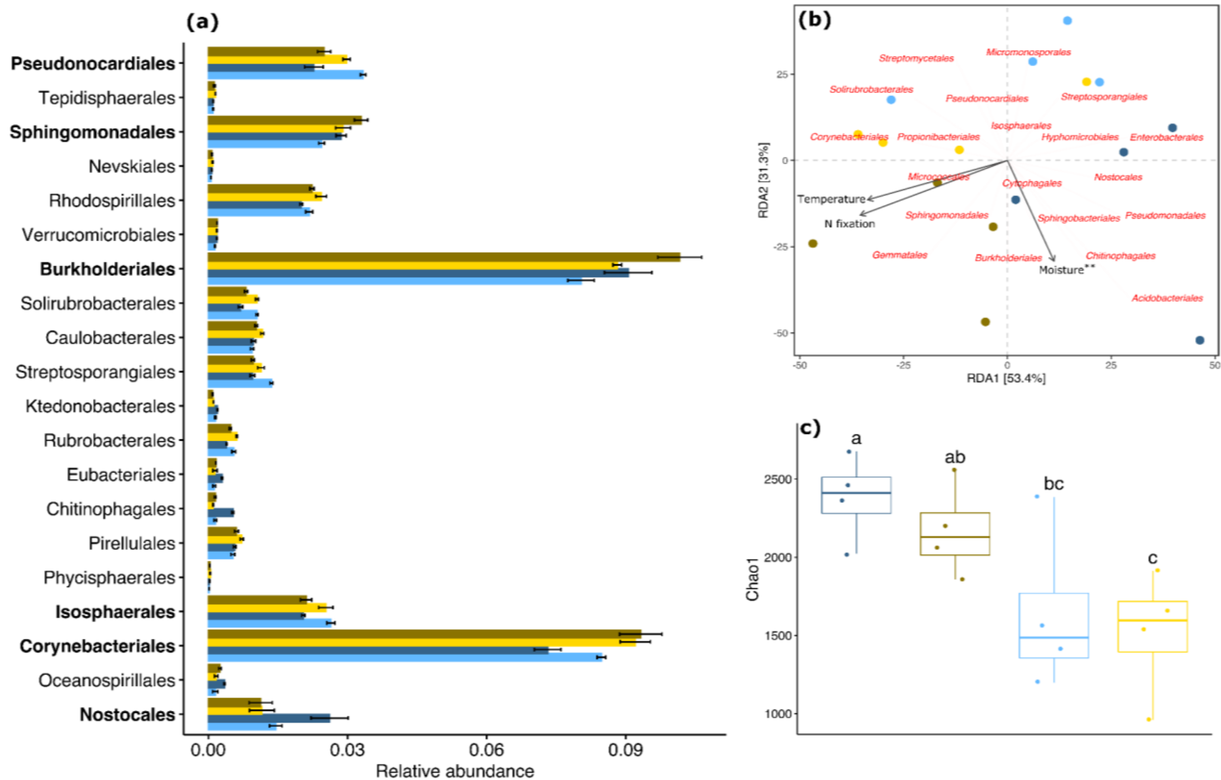
334 20 °C, with overall higher richness observed in saturated BSC than 75% (Figure 4c).



335

336 **Figure 4.** Microbial community composition and richness in a subarctic BSC under
337 different temperature and moisture treatments. Blue and yellow indicate 10 and 20 °C,
338 and light and dark tones indicate 70 and 100% moisture content, respectively. a) Relative
339 read abundances of the 20 most important Orders in our study system, identified by
340 Random Forest classification (see Figure S7). Taxa belonging to the 20 most abundant
341 Orders in the BSC are highlighted in bold. b) dbRDA ordination plots of the community-
342 treatment relationships of microbial communities. The top 20 most abundant Orders are
343 shown in red. The stars (r=0.30, $P < 0.01$) represent significant Pearson correlations
344 between the Bray–Curtis dissimilarity score and moisture content using mantel test. c)
345 The α-diversity Chao1 richness index across treatments. Different letters above data
346 points indicate statistically significant differences ($P<0.05$).

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347

348 Fungal:bacterial ratios were slightly affected by a combination of warming and
349 wetting (Figure S8). Total gene numbers indicate that bacteria grew faster than fungi in
350 the saturated BSC at 20 °C, resulting in a decrease in fungal:bacterial ratios (Figure
351 S7). Total DNA did not change significantly across environmental treatments ($P>0.05$),
352 but it tendended to increase with moisture, especially under warming (Figure S8).

353

354 **3.5 BSC microbial functions.**

355 The LEfSe results show the effect size of the top 15 differentially abundant MetaCyc
356 metabolic pathways between temperature (Figure 5a) and moisture (Figure 5b). At 10
357 °C, pathways linked to autotrophy and anaerobic respiration such as nucleoside and
358 nucleotide biosynthesis/degradation, cofactors, carrier and vitamin biosynthesis,
359 glycolysis and photosynthesis were significantly enriched. At 20 °C, central metabolism
360 pathways associated with cellular respiration and fermentation were more abundant
361 such as the pentose phosphate pathway, TCA cycle and electron transport chain.
362 Pathways associated with fermentation and TCA cycle were found enriched at 75%
363 water content compared to saturated water content which was characterized by an
364 enrichment in cellular respiration related pathways as well as carbohydrate, vitamin,
365 nucleotide, fatty acid and lipid biosynthesis.

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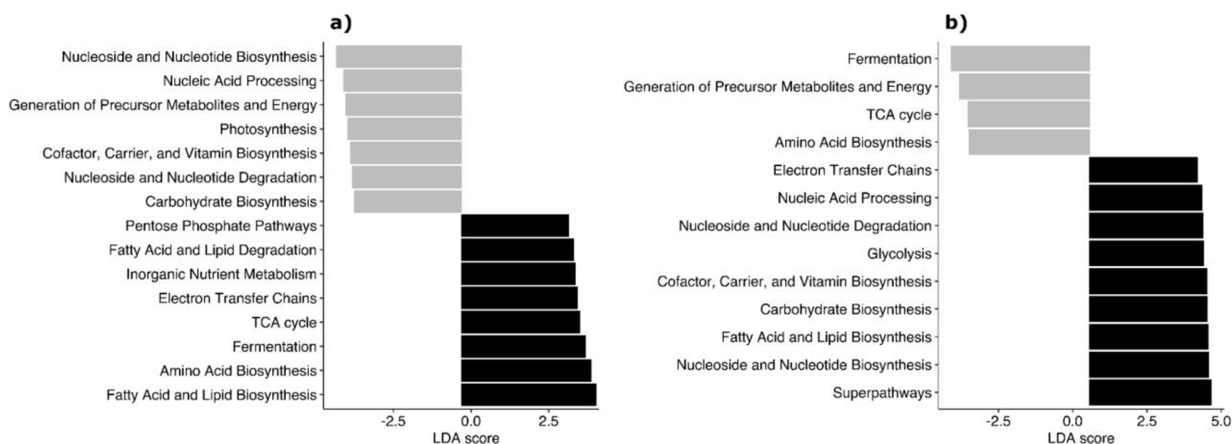

366

367

368 **Figure 5.** Top 15 MetaCyc pathways to be differentially abundant at a) temperature (10
369 °C in grey vs 20 °C in black) and b) moisture (70% in grey vs 100% in black). LDA scores
370 were calculated using LEfSe analysis.

371

372 ## 4. Discussion

373 Climate-driven shifts in the cover, abundance, composition and activity of BSC
374 have the potential to alter the biogeochemistry of terrestrial ecosystems (Elbert et al.,
375 2012; Porada et al., 2014; Weber et al., 2015; Ferrenberg et al., 2017). In hot drylands,
376 warming alone may have little or no effect on the bacterial communities of BSC, wetting
377 alone can increase cyanobacterial abundance, and wetting and warming combined can
378 decrease it (Steven et al., 2015). This shows how bacterial communities from dryland
379 BSC can buffer moderate levels of warming to some extent, but that when combined with
380 altered precipitation the effects of warming can be noticeable (Steven et al., 2015). In our
381 experiment with a BSC adapted to cold and mesic conditions, wetting did not have an
382 effect on the surface cover (a proxy for abundance) of cyanobacteria, and warming
383 increased it. This suggests that the growth of cyanobacteria in our system may be more

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384 limited by low temperatures than by lack of moisture. Alternatively, this may reflect the
385 wider range of temperature than moisture levels in our experimental design. Mean annual
386 temperature and precipitation are projected to increase in the high north in the coming
387 decades (IPCC, 2021). If the Icelandic BSC used in this study is representative of BSC
388 from cold and mesic climates in other high latitudes, our results suggest that the
389 abundance of cyanobacteria in this type of BSC could be affected more by warming than
390 by increased precipitation.

391 The time-scales at which BSC responds to the environment varies with stages of
392 ecological succession (Pushkareva et al., 2017). In the Arctic, temperature and light
393 intensity can more rapidly affect N fixation rates in less-developed BSC dominated by
394 cyanobacteria than in more-developed BSC with dense lichen covers (Pushkareva et al.,
395 2017). The BSC used in this study has the signatures of an early successional stage,
396 including abundant cyanobacteria, being virtually free of lichen patches, having
397 fungal:bacterial ratios closer to those found in deserts than in the tundra (Fierer et al.,
398 2009), and having a mean Chl *a* content closer to those reported for cyanobacterial soil
399 crust than for lichen crusts (Wu et al., 2017). Ecosystems dominated by BSC similar to
400 the one used in this study could be particularly sensitive to climate change.

401 Our correlation analysis suggests that the positive relationship between
402 cyanobacterial cover and N fixation in our study system is in part related to net abundance
403 of cyanobacteria on the soil surface, and in part to the level of their metabolic activity.
404 Between 10 and 20 °C, warming increased both cyanobacterial cover and N fixation,
405 suggesting that the increase in N fixation within this temperature range may have been
406 caused by an increase in the number of heterocysts — the N fixing cells of cyanobacteria.

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407 If total DNA is assumed to be an indicator of microbial biomass (Semenov et al., 2018;
408 but see Leckie et al., 2004), the previous interpretation is challenged by the lack of
409 difference in total DNA between environmental treatments. However, it is plausible that
410 warming caused a growth of cyanobacteria and a reduction of other microbial groups,
411 resulting in no change in total DNA. A comparison of long-term climatic experiments in
412 drylands in the USA, for example, showed that wetting BSC increased the abundance of
413 cyanobacteria while reducing the abundance of other photosynthetic organisms (Steven
414 et al., 2015).

415 Alternatively, warming may have altered the microbial composition of the BSC
416 profile, which varies with depth (Maire et al., 2014). Many cyanobacteria commonly found
417 in BSC such as *Microcoleus vaginatus* are motile (Campbell, 1980), and can use this
418 motility to reach out for light (Biddanda et al., 2015; Schuergers et al., 2016). In a
419 laboratory experiment with cultured *Oscillatoria*-like cyanobacteria, warming increased
420 the speed at which cells moved towards light (~50 μm min^{-1} at 10°C and ~215 μm min^{-1}
421 at 35°C; Biddanda et al., 2015). An increase in cyanobacterial cover with no change in
422 total DNA could have been caused by a temperature-accelerated migration of
423 cyanobacteria to the BSC surface, where there is more light. There, they could have fixed
424 more N than in deeper and darker BSC layers. Overall, our results suggest that between
425 10 and 20°C, increases in N fixation rates in our cold- and mesic-adapted BSC were
426 caused by increases in cyanobacterial abundance, migration of cyanobacteria to the BSC
427 surface, or a combination of both.

428 Between 20 and 25°C, we observed an increase in N fixation rates with no increase
429 in cyanobacterial cover, suggesting that it may have been caused by changes in the

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

439 Much of the research on the effects of climate change on ecosystem function
440 focuses on temperature and moisture (Zelikova et al., 1012; Hu et al., 2014; Salazar-
441 Villegas et al., 2016; Rousk et al., 2018). The comparatively lower number of studies that
442 have included light intensity have found, as in our own study, clear interactions between
443 light, temperature and moisture on the cycling of elements through BSC (Lange et al.,
444 1998; Grote et al., 2010). Interestingly, we did not find a relationship between N fixation
445 rates and Chl *a*, as hypothesized. At the short temporal scale of our experiment, the
446 effects of light on C fixation may be more dependent on the activity than on the abundance
447 of photosynthetic cells and pigments. Because of the great dependence of N fixation on
448 photosynthetically fixed C, the projected decreases in downward solar radiation in the
449 high north by 2100 (as much as -10 W m^{-2} relative to the reference period of 1986-2005,
450 under the RCP4.5 scenario; KNMI, 2021), could reduce N fixation rates in cold-adapted
451 BSC.

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

461 It is well known that warming and moisture can induce significant compositional
462 change in the bacterial communities of biocrust (Garcia-Pichel et al., 2013; Steven et al.,
463 2015; Delgado-Baquerizo et al., 2018), with associated effects on bacterial functions
464 (Steven et al., 2015). This can happen, for example, by environmental change
465 differentially altering the growth rate and survival of different taxonomic groups (Lürling et
466 al., 2013). Our results support the idea that warming can exert a selective pressure on
467 warm-adapted microbial groups over those adapted to colder regimes (Muñoz-Martín et
468 al., 2018). Moreover, our findings show that this selective pressure can manifest within a
469 few days after environmental change. This was observed in our metagenomic
470 investigations where photosynthesis-related genes are enriched at 10 °C but not at 20
471 °C, which corresponds to a decreased relative abundance of Nostocales. In addition,
472 pathways related to a heterotrophic lifestyle are enriched at higher temperature and
473 moisture, which correspond to a shift in the community composition with
474 Alphaproteobacteria (e.g., Burkholderiales and Sphingomonadales) becoming more

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475 dominant in those conditions. In a broader sense, this suggests that climate change could
476 lead to a replacement of the photosynthetic and N fixing Nostocales by non-
477 photosynthetic N fixing bacteria. Such alteration could affect photosynthetic activity
478 supported by cold adapted biocrust, and thus C cycling, as well as N cycling. A deeper
479 understanding of the contribution of microbial community composition to Nfixation activity
480 appears to be critical to predicting the productivity of cold biocrusts.

481 Short-term ecosystem responses to the environment may not necessarily reflect
482 long-term trends (Alatalo et al., 2015). More multi-year, in situ experiments are needed at
483 high latitudes (e.g. Rousk et al., 2018; Salazar et al., in progress) to better understand
484 the effects of climate change on cold- and mesic-adapted BSC over large spatial and
485 temporal scales. However, our short-term laboratory experiment 1) highlights direct and
486 interacting effects of environmental factors on BSC composition and functioning, which
487 could be useful to inform the structure and/or parameterizarion of ecosystem models that
488 explicitly take into account the inherent dynamics of BSC (Rodríguez-Caballero et al.,
489 2015); 2) provides data that can help with BSC characterization (Pietrasiak et al., 2013),
490 which could further serve as reference when assessing the impacts of human
491 disturbances on BSC (Szyja et al., 2018) and/or with the design and implementation of
492 BSC-based restoration practices on degraded land (Bowker, 2007; Velasco-Ayuso et al.,
493 2017; Tucker et al., 2020); and 3) contributes to the understanding of pulses of BSC
494 activity in response to short-term climatic anomalies such as increasingly frequent and
495 intense heat waves.

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502 Faculty of Environmental and Forests Sciences, Agricultural University of Iceland.

503

504

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