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Vertical movement of soluble carbon and nutrients from biocrusts to subsurface mineral soils

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Abstract

Dryland ecosystems can be constrained by low soil fertility. Within drylands, the soil nutrient and organic carbon (C) cycling that does occur is often mediated by soil surface communities known as biological soil crusts (biocrusts), which cycle C and nutrients in the top ca. 0 – 2 cm of soil. However, the degree to which biocrusts are influencing soil fertility and biogeochemical cycling in deeper, subsurface mineral soils is unclear. The movement of dissolved resources from biocrusts to deeper soil layers in leachate may be one of the main mechanisms through which biocrust fertility is transferred downward towards deeper microbial communities and plant roots occurring within mineral soil. Here we examined the role of biocrust leachate in contributing to subsurface nutrient and soluble C pools and subsurface microbial cycling. We collected biocrusts from three biocrust successional stages and explored resource pools *in situ* at multiple soil depths, while collecting leachate and measuring nutrient and organic C concentrations and metabolite composition from each successional stage in the laboratory. After four leachate collections, we conducted an incubation of mineral soil collected from below each biocrust successional stage to measure heterotrophic microbial CO_2 flux and biomass. Overall, our findings observed that the degree of nutrient and C connectivity between biocrusts and the sub-crust mineral soil depended on the biocrust successional stage and the element being considered, and the influence of biocrust successional stage on mineral soil CO_2 flux is likely related to long-term resource build up. Together, our results suggest that the influence of biocrust leachate on subsurface mineral soil is complex and context dependent, but, over longer time periods and at later successional stages, can have measurable effects on dryland soil biogeochemical cycling with feedbacks to resource availability and CO_2 flux.

1. Introduction

Within drylands, productivity and function can be co-limited by water, nutrients, and carbon (C) (Austin, 2011). Understanding the pathways through which nutrients and C enter and are retained within dryland soils is th.erefore essential for understanding ecosystem processes in this expansive biome (Hartley et al., 2007; Rudgers et al., 2018). Yet, we have a limited understanding of how common soil surface communities in drylands, known as biological soil crusts (biocrusts), are biogeochemically connected with the mineral soil below (deeper than 2 cm) (Barger et al., 2016). Biocrusts contain varying levels of lichens, mosses, cyanobacteria, fungi, algae, and other macro- and micro- organisms often occurring in successional stages (Belnap & Lange 2001). These biocrusts can influence surface soil fertility through their roles as soil stabilizers (Chaudhary et al., 2009), CO_2-fixers (Wertin et al. 2012; Darrouzet-Nardi et al., 2015; Sancho et al., 2016), contributors of nitrogen (N) via N_2 fixation (Barger et al., 2016; Torres-Cruz et al., 2018), and regulators of soil microbial communities (Baran et al., 2015), in addition to contributing to plant nutrients, as shown through isotopic labeling (Mayland and McIntosh, 1966; Stewart, 1967). Contributions from biocrust have the capacity to be particularly significant in ecosystems notable for their low soil organic matter reservoirs and overall low levels of soil fertility (Collins et al., 2014).

One mechanism through which nutrients and C move from biocrusts into the mineral soil is downward transport during and following pulsed precipitation events (Barger et al., 2016). Water leached through the biocrust layer can carry with it ammonium (NH_4^+) and nitrate (NO_3^-) biogenic phosphorus (P) and a wide variety of metabolites (Barr, 1999; Johnson et al., 2005; Porada et al., 2014; Baran et al., 2015; Swenson et al., 2018). However, studies of the chemical makeup and the fate of biocrust-sourced dissolved compounds are rare and contradictions exist

within the literature as to biocrusts' contribution to subsurface soil nutrients and organic matter. In some cases, biocrusts have been shown to increase the levels of subsurface nutrients and organic C, such as inorganic N (Guo et al., 2008; Barger et al., 2016; Chamizo et al., 2012; Ferrenberg et al., 2018), while in others, some subsurface nutrients and organic C were similar beneath biocrusts types, plants, and bare ground (Delgado-Baquerizo et al., 2013; Moreira-Grez et al., 2019). Further, biocrust communities are comprised of different morphological groups and species, which can differ across desert and soil types (Colesie et al., 2016) and along successional gradients within a given area (Housman et al., 2006), with consequences for the amounts and forms of compounds leached into subsurface soil (Johnson et al., 2005; Tucker et al., 2020).

Biocrust-derived leachate may also influence subsurface microbial communities, with implications for nutrient retention and gaseous release from mineral soil layers, as seen with leaf litter in other ecosystems (e.g., Cleveland et al., 2010). Exudates from biocrust can structure soil microbial communities and soil food webs adjacent to biocrusts (Baran et al., 2015). However, the extent to which leachate influences deeper soil microbial communities is unclear. Microbial operational taxonomic units (OTUs) below biocrusts can be similar across different biocrust and soil types (Moreira-Grez et al., 2019; Steven et al., 2013), with the notable exception of moss crust, which can have higher microbial biomass and community diversity in the mineral soil below the moss compared to earlier successional, cyanobacteria-dominated biocrust stages (Bao et al., 2019; Delgado-Baquerizo et al., 2015) possibly due to the large amounts of organic C released from moss crusts, which can structure microbial communities (Baran et al. 2015). The variability in resource inputs from different biocrust types and differences in the microbial communities below biocrust types underscore the likelihood of varying levels of connectivity

between the biocrust layer and the mineral soil below. Connectivity between the biocrust and the microbial communities and functions in the mineral soil layer has implications for microbial nutrient turnover, resource storage, and CO_2 respiration in both the short and long term (Cleveland et al., 2010).

Here, we present a novel experimental design that sought to assess biocrusts' connectivity with mineral soil using multiple successional stages of biocrust. Specifically, we examined lightly pigmented, darkly pigmented, and moss dominated biocrust that represent a generalized gradient of succession in our study system from least to most developed (Belnap et al. 2001, Belnap & Eldridge 2001). Lightly pigmented cyanobacterial crusts are early colonizers and are generally dominated by the cyanobacterial *Microcoleus spp.* (Rosentreter & Belnap 2001, Couradeau et al., 2016). Darkly pigmented crusts, generally dominated *Microcoleus spp.* and *Scytonema* spp., (Rosentreter & Belnap 2001, Couradeau et al., 2016) and moss dominated crusts are considered more developed, later successional forms of biocrust. Darkly pigmented cyanobacteria crusts generally have high rates of N fixation, while darkly pigmented and moss crusts have high rates of C fixation when compared to lightly pigmented crusts (Housman et al. 2006; Tucker et al. 2019). For this experiment, we located an area with a relatively homogenous sandy soil type and assessed the differences in connectivity among the three biocrust successional stages and the mineral soil below. We addressed the following questions: (1) What compounds are leached from the biocrust layer during wet-up events and do the compounds differ among biocrust types? and (2) Do compounds leached from the different biocrust successional stages regulate short-term microbial activity in sub-surface soils? In addressing these two questions, this experiment lends insight into the role of dissolved resources moving from biocrusts into the mineral soil and the short-term consequences of these inputs for

heterotrophic respiration, with implications for a larger understanding of nutrient and C cycling in dryland soils.

2. Material and Methods

This study consisted of three complementary components that each assessed soil or leachate associated with different biocrust successional states on the Colorado Plateau, USA. These three components consisted of a field assessment of nutrient and C concentrations in *in-situ* biocrust and the below-biocrust mineral soil, a laboratory assessment of potential leachate chemistry, and a soil incubation with biocrust leachate. Biocrust types consisted of an early successional, lightly pigmented cyanobacterial biocrust (i.e., likely dominated by *Microcoleus vaginatus,* (Rosentreter & Belnap 2001, Garcia-Pichel et al. 2013)), a mid-successional darkly pigmented cyanobacterial biocrust (likely dominated by *Microcoleus vaginatus and Scytonema* spp., (Rosentreter & Belnap 2001, Couradeau et al., 2016)), and a mid to late successional moss dominated biocrust (dominated by *Syntrichia caninervis*, Weber et al., 2016). Biocrust samples were collected in January 2017 in a 25 m^2 area of semiarid desert outside of Moab, UT (38°41' 02.31" N, 109°43'11.60" W, 1,529 m above sea level). The soil type was visually homogenous, with the soils characterized as a well-drained, fine sandy loam on average 86 cm deep in the Begay-Sazi-Rizo complex (Soil Survey Staff, NRCS). Inorganic C varied from 0.16% to 0.67% in the crust layer and 0.07% to 0.97 % in the mineral soil layer (Supplemental Table 1). Soils in the region are generally neutral to alkaline, pH ranged from 7.26-7.84 in the biocrust layer and 7.67-8 in the mineral subsoil (Supplemental Table 1). The ecological site was a Four-Wing Saltbush semidesert with a mean annual precipitation of 30 cm and a mean annual temperature of 10 °C. Parent material was alluvium and eolian deposits derived from sandstone (Soil Survey Staff, NRCS). The three different successional stages were co-occurring within the 25 m^2 area.

136 The co-occurrence of different biocrust successional stages was likely due to past physical
137 disturbance, potentially the historic presence of cattle, which disturb biocrusts in discrete patches
138 and can leave other patches intact. However, the site was visually undisturbed during biocrust
139 collection.
140 *2.1 Assessment of biocrust and mineral soil in the field*
141 To determine the *in-situ* C and nutrient concentrations both within and below the different
142 biocrust types, we collected biocrust samples using a 10 x10 cm metal square core down to 2 cm
143 depth. A flat metal sheet was slid under the square core to remove the biocrust (n = 10 for each
144 crust type). On the exposed soil in the 10 x 10 cm area, we took three 2.54 cm diameter cores of
145 8 cm length (2-10 cm below crust surface), which were combined into one sample (n = 10 for
146 each crust type). We sieved the soil through a 4 mm sieve, removed roots and visible organic
147 matter, and homogenized before subsampling for extractions.
148 To determine total C and N concentrations, we dried a subset of each sample at 60 °C,
149 ground the samples, and measured for total C and N on an elemental analyzer (Elementar Vario
150 Micro Cube, Elementar Inc., Langenelsbold, Germany). Many dryland soils contain carbonates;
151 thus, determining total organic C concentrations from total C value requires assessment or
152 removal of inorganic C. Soil inorganic C concentrations were measured using a modified
153 pressure calcimetry assay (Sherrod et al. 2002). Briefly, a homogenized soil sample was sealed
154 in a 20 mL amber jar and exposed to hydrochloric acid in excess. A pressure transducer was then
155 used to measure the pressure from the resultant CO_2. Organic C was then calculated as total C
156 with inorganic C subtracted. To determine the pigment concentrations of lightly and darkly
157 pigmented biocrust samples, we extracted chlorophyll *a* (Chl_a) and scytonemin (Scy) with 90 %
158 acetone for 12 hours in the dark at 4 ºC after being finely ground (Castle et al., 2011). The

159 supernatant was decanted, and pigment concentrations were measured spectrophotometrically
160 (GENESYS 10S UV-VIS, Thermo Scientific, Waltham, MA) at 665 nm and 394 nm for Chl_a and
161 Scy, respectively. The equation to convert the A_{665} value to [Chl_a] was taken from Ritchie 2008
162 and conversion of the A_{394} values to [Scy] was performed as in Garcia-Pichel and Castenholz
163 (1991). We measured organic matter concentrations of the lightly pigmented, darkly pigmented,
164 and moss biocrusts by loss-on-ignition of an oven-dried (105 °C) sample in a muffle furnace at
165 550 °C for 4 hr ((Davies, 1974); ThermoScientific Thermolyne, Waltham, MA, USA).
166 We extracted inorganic N pools, NH_4^+ and NO_3^-, using 2 M KCl and fresh soil. The soil
167 slurry was shaken for 1 hour then allowed to settle overnight (Robertson et al., 1999). Inorganic
168 N concentrations (NH_4^+ and NO_3^-) were quantified colorimetrically using the indophenol blue
169 method for NH_4^+ and using a Cd-column reduction followed by the Greiss-Ilosvay method for
170 NO_3^- on a Smartchem 200 Discrete Autoanalyzer (Unity Scientific, Milford, MA). Soil PO_4^{3-} was
171 extracted using Olsen's method, with a 0.5 M $NaHCO_3$ solution and a shaking time of 16 hr
172 (Olsen, 1954). Soil extractable PO_4^{3-} and microbial PO_4^{3-} concentrations were quantified using a
173 modified ascorbic acid molybdate analysis (Kuo, 1996) on a Smartchem 200 Discrete
174 Autoanalyzer (Unity Scientific, Milford, MA). Limit of quantification was 0.02 mg PO_4^{3-}-P/l for
175 all P measurements. Microbial C, N, and P concentrations were estimated with a chloroform cell
176 lysis method by adding 1 ml of amylene-stabilized $CHCl_3$ to soil in a 125 ml flask that was
177 stoppered with neoprene and allowed to sit in the dark for 16 hr before being ventilated and
178 extracted with 0.5 M K_2SO_4 (for microbial biomass C and N, Beck et al., 1997; Brookes et al.,
179 1985) or 0.5 M $NaHCO_3$ (for microbial biomass PO_4^{3-}) and shaken for 1 hr (Weintraub et al.,
180 2007). Microbial biomass C, N, and P were calculated as the amount extracted from
181 nonfumigated soil subtracted from the amount extracted from fumigated soil. No microbial

biomass correction factors were applied (Weintraub et al., 2007). All extracts were filtered through Whatman #1 filter paper (GE Healthcare, Chicago, IL). Extractable organic C, total dissolved N, and microbial biomass C and N were analyzed on a Shimadzu TOC-V_{CPN} with the TNM-1 attachment (Shimadzu Corporation, Kyoto, Japan). Determining the concentrations of the biomass, nutrient, and C concentrations both within and below biocrusts provides a point-in-time assessment of biologically available pools in the field.

2.2. Assessment of leachate chemistry

On the same day and at the same site as the collections described above, we collected intact cores of each biocrust successional state (n = 15 for each stage), 2 cm deep and 4.6 cm in diameter. We returned the cores to the laboratory and used them to determine the nutrient concentrations of potential leachate from different biocrust successional states over a four-week period. Once in the lab, we used a razor blade to carefully scrape the subsoil from the biocrust. The subsoil could be differentiated from the biocrust layer by the lack of cohesion between soil particles and the lack of visible cyanobacterial filaments or rhizomes. Because the crust layer varies in thickness, specifically between successional stages, the biocrust thickness was different for each sample and ranged from 6 mm – 12 mm for lightly and darkly pigment cyanobacterial crusts and 10 mm – 15 mm for moss (Supplemental Table 1). We seated the cores in plastic cylinders that were open on the top and had mesh screen on the bottom. Below the mesh screen was a second cylinder with a layer of marbles resting on top of a second layer of mesh screen. The marbles were to ensure sediment did not pool on the bottom of the mesh, to control the flow of leachate during extractions, and to improve connectivity for liquid movement between the mesh layers (Figure 1).

Once a week for four weeks, we added 30 ml of deionized water to lightly pigmented and darkly pigmented crusts, 35 ml of deionized water to moss crusts, and 25 ml to blank controls that did not have any biocrust but maintained all other aspects of the infrastructure. These volumes corresponded to 15-21 mm of rainfall and were chosen to ensure enough liquid moved through the sample and was available for C and nutrient analyses. Differences in watering amount were accounted for in the final µg/ml calculations. After allowing the liquid to saturate the biocrust and move downward with gravity for 10 minutes, we placed the biocrust cores onto a vacuum filtration system to pull remaining liquid through the biocrust system and we collected the liquid in vials below the samples. We analyzed the collected liquid leachate for NH_4^+, NO_3^-, PO_4^{3-}, extractable organic C and total dissolved N as described above. The concentrations in the leachate were summed across the four time points to compare across biocrust types. To examine differences in the nutrients within leachate and the nutrients with the soil crust layer between biocrust types, we standardized nutrients "lost" in leachate to those occurring in the biocrust layer using the equation: ((sum nutrient leached)/(nutrient amount in biocrust)) × 100

To assess metabolites in the leachate, we combined leachate from each sample across the four-time points of the experiment and then compiled three replicates from the same biocrust type together into one sample, so that the total number for each biocrust type was $n = 3$. We did this to ensure we had enough sample to perform the analysis. The relative concentrations of key metabolites were profiled using normal phase liquid chromatography (Merck SeQuant ZIC-HILIC column, 150_1 mm, 3.5 mm, 100 Å) coupled to an Agilent 6520 ESI-Q-TOF at the Lawrence Berkeley National Laboratory (Sparks et al., 1996). For metabolomics, approximately 30 ml liquid leachate were lyophilized (FreeZone 2.5 Plus, Labconco) and resuspended in 200 µl methanol containing internal standards (5-50 µM of ^{13}C-^{15}N Cell Free Amino Acid Mixture,

227 Sigma). Samples were vortexed for 20 seconds, filtered through 0.2 µm centrifugal filters and
228 placed into LC-MS vials for analysis. LC-MS data were acquired using an Agilent 1290 LC
229 stack with a HILIC column (Merck SeQuant ZIC-HILIC column, 150_1 mm, 3.5 mm, 100 Å)
230 coupled to a Q Exactive Orbitrap MS (Thermo Scientific). Metabolites were identified using the
231 Metabolite Atlas and verified based on exact mass and retention time (< 1 min difference) and
232 MS/MS fragmentation spectra matching to known standards (Supplemental Table 2 &
233 Supplemental Table 3). Differences between relative amounts of metabolites were determined by
234 normalizing the peak area for each metabolite to the high peak value across biocrust types.
235
236 *2.3 Assessment of CO_2 flux and microbial biomass after leachate addition*
237 To answer our question exploring the relationship between leachate and heterotrophic
238 microbial activity, we conducted a soil incubation experiment where we added leachate from
239 each biocrust successional stage (collection described in 2.2. above) to the mineral soil collected
240 from beneath biocrusts in a full-factorial design. Specifically, mineral soil samples from the
241 same site described above were collected in July 2019 from beneath lightly pigmented
242 cyanobacterial, darkly pigmented cyanobacterial, and moss dominated biocrusts and soils were
243 given leachate collected from each of the crust types (n = 3 for each below-crust soil–leachate
244 pairing). We collected mineral soil by removing the 0-2 cm layer of lightly pigmented, darkly
245 pigmented, and moss-dominated biocrusts using a 10 x 10 cm square core and collected soil
246 beneath by taking 3, 4.6 cm diameter cores at a depth of 2-5 cm. For our incubation, we added
247 15 g of the below-biocrust mineral soil to 120 ml gas-tight glass Mason jars fitted with rubber
248 septa and brought the soil to 50 % of water holding capacity (around 2 ml per sample) with
249 leachate. We sealed the jar for 24 hours in the dark and then used a syringe to mix and collect 8

ml of headspace without exposing the headspace to the atmosphere. We analyzed CO_2 concentration of the headspace using a benchtop infrared gas analyzer (IRGA; CA-10, Sable Systems International, North Las Vegas, NV). Soil respiration rate was calculated as μmol CO_2 g^{-1} hr^{-1}. Before and after the 24-hr incubation we extracted the samples for microbial biomass C concentration assessment as described above.

2.4 Statistics

We checked the data for normality and homogeneity of variance and found that many of the response variables were non-normal and heteroscedastic. We used permutational ANOVAs, which do not assume data normality or homogeneous variance, to determine how strongly response variables differed across biocrust successional states. We conducted a pairwise permutational test to determine how different the response variables were from one another among the biocrust successional states. The permutational ANOVAs were conducted using the package 'VEGAN' in R (Oksanen et al., 2019) and the permutational pairwise test was conducted using the package 'pairwiseAdonis' in R (Arbizu, 2017). We also calculated the differences in the magnitudes among the crust types for each response variable. The differences in magnitude are reported as ratios such as $\bar{X}_{light} : \bar{X}_{dark}$ where $\bar{X}_{light}$ is the mean of the lightly pigmented cyanobacterial biocrusts for a given variable and $\bar{X}_{dark}$ is the mean of the darkly pigmented cyanobacterial biocrust for the same variable. The data were non-negative, showed some degree of log normality and contained zeroes. As such, we calculated 95 % confidence intervals for the ratios using a maximum-likelihood method designed for data with these features (Zhou and Tu, 2000). Confidence intervals not containing 1 are considered statistically significant for $H_0 = 1$, which would correspond to a 1:1 ratio, or no difference among crust types (likelihood ratio test; Zho and Tu, 1999; Zhou and Tu, 2000). The calculations were conducted

using the package 'treateffect' in R (Darrouzet-Nardi, 2020). To compare relative intensify of detected metabolites, we created a heat map using the function "heatmap.2" in the package "gplots v3.1.1" in R and the hierarchical clustering with the package used the complete argument in the "hclust" function (Figure 3). A two-way ANOVA and Tukey HSD test at an alpha level of < 0.05 was used to indicate the normalized metabolite amounts that differed significantly among biocrust types.

3. Results

3.1 Characterization of biocrust successional states

The Chl_a and Scy concentrations were higher in darkly pigmented cyanobacterial, mid-successional biocrusts than in the early successional lightly pigmented cyanobacterial biocrusts (Figure 2, Supplemental Table 4). Chlorophyll *a* concentrations were 5.5 times [4.11, 7.45; 95 % CI] as high in darkly pigmented as in lightly pigmented cyanobacterial crusts ($p = 0.001$, $F = 143.84$). Scytonemin concentrations were 9.8 times [6.24, 15.5; 95 % CI] as high in darkly pigmented as in lightly pigmented cyanobacterial crusts ($p = 0.001$, $F = 90.70$). The percent organic matter increased across biocrust successional states ($p = 0.001$, $F = 59.78$), increasing 2.3 times [1.87, 2.67; 95 % CI] from lightly pigmented biocrust to darkly pigmented cyanobacterial crust ($p < 0.001$), and then 1.8 times [1.52, 2.34, 95 % CI] from darkly pigmented cyanobacterial to moss dominated biocrust ($p < 0.001$).

3.2 Nutrients and organic C

3.2.1 N concentrations

295 Generally, N concentrations in the biocrust layer were higher within darkly pigmented
296 cyanobacterial and moss biocrusts than in lightly pigmented cyanobacterial crusts (Figure 3 A.,
297 B., C., Supplemental Table 4). For example, total soil N and extractable NH_4^+ concentrations
298 were around twice as high in darkly pigmented cyanobacteria and moss dominated biocrusts than
299 lightly pigmented cyanobacterial crusts (NH_4^+ dark:light = 2.6 [1.99, 3.42; 95 % CI], moss:light
300 = 2.8 [1.91,4.45; 95 % CI]) (% Total N dark:light = 1.92 [1.56, 2.37; 95 % CI], moss:light = 2.26
301 [1.73, 2.93; 95 % CI]). Extractable NO_3^- concentrations were the exception, with NO_3^- values
302 highest in the darkly pigmented cyanobacterial crust but similarly low in the other two crust
303 types ($p = 0.011$, F = 3.61). The ratio of organic C to total N was 1.4 times [1.23, 1.67; 95% CI]
304 higher in moss crusts than in the lightly pigmented cyanobacterial crusts than and 1.2 times [1.1,
305 1.44; 95% CI] higher in darkly pigmented crusts than in lightly pigmented crusts (Supplemental
306 Table 4).
307 For the leachate, total dissolved N concentration was highest in lightly pigmented
308 cyanobacterial crusts compared to moss or darkly pigmented cyanobacterial biocrusts, with the
309 latter having the lowest N concentration overall. For example, total dissolved N was 2.34 times
310 [1.34, 3.92; 95% CI] higher in leachate from the lightly pigmented cyanobacterial crusts as it was
311 from darkly pigmented cyanobacterial crusts ($p = 0.02$), while NO_3^- concentrations were 3.98
312 times [2.27, 6.97; 95 % CI] higher in lightly pigmented cyanobacterial biocrusts as it was in
313 moss crusts ($p < 0.001$). When comparing the N leached from the biocrust to the N found in the
314 biocrust layer, lightly pigmented cyanobacterial crusts lost more NH_4^+ (13.04%) and NO_3^-
315 (55.25%) relative to the amount within the biocrust layer, than either the moss (NH_4^+ = 3.54,
316 NO_3^- = 8.74) or darkly pigmented cyanobacterial crust (NH_4^+ = 1.77, NO_3^- = 9.02). The ratio of
317 extractable total dissolved organic C to total dissolve N in leachate grew substantially larger

318 along the successional gradient, with the C:N ratio 4.3 times [3.56, 5.31; 95% CI] greater in
319 leachate from mosses than leachate from lightly pigment cyanobacterial crusts and 1.7 times
320 [1.09, 2.73; 95% CI] greater than leachate from darkly pigment cyanobacterial crusts
321 (Supplemental Table 4).

322 There were few generalizable patterns for N forms within the mineral soil beneath the
323 biocrust, and the overall patterns of N concentrations in the soil layer did not reflect those in the
324 biocrust layer or its leachate. The largest differences were seen in the extractable NO_3^- and total
325 dissolved N concentrations in the soil, which were lower beneath lightly pigmented
326 cyanobacterial crusts compared to below darkly pigmented and moss crusts. Extractable NH_4^+
327 concentrations in the soil were 1.5 [1.08, 2.15; 95 % CI] times higher below lightly pigmented
328 crusts and 2.2 [1.56, 3.04, 95 % CI] times higher below moss crusts than below darkly
329 pigmented cyanobacterial crusts (Figure 3C).

330

331 *3.2.2 P concentrations*

332 The extractable PO_4^{3-} concentrations in the biocrust layer increased from lightly
333 pigmented to darkly pigmented cyanobacterial to moss crusts ($p = 0.002$, F = 10.16) (Figure 3D).
334 The PO_4^{3-} in the leachate had a similar pattern to extractable PO_4^{3-} in the biocrust layer, but the
335 variation among the biocrust successional states was larger ($p = 0.16$, F = 1.99) (Figure 3E). The
336 PO_4^{3-} concentrations in the soil layer below the biocrusts were about 1.5 times lower below
337 darkly pigmented cyanobacterial crust compared with the other two crust successional states
338 ([light:dark 1.4 [1.02, 1.79; 95% CI], moss:dark 1.62 [1.29, 2.03; 95% CI]), while microbial
339 biomass PO_4^{3-} concentrations were similar in soils below all three crust successional states ($p =$
340 0.53, F = 0.94) (Figure 3F).

341

342 *3.2.3 C concentrations*

343 Extractable total dissolved organic C in the biocrust layer increased from lightly
344 pigmented to darkly pigmented cyanobacterial to moss crusts ($p = 0.004$, $F = 6.49$) (Figure 3G).
345 Dissolved organic C in the leachate from moss crusts was 2.5 times [1.7, 3.87; 95% CI] higher
346 than lightly pigmented crusts and 4 times [3.01, 5.54; 95% CI] higher than darkly pigmented
347 cyanobacterial crusts (Figure 3H). In the mineral soil below the biocrust, extractable total
348 dissolved organic C and microbial biomass C were much higher below moss crusts than below
349 lightly and darkly pigmented cyanobacterial crusts. For example, total dissolved organic C was
350 11.6 times [5.14, 26.37; 95% CI] higher in moss than in lightly pigmented cyanobacterial crusts.
351 Soil total organic C was similar between moss crusts and darkly pigmented cyanobacterial crusts
352 ($p = 0.07$) but was 2.47 [1.59, 3.82; 95% CI] times higher in moss crusts than in lightly-
353 pigmented crusts and 1.22 times [0.97, 1.55; 95% CI] higher in darkly-pigmented crusts than
354 lightly pigmented crusts (Figure 3I).

355

356 *3.3 Metabolites in leachate*

357 The LC-MS analysis showed a wide range of metabolites in the leachate from each
358 biocrust successional state. Most of the metabolites were verified with authentic standards, and
359 those that were not were considered putative and not included in the list of present metabolites.
360 Moss dominated crusts seemed to have the highest relative abundance of metabolites compared
361 to lightly and darkly pigment crusts. Hierarchical clustering grouped lightly pigmented and
362 darkly pigmented crusts together, separate from moss dominated crusts. Some, but not all,
363 metabolites differed strongly among biocrust successional stage (Figure 4, Supplemental Table

5). Many present metabolites were osmolytes (Figure 5). Of the five commonly recognized osmolytes found with the leachate (ectoine, proline, betaine, choline, and trigonelline) only the relative abundance of betaine and choline differed strongly among successional stages. Betaine: moss:dark 3.49 [1.71, 7.12; 95% CI], moss:light 4.342 [1.13, 16.68; 95% CI], dark:light 1.24 [0.27, 5.7; 95% CI].

3.4 Soil respiration and microbial C

A full-factorial design crossing soils from beneath the different biocrusts with leachate collected from the different biocrusts revealed that soil CO_2 respiration changed significantly as a function of the successional state beneath which the mineral soils were collected ($p = 0.001$, F = 54.440), but not as a function of the successional stage from where leachate was sourced ($p = 0.452$, F = 0.89) (Figure 6, Supplemental Table 4). Because respiration rates did not differ significantly among leachate sources on a given subsoil, we treated the four different leachates as replicates when examining the relationship between CO_2 respiration and subsoils. Soil CO_2 respiration rates were similar in soils collected from beneath lightly pigmented and darkly pigmented cyanobacterial crusts ($p = 0.92$) but were around 1.7 times higher in soils collected from beneath moss biocrust (moss:light = 1.76 [1.52, 2.04; 95 % CI], moss:dark = 1.74 [1.56,1.95; 95 % CI]; Figure 6). Microbial biomass C concentrations were also significantly different across mineral soil types ($p = 0.001$, F = 38.03) but were similar among leachate treatments ($p = 0.86$, F = 0.24). We again treated leachate types as replicates within mineral soil types, since the differences among leachate types were small and not significant. Microbial biomass C was around 2.3 times higher in mineral soil beneath darkly pigmented cyanobacterial and moss crusts than below lightly pigmented crusts (dark:light = 2.28 [1.93, 2.7; 95 % CI],

387 moss:light = 2.31 [1.87, 2.85; 95% CI]). Mineral soil collected beneath lightly pigmented
388 cyanobacterial crust had the highest soil respiration to microbial biomass C ratio (activity :
389 biomass = 0.102) compared to darkly pigmented crust soil (activity : biomass = 0.045) and moss
390 crust soil (activity : biomass = 0.077). Note that the microbial biomass in the incubation
391 experiment were distinct from the *in-situ* microbial biomass measurements and not comparable
392 due to the differences in timing and method of collection.
393
394 **4. Discussion**
395
396 Here, we explored the connectivity between biocrusts and the subsurface mineral soil.
397 We found that biocrusts released a wide range of nutrients and organic C compounds during
398 leaching events and that the concentrations of C, N, and P in leachate differed widely among the
399 three biocrust successional stages, as did the degree to which the resources accumulated in the 2-
400 10 cm soil layer (Figure 3). Further, we found that leachate concentration did not appear to affect
401 short-term microbial heterotrophic CO_2 fluxes in mineral soil (Figure 6) as it has in other
402 ecosystem types (e.g., Cleveland et al., 2010). Instead, the provenance of mineral soil, with
403 regard to which biocrust successional state occurred on the surface where the soil was collected,
404 was the main driver of differences in CO_2 flux, suggesting a longer-term effect of biocrust type
405 on sub-surface microbial respiration. Overall, we observed that the degree of connectivity
406 between biocrusts and the mineral soil depends on the biocrust successional stage and the
407 resource being considered, and that changes to successional stage may have significant influence
408 on the biogeochemical connectivity between biocrusts and mineral soil.
409
410 *4.1 Nutrients and organic C in biocrust leachate*

411 The patterns in leached nutrients and C varied among biocrust type and element. For
412 example, lightly pigmented cyanobacterial crusts lost the most N in leachate of the three biocrust
413 types. This finding is surprising, given the dominant species of this area's lightly pigmented
414 crusts, *M. vaginatus*, is not a N-fixer; although, N fixation by free-living organisms tightly
415 associated with *M. vaginatus* is common, these rates are typically relatively low (Belnap, 2001;
416 Steppe et al., 1996). However, its known that cyanobacteria secrete a large fraction of their
417 photosynthate into their surrounding environment (Baran et al., 2015; Thomazo et al., 2018), and
418 there is emerging evidence for a 'cyanosphere,' in which the pioneering soil cyanobacteria, *M.*
419 *vaginatus,* concentrates N-fixing bacteria around cyanobacterial bundles through organic C
420 exudation (Couradeau et al., 2019, Nelson et al. 2021). Our results build on these findings to
421 suggest the early-successional cyanosphere is less able to retain N than later-successional
422 cyanobacterial communities, and notably, that this cyanobacterial-dominated biocrust loses more
423 N relative to the N it stores in the biocrust layer. Similar patterns of N loss in leachate, including
424 large amounts of organic N loss, were observed in a separate experiment examining leached N
425 from lightly and darkly pigmented biocrusts collected on the Colorado Plateau (Johnson et al.,
426 2005). The ability of darkly pigmented crusts to retain more N and C than lightly pigmented
427 crusts suggests structural and/or species differences between lightly and darkly pigmented
428 biocrusts that allows microbial communities within darkly pigmented cyanobacterial biocrusts to
429 better retain resources. This could be related to the more complex species compositions that bind
430 and resorb nutrients (Garcia-Pichel et al., 2001; Garcia-Pichel and Belnap, 1996, Courdeau et al.
431 2019) and suggests another differences among the three biocrust types is the ability to retain
432 nutrients, specifically N. Our findings suggest lightly pigmented biocrusts maintain lower C and
433 nutrient stocks and are less able to maintain soil fertility in the 0-2 cm soil layer than later

successional crust types. But, surprisingly, may promote N fertility in deeper soil layers disproportionate to their N stocks, as indicated by the larger amounts of N leached downward from the surface.

The large stoichiometric differences in the leachate (for example, the large differences in C:N between lightly and darkly pigmented cyanobacterial crust leachate) likely influences the ultimate fate of the leachate, as well as the microbial communities that utilize and recycle it. Nutrients and organic C in leachate may be resorbed, taken up by vascular plants, fungi, archaea, or other bacteria, flushed to deeper soil layers, rapidly oxidized, or transformed and lost in gaseous form (Barger et al., 2016), all of which could be influenced by changes in leachate stoichiometry. Here, the dissolved C:N ratio in leachate doubled among successional stages, suggesting that disproportionate amounts of dissolved organic C are being released into the soil below late-successional crusts relative to N, likely structing the complex communities found there (Baran et al. 2015) but leading to a larger potential for N limitation in soils below late-successional crusts. Because biocrust types are anticipated to change under global change scenarios (Ferrenberg et al., 2015; Reed et al., 2016) understanding these stoichiometric differences in biocrust leachate, as well as the connectivity among biocrust types and mineral soil, is important for understanding the fate of these nutrients and C in transitioning drylands (Ferrenberg et al., 2018b; Maestre et al., 2013; Reed et al., 2012). Further, changes in precipitation patterns and increasing aridity will likely influence the degree of connectivity between biocrust and the mineral soil. This is due to the predominant role of precipitation in controlling the downward movement of water and therefore the degree to which the biocrust and subsoil are connected (Collins et al. 2014). Less precipitation or smaller precipitation events may decrease the degree to which biocrust contribute to subsoil nutrients.

The metabolite content in leachate also varied among biocrust successional stages. The large amounts of organic C found in leachate from moss crusts is similar to other studies examining biocrust leachate (Zhao et al., 2016) and contained a correspondingly large concentration and diversity of metabolites. Osmolytes, specifically betaine, choline, ectoine, trigonelline, and proline, were found in leachate from each biocrust type and most commonly in moss crusts. There is evidence that these osmolytes are essential components of the desiccation and rehydration of biocrusts (Swenson et al., 2015). The long list of other metabolites detected, including amino acids, nucleotides, nucleobases, sugars, and vitamins, suggests additional functions related to microbial activity. Metabolites are important components of microbial food webs within biocrusts, with heterotrophs specializing in specific metabolites released from cyanobacteria as substrates (Baran et al., 2015). The differences in metabolite content from different biocrust types is likely related to the different complexities and structures within each biocrust type. For example, extracellular polymeric matrixes (EPM) released as microbially-produced exopolysaccharides from biocrusts can bind and capture metabolites, helping to retain them in the biocrust layer (Swenson et al., 2018). Different amounts of EPM in the three crust types (Rossi et al., 2018), as well as the large amounts of organic C derived from tissue and rhizomes of moss crusts (Dümig et al., 2013), may help explain differences in leachate metabolite content. Future work quantifying the various metabolites and directly comparing concentrations of the observed metabolites with microbial activity would further our understanding of these pools and their role in microbial activity in the soils below biocrust.

4.2 Nutrient pools within and below biocrusts

The nutrient and organic C concentrations of the 2-10 cm soil layer did not strongly reflect the concentrations of the biocrust layer or the leachate, with the exceptions of moss crusts, which leached large amounts of total dissolved organic C and had high total dissolved organic C pools in the mineral soil (Figure 3). Some studies have observed a difference in nutrient and C pools below biocrusts (Barger et al., 2013; Guo et al., 2008; Brankatschk et al., 2013) while others have not (Beraldi-Campesi et al., 2009; Moreira-Grez et al., 2019; Yang et al., 2019) reflecting a lack of consensus on the degree of connectivity between biocrusts and the mineral soil below. This is not entirely surprising, as nutrient pools can change dramatically and dynamically through time and, because they represent the net effect of multiple inputs and types of uptake/loss (Hart et al., 1994), they may not correlate with inputs or be dissimilar across crust types at a given time point. For example, in a separate experiment conducted on the Colorado Plateau, soil NO_3^- concentrations in the 0-10 cm soil layer almost doubled between winter and spring, while resin-extractable NO_3^- decreased around 17 times during the same time period (Zelikova et al., 2012). A separate study measuring 0-5 cm below lightly pigmented and darkly pigmented cyanobacterial crust on the Colorado Plateau did not observe large differences in inorganic N amounts among crust types over time (Barger et al., 2005). To more fully explore connectivity between biocrusts and the mineral soil, more studies examining resource pools within the biocrusts and mineral soils across time are needed.

4.3 Microbial CO_2 flux from sub-crust soils

Nutrients leached from the different biocrust types did not change short-term heterotrophic activity in sub-surface soils (Figure 6). This was unexpected, as we did see significant differences in leachate chemistry across biocrust successional states (e.g., total

dissolved organic C concentrations were more than twice as high in leachate from late successional moss dominated biocrust than in either of the earlier successional states) and as both microbial respiration and biomass responded to differences in leachate concentrations in other ecosystems (Cleveland et al., 2006; Qiu et al., 2005). While we observed short-term responses here, it is possible we would see larger differences with longer incubation times. Soil respiration rates in the mineral soil were relatively low compared with other systems, reinforcing the notion that subsurface soils have lower microbial activity (Miralles et al., 2012). When looking at the role of biocrust community type, the higher respiration rate coming from below the moss biocrusts suggests a longer-term influence of crust leachate on sub-surface microbial activity. The large amounts of organic C leached from moss crusts and the large total dissolved organic C pools found in the mineral soil below moss crusts may serve as an easily accessible source of C for heterotrophs in the mineral soil when water is available. These findings suggest a longer-term influence of moss crust on the mineral soil and microbial cycling (Dümig et al., 2013), namely through the accumulation of organic C in the soil over time, with consequences for the amount of C being released from dryland soils.

5. Conclusion

The vertical movement of soluble C and nutrients from the biocrust layer to the mineral soil may be one of the main mechanisms through which biocrusts contribute to mineral soil fertility. Here, we observed that the degree of connectivity between biocrusts and the mineral soil depends on the biocrust type and the resource being considered. The contrasting findings in the literature as to the role biocrusts play in providing fertility to the deeper soil layers further highlights how differences in biocrust type, element, soil depth, seasonality, and water inputs can

change the degree of connectivity between biocrusts and deeper soils. This study adds to our understandings of how different biocrust types and deeper mineral soil exchange fertility and provides nuance to the outcomes of nutrients and C cycling along successional gradients in dryland regions. Future studies manipulating multiple abiotic variables, such as soil texture and precipitation amounts, would further our understanding of connectivity and allow for improved predictions of large scale biocrust contributions to the mineral soil.

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Figures

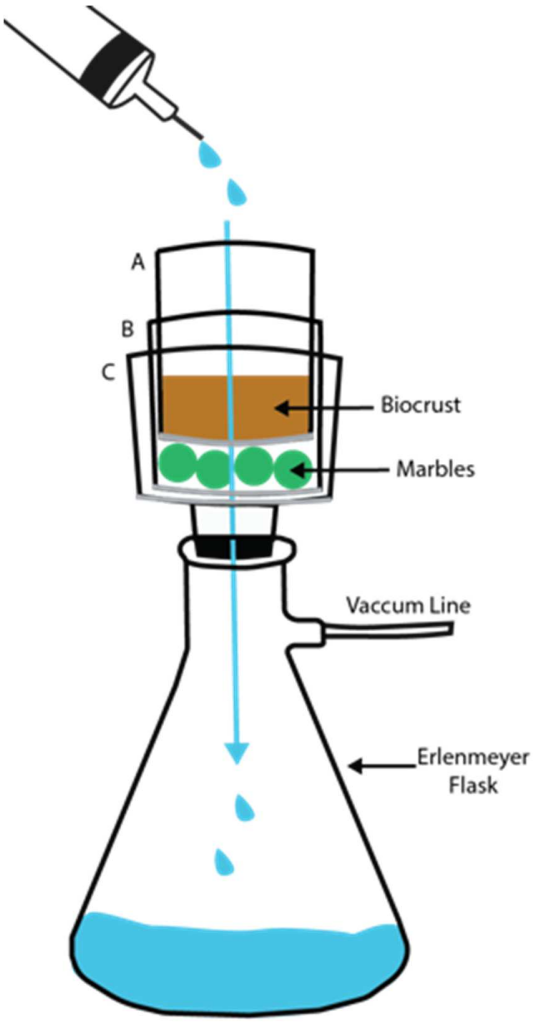

Figure 1. The collection system used to collect leachate from biocrusts. Biocrusts were placed in a plastic cylinder (A), that was open on the top with a 1.18 mm mesh screen (shown in grey) at the bottom. Cylinder A was placed within a second plastic cylinder (B) open on the top and with a layer of marbles resting on a mesh screen (shown in grey). Marbles were used to ensure sediment did not pool over the filter. Cylinders A and B were placed in a Buchner funnel (C) with a 55 mm Whatman #1 filter on the bottom (show in grey). Watering treatments were administered across the biocrust using a syringe. Water moved through cylinders A and B and

805 through the funnel and filter into an Erlenmeyer flask. Water was pulled through the system
806 using vacuum filtration and immediately collected and frozen until analysis.
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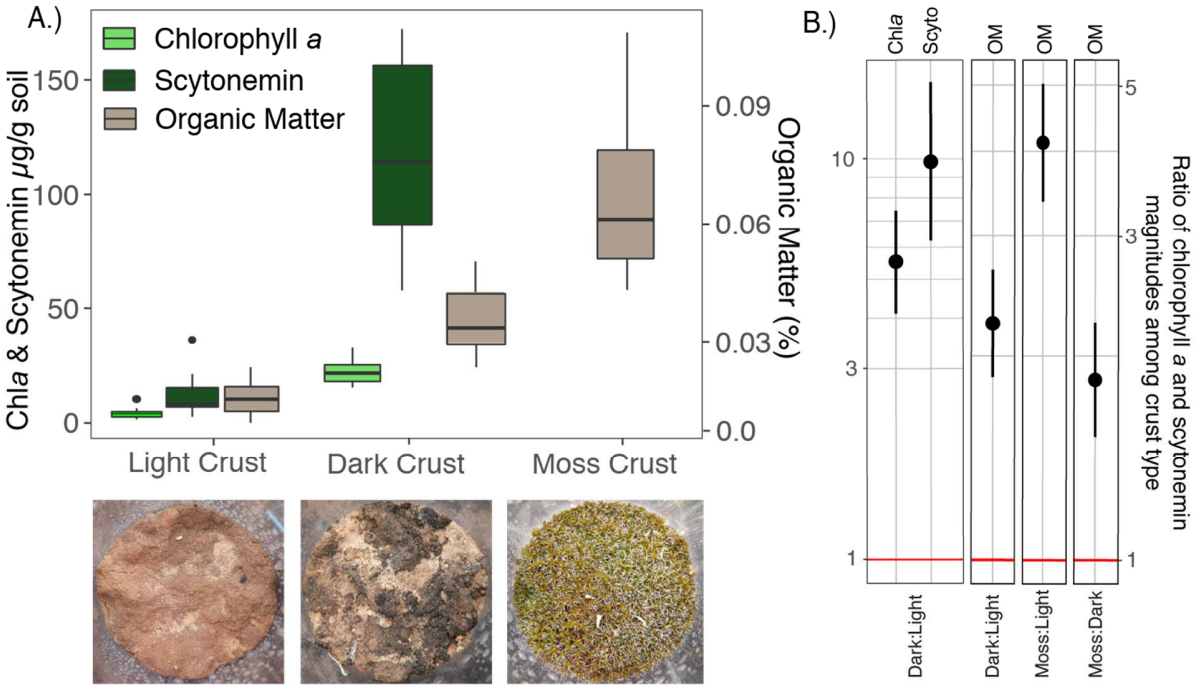

Figure 2. A.) The amount of chlorophyll *a* and scytonemin in µg per g of soil in lightly pigmented and darkly pigmented cyanobacterial crusts and the percent organic matter in all three biocrust types pictured in the figure (chlorophyll *a* and scytonemin were not measured in moss dominated crusts). Vertical bars on the boxplots represent median values and the vertical lines represent minimum and maximum values. B.) Ratios with 95 % confidence intervals among biocrust successional stage comparing chlorophyll *a*, scytonemin, and organic matter concentration. Confidence intervals not crossing 1 would be considered statistically significant.

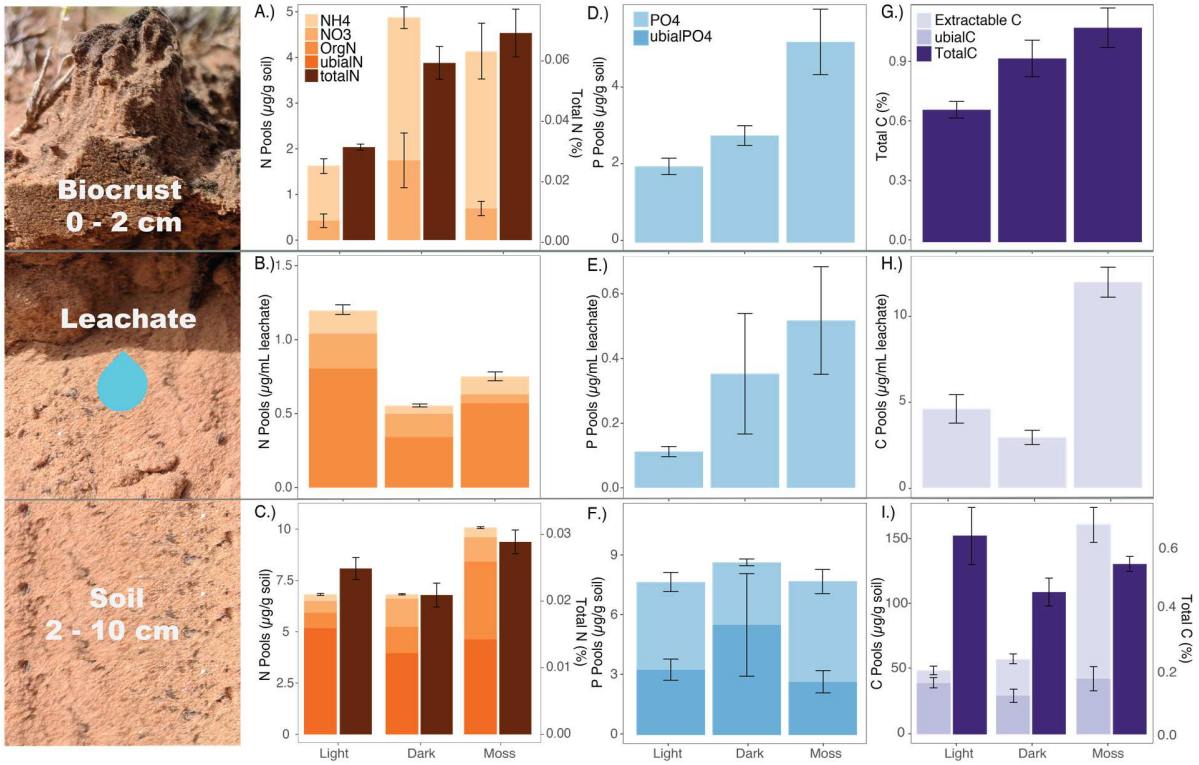

Figure 3. Top row: Nutrient and C concentrations in field collected biocrust (0-2 cm) (A., D., G.), middle row: nutrient and C concentrations released in leachate (B., E., H.) and bottom row: nutrient and C concentrations in the field collected soil directly beneath biocrust (2-10 cm) (C., F., I.). In the leachate layer, N, P, and C values are summed across the four time points of the experiment. Error bars represent SE. Overlapping error bars were removed to reduce confusion and are listed as follows: B). NO_3^-: light SE ± 0.022, dark SE ± 0.023 moss SE ± 0.018, TDN: light SE ± 0.14, dark SE ± 0.06, moss SE ± 0.07 C). NO_3: light SE ± 0.19, dark SE ± 0.27, moss SE ± 0.14, TDN: light SE ± 0.16, dark SE ± 0.14, moss SE ± 0.48, microbial N: light SE ± 0.54, dark SE ± 0.62, moss SE ± 0.96).

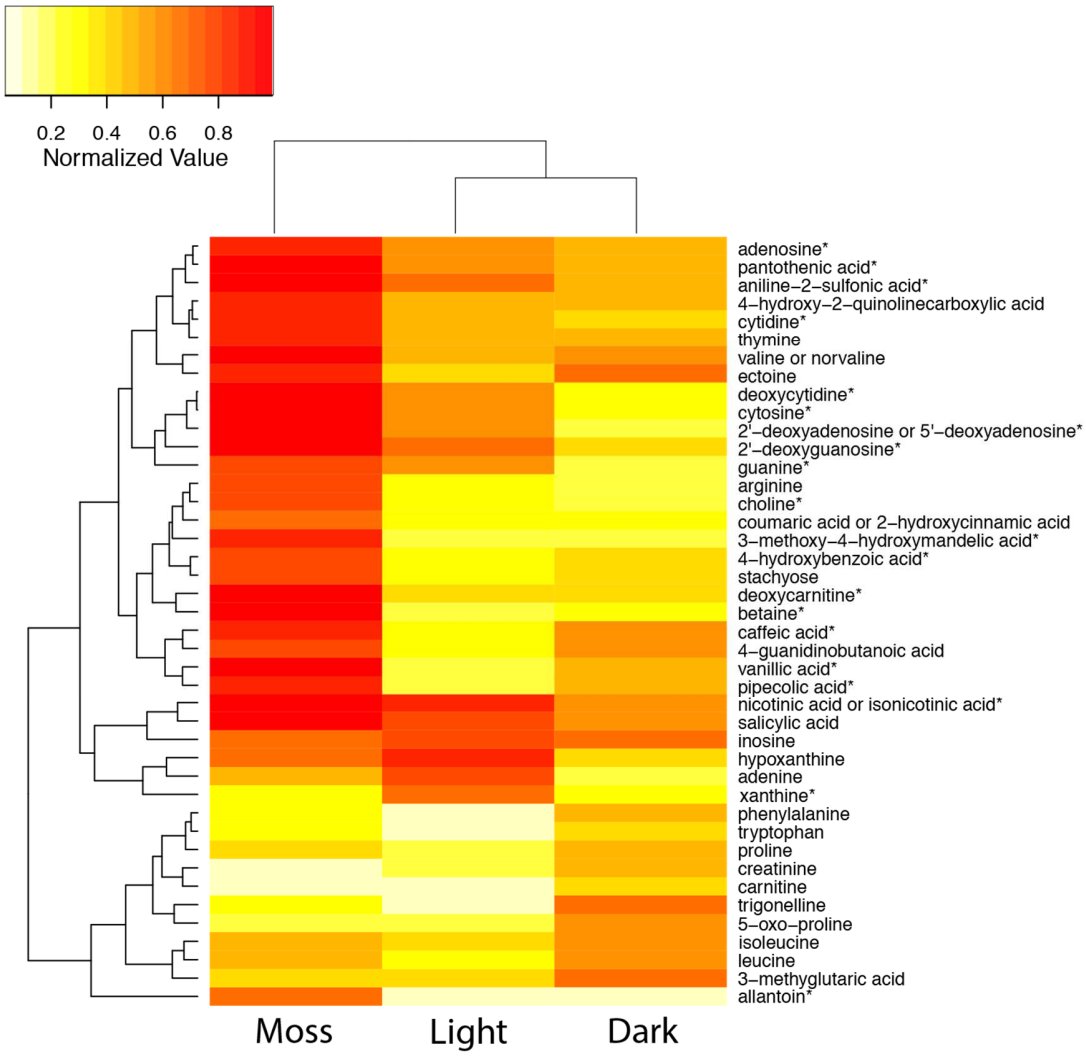

Figure 4. Comparison of relative intensity of detected metabolites from the three biocrust successional stages: moss dominated crust (Moss), lightly pigmented cyanobacterial crust (Light), and darkly pigmented cyanobacterial crust (Dark). Peak values were normalized to the largest peak value for each metabolite across biocrust successional stages. 0 indicates the lowest relative abundance and 1 represents the highest relative abundance. The dendrogram on the left and top clusters similarly extracted metabolites based on hierarchical clustering and the heat map displays the intensity of metabolites normalized to the most intense peak within each row

(metabolite). * indicates metabolite intensities that are statistically different (based on an alpha level of 0.05) among the three biocrust types. Only confirmed metabolites were included.

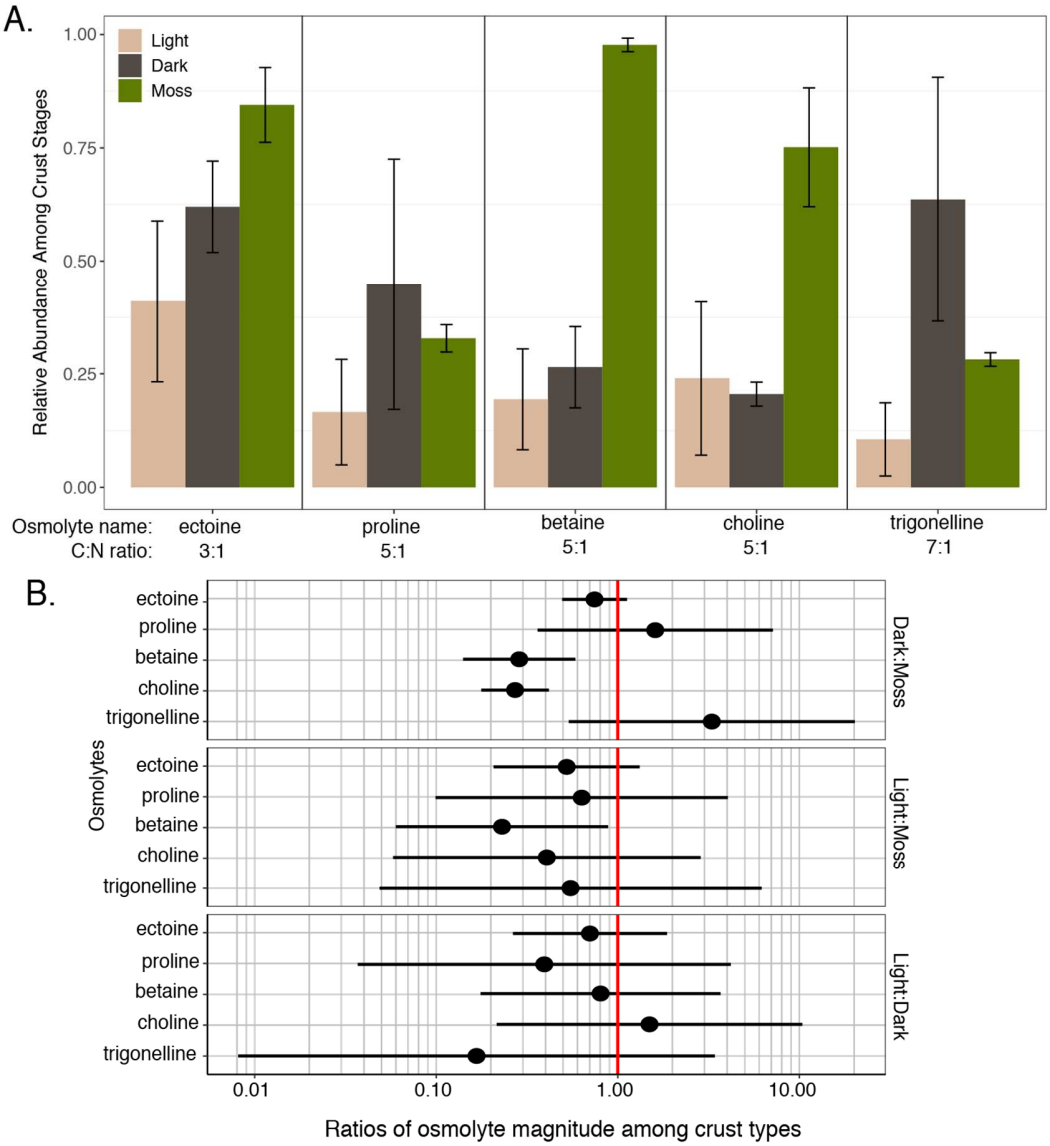

Figure 5: A. The normalized abundance among biocrust types of common, confirmed osmolytes found within the leachate in order of increasing C:N ratio. While comparisons across osmolyte types cannot be made, the order of magnitude of the average peak area across biocrust successional stages for each osmolyte is included to show potential differences in the quantity of the different osmolytes. B. Ratios with 95 % confidence intervals among biocrust successional

857 stages for each osmolyte. Confidence intervals not containing 1 would be considered statistically
858 significant.
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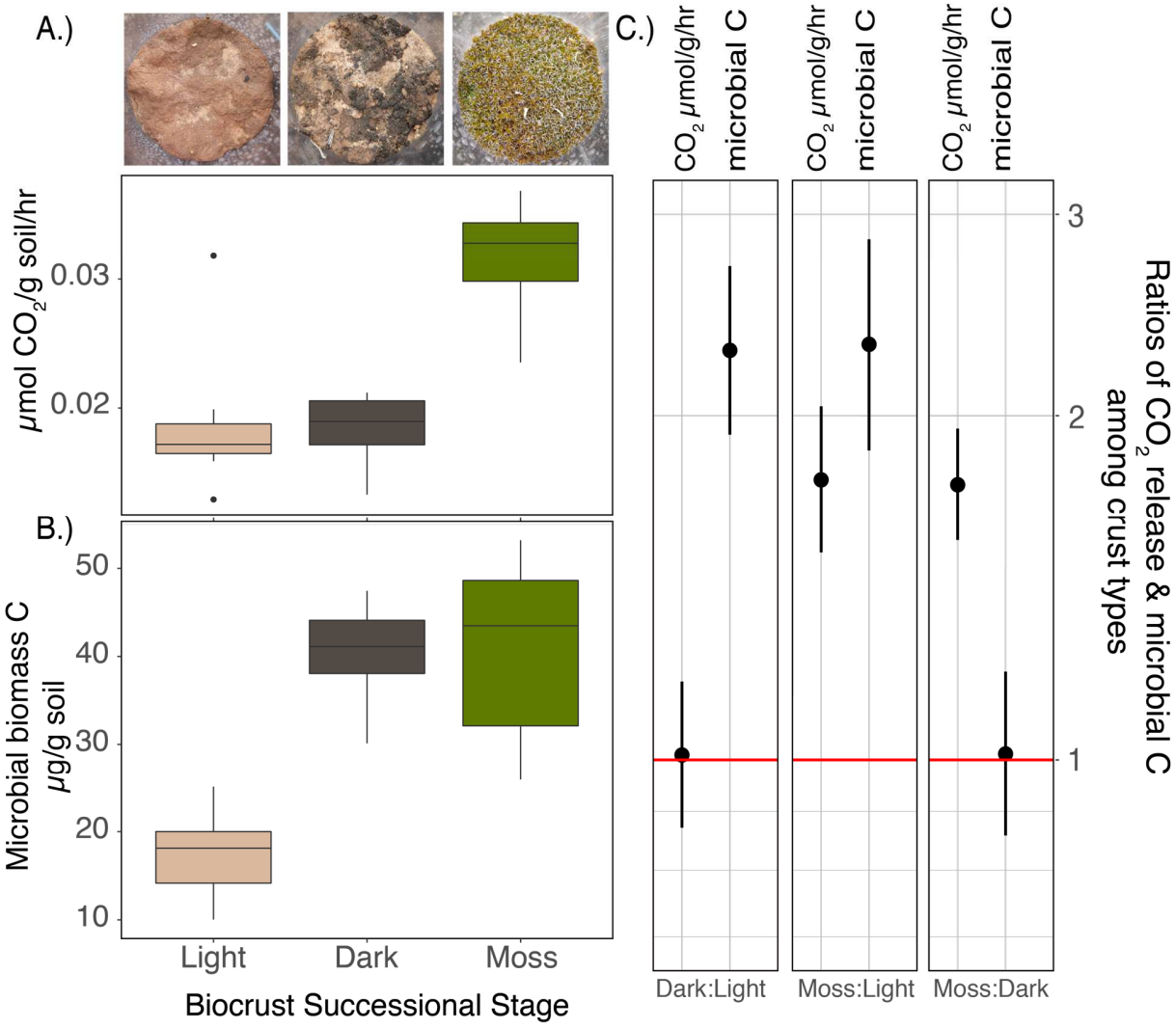

Figure 6. A). Respiration rates (µmol CO_2/g dry soil/hr) of soils collected from beneath the three biocrust successional states during a 24 hr incubation B). Changes to microbial biomass C concentrations within mineral soil collected from beneath the three different biocrust successional states after a 24 hr incubation. Vertical bars on the boxplots represent median values and the vertical lines represent minimum and maximum values. C). Ratios with 95 % confidence intervals among biocrust successional stages for soil CO_2 respiration (CO_2 µmol/g/hr) and microbial biomass C. Confidence intervals not crossing 1 would be considered statistically significant.