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Authors

Swenson, Tami L
Couradeau, Estelle
Bowen, Benjamin P
et al.

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REGULAR ARTICLE

A novel method to evaluate nutrient retention by biological soil crust exopolymeric matrix

Tami L. Swenson · Estelle Couradeau · Benjamin P. Bowen · Roberto De Philippis · Federico Rossi · Gianmarco Mugnai · Trent R. Northen

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Abstract
Aims Biological soil crusts (biocrusts) are microbial communities commonly found in the upper layer of arid soils. These microorganisms release exopolysaccharides (EPS), which form the exopolymeric matrix (EPM), allowing them to bond soil particles together and survive long periods of dryness. The aim of this work is to develop methods for measuring metabolite retention by biocrust EPM and EPS.
Methods We report new methods for the investigation of metabolite sorption on biocrusts compared to the underlying unconsolidated subcrust fraction. A ^{13}C–labeled bacterial lysate metabolite mixture was incubated with biocrust, subcrust and biocrust-extracted EPS. Non-sorbed metabolites were extracted and analyzed by liquid chromatography/mass spectrometry.
Results This simple and rapid approach enabled the comparison of metabolite sorption on the biocrust EPM or EPS versus mineral sorption on the underlying soils. Our results suggest that the biocrust (and its extracted EPS) sorb more metabolites, especially amino acids and organic acids, than the underlying subcrust.
Conclusions This study demonstrates a useful method to highlight the essential role of biocrust (especially the EPM), which acts as a passive nutrient filter, sequestering metabolites released by microbes during wetting events. This may facilitate recovery of the community upon wetting and further enhance biocrust survival and nutrient retention.

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T. L. Swenson (✉) · E. Couradeau · B. P. Bowen · T. R. Northen (✉)
Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA
e-mail: tlswenson@lbl.gov
e-mail: TRNorthen@lbl.gov

R. De Philippis · F. Rossi · G. Mugnai
Department of Agrifood Production and Environmental Sciences, University of Florence, Florence, Italy

R. De Philippis
Institute of Ecosystem Study, CNR, Sesto Fiorentino, FI, Italy

T. R. Northen
Department of Energy, Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA

Keywords Biological soil crusts · Exopolymeric matrix · Exopolysaccharides · Metabolomics · Sorption

Abbreviations

EPM	exopolymeric matrix
EPS	exopolysaccharides
LC/MS	liquid chromatograph-mass spectrometry
XRPD	X-ray powder diffraction

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Introduction

Biological soil crusts (biocrusts) are a major component of arid lands ecology, where they colonize plant-free patches of land encompassing 20% of Earth's continental area. These millimeter-thick top soil formations result from the intricate relationship between the soil material and the microbial communities inhabiting it (Garcia-Pichel 2003). Biocrust microbial communities are a major contributor to global nitrogen and carbon cycling (Elbert et al. 2012), fertilizing arid lands and sustaining desert ecosystems. Although essential to arid lands ecology, the fate of biocrusts is susceptible to warming effects (Maestre et al. 2013) and alterations in precipitation frequency (Belnap et al. 2004; Johnson et al. 2012) which are predicted under the current climate change scenario (Reed et al. 2016).

Understanding how biocrust communities undergo extended periods of dryness and are able to resume high levels of metabolic activity upon wetting (Garcia-Pichel and Belnap 1996) might be crucial to better predict their response to climate change. The primary ecosystem engineers for many biocrusts are the Cyanobacteria *Microcoleus* spp. (Gundlapally and Garcia-Pichel 2006; Zheng et al. 2010). Biocrust succession starts with colonization of bare soils by *Microcoleus* and subsequently by other biocrust organisms, increasing its stability and deeply modifying soil hydrological, physical and chemical properties (Reynolds et al. 2001; Belnap 2006; Rossi et al. 2012; Couradeau et al. 2016). Ultimately, this leads to biocrust layers that are enriched in C and N and depleted in Ca, Cr, Mn, Cu, As and Zr compared to the underlying unconsolidated subcrust soils (Beraldi-Campesi et al. 2009).

Biocrust communities exhibit cyclic active and dormant states based on the availability of water (Garcia-Pichel and Pringault 2001), implying highly specialized physiological traits enabling switching between these two states (Rajeev et al. 2013). Many biocrust organisms have adaptations for these desiccation cycles including bacteria, fungi, lichens and mosses (Rajeev et al. 2013; Murik et al. 2016). Specifically, some microbes may survive desiccation through vitrification of their cytoplasm with compatible solutes (Sun and Leopold 1997). Wetting is thought to cause membrane leakage and release of compatible solutes and other intracellular metabolites resulting in either mineralization or in the loss of essential nutrients that are critical for community survival (Morbach and Krämer 2002; Schimel et al. 2007).

The biocrust exopolymeric matrix (EPM), mainly of polysaccharidic nature (Al-Thani 2015), supports the action of bacterial filaments on conglomerating soil particles into coherent crusts (Garcia-Pichel and Wojciechowski 2009) and creates a favorable microenvironment in terms of nutrient and moisture distribution. Due to its hygroscopic nature, the EPM absorbs and retains water (Mager and Thomas 2011), helping cells adapt to desiccation cycles by reducing hydration rates. The EPM is a suprastructure of exopolysaccharides (EPS), that are either loosely bound to cells and sediments or more "condensed" as tightly bound material or as cyanobacterial sheaths (Rossi et al. 2017). Although polysaccharidic material is prominent in the EPM, other organic molecules are associated with cyanobacterial EPS. For instance, the analysis of *Microcoleus vaginatus* (*M. vaginatus*) EPS revealed that peptides constituted 50% of its EPS dry weight (Pereira et al. 2009). EPS provide for a variety of essential functions for biocrusts including water retention, structural stability, gliding motility, UV protection and a nutrient source (Mager and Thomas 2011; Colica et al. 2014; Rossi and De Philippis 2015; Rossi et al. 2017). Biocrust pH is circumneutral and turns alkaline as a result of photosynthetic activity (Garcia-Pichel and Belnap 1996), therefore EPS whose isoelectric points are acidic (under pH 4–6) are typically negatively charged under daytime physiological conditions. EPS are indeed known to complex divalent cations such as Ca^{2+} and Mg^{2+} (Braissant et al. 2007; De Philippis et al. 2011) and in the context of biocrusts, they may also interact with positively charged metabolites. We hypothesized that EPS, or more generally the EPM and associated biologically-inactive biocrust components, might act as a passive trap that may retain nutrients (i.e. metabolites), facilitating recovery of biocrusts upon wetting. Yet, we are not aware of existing methods to measure these processes.

Metabolomics is a useful tool to gain insights on functional processes including those occurring within natural environments and microbial communities. Specifically, exometabolomics has been used to examine biocrust soil water metabolite dynamics following a wetting event (Baran et al. 2015) and to evaluate how individual bacterial isolates transform this small molecule environment based on substrate preferences (Baran et al. 2015). Mechanisms of abiotic metabolic dynamics have been evaluated by analyzing the competitive sorption of ^{13}C–labeled bacterial metabolites on minerals

(Swenson et al. 2015a) and on a sandy loam soil (Swenson et al. 2015b). Metabolomics approaches such as these are invaluable in furthering our understanding of how both active (microbes) and passive (minerals, EPM) factors can impact accessible nutrients in environments, particularly biocrusts, where the EPM accounts for a large fraction of the soil surface. These processes may contribute substantially to nutrient dynamics, facilitating the survival of this ecosystem.

Here, we expand on our ^{13}C–labeled bacterial metabolite sorption method using liquid chromatography-mass spectrometry (LC/MS) with biocrust to examine the degree to which organic matrix components (primarily EPS and associated cell components) sorb metabolites compared to the underlying unconsolidated subcrusts (Fig. 1). Here we define sorption as the process by which the material (biocrust, subrust or EPS) adsorbs and absorbs metabolites. We then integrate these methods with those for extraction of EPS from biocrust to examine metabolite sorption on this specific EPM component. To the best of our knowledge, this is the first approach to explore sorption of specific metabolites from a complex bacterial metabolite pool on biocrust.

Materials and methods

Materials

LC/MS-grade water and LC/MS-grade methanol (CAS 67-56-1) were from Honeywell Burdick & Jackson (Morristown, NJ), LC/MS-grade OmniSolv acetonitrile (CAS 75-05-8) was from EMD Millipore (Billerica, MA) and ammonium acetate (CAS 631-61-8) was from Sigma (St. Louis, MO). LC/MS internal standards included: MOPS (CAS 1132-61-2), HEPES (CAS 7365-45-9), ^{13}C-15 N-L-phenylalanine (CAS 878339-23-2), 4-(3,3- dimethyl-ureido)benzoic acid (CAS 91880-51-2), d5-benzoic acid (CAS 1079-02-3), 9-anthracene carboxylic acid (CAS 723-62-6) all from Sigma (St. Louis, MO).

Soil collection and preparation

Cyanobacterial-dominated biocrust samples were cored and collected using multiple Petri dishes (28 cm^2 × 1 cm depth) from the Green Butte Site near Canyonlands National Park (38°42'54.1"N, 109°41'27.0"W, Moab, UT, USA). Soils were brought back to the laboratory and maintained in a dark desiccation chamber, at room temperature. For this study, biocrusts (from two Petri dishes) were selected from an apparent late/mature successional stage typically seen at the field site (Fig. 2a). Biocrusts were physically separated from subcrusts by gently lifting up the adhered topsoil fraction (Fig. 2b). Biocrusts and subcrusts were homogenized to control for spatial variation using a tissue homogenizer (TissueLyser II, Qiagen) with stainless steel beads for 2 × 30 s. These were then autoclaved four times to eliminate biological activity. Sterility was checked by extracting soil (1 g) with sterile water (4 mL) for 1 h, centrifuging at 3220 x g for 15 min and streaking out an

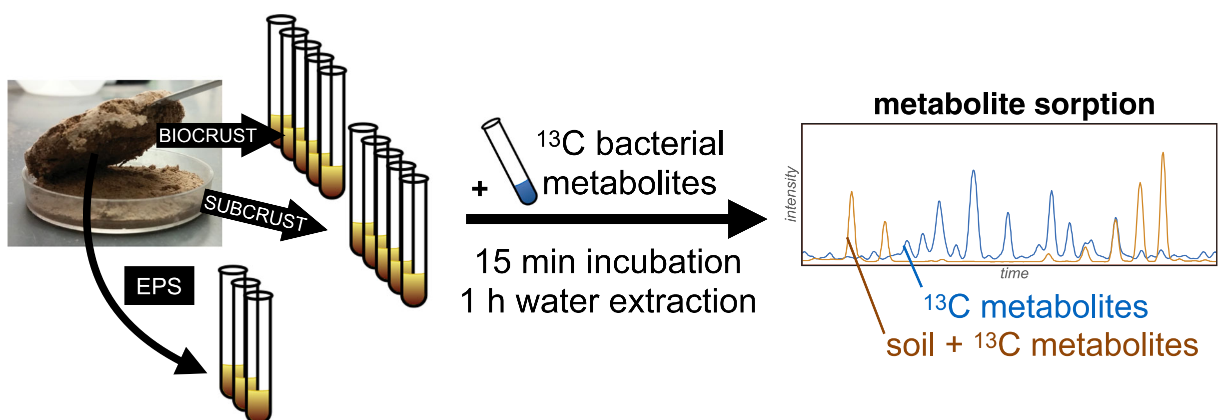

Fig. 1 Workflow used to examine sorption of bacterial metabolites on biocrust, subcrust and EPS. Biocrust from a dark successional stage was separated from the underlying subcrust layer and EPS was extracted from the biocrust. Samples were incubated with ^{13}C bacterial metabolites for 15 min and non-sorbed metabolites were extracted with water and analyzed by LC/MS. Data were compared to controls containing either ^{13}C bacterial metabolites only or soils/ EPS only to determine (percent) metabolite sorption

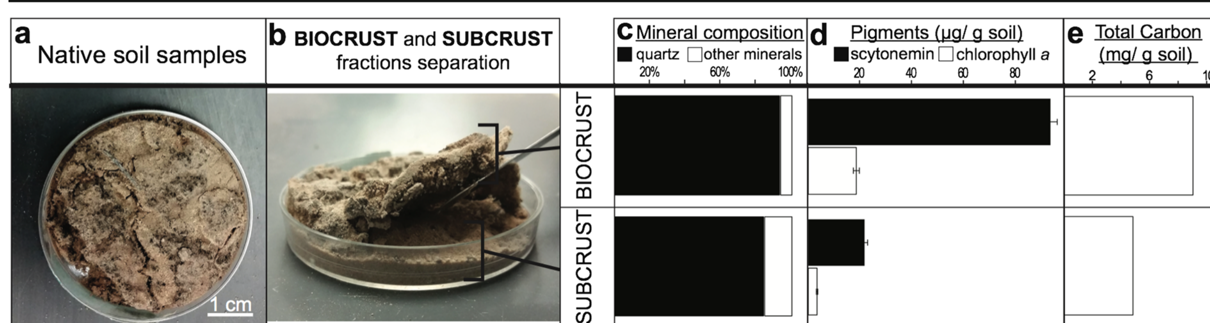

Fig. 2 Characteristics of the two biocrust fractions compared for metabolite sorption. Biocrusts were collected (**a**) and separated into biocrust and subcrust fractions (**b**). The biocrust contained higher quartz content ($n = 1$) (**c**) and pigment concentrations (scytonemin and chlorophyll *a,* $n = 3$) compared to the subcrust (**d**). The biocrust also had a higher total carbon content ($n = 1$) (**e**) compared to the subcrust. The detailed mineral composition can be found in Supplementary Table 3

aliquot (100 uL) of the supernatant on LB agar plates. No growth was observed after incubation at 30 °C for at least two weeks.

Soil property measurements

The quantitative phase analysis of the mineralogical composition of the soils ($n = 1$) was carried out using X-ray powder diffraction (XRPD). Samples were ground into a powder using an agate mortar and then loaded onto a glass slide to be measured in a Rigaku SmartLab X-ray diffractometer, under a bragg-Brentano geometry in a theta-theta configuration. Data were collected from 4° to 70° of 2θ, using Kα radiation. After manual identification of the phases present, a Rietveld refinement analysis of the collected XRPD profile (Snyder 1993) using the MAUD software (Lutterotti et al. 1999) was performed to obtain their weight fractions. The percentage of clay minerals may have been underestimated by a few percentages in this bulk analysis.

To measure soil pH, dry soil (1 g) was mixed with double deionized water (2 mL). Sample pH was also measured on the sorption reactions before and after the 1 h water extraction ($n = 1$). Total pigments were extracted by grinding soil samples (approximately 0.5 g) with a mortar and pestle in 2 mL 90% acetone for 3 min ($n = 3$). Samples were then transferred to 15 mL polystyrene tubes, brought up to 4 mL with 90% acetone and incubated in the dark at 4 °C for 24 h. Samples were then centrifuged at 7000 rpm for 5 min and supernatant aliquots (200 uL) were transferred to a clear 96-well flat-bottom plate. Absorbances were measured at 384 nm, 490 nm and 663 nm. Pigment analysis was performed in triplicate for each soil. Chlorophyll *a* and scytonemin concentrations were calculated using trichromatic equations (Garcia-Pichel and Castenholz 1991) using an extinction coefficient of 89.7 g^{-1} cm^{-1} for chlorophyll *a* (Ziegler and Egle 1965) and 112.61 g^{-1} cm^{-1} for scytonemin (Brenowitz and Castenholz 1997). Total carbon was measured on soils (0.5 g) using catalytically-aided combustion with a Shimadzu SSM-5000A ($n = 1$).

EPS extraction

EPS (combined soluble, sheaths and capsules) were collected following the methods of Mugnai et al. (2017) with modifications. Homogenized and autoclaved biocrust (1 g per replicate) was transferred to a 15 mL conical tube and extracted with 3 mL LC/MS-grade water by vortexing for 15 min. Soils were pelleted by centrifuging at 4000 x g for 30 min at 4 °C and transferring the supernatant to a new 15 mL tube. This process was repeated two more times (with 5 mL water each time) for a total of 13 mL of extracted soluble EPS. Bound EPS (likely containing cyanobacterial sheaths) were extracted by adding 5 mL water to the pellet from above, vortexed and incubated at 80 °C for 1 h. Soils were pelleted by centrifuging at 4000 x g for 30 min at 4 °C and the supernatant added to the soluble EPS fraction. Extracts were frozen at −80 °C and lyophilized until dry.

Sorption assay

The sorption assays for biocrusts, subcrusts and EPS were all performed separately with their own sets of

controls. Heavy (^{13}C–labeled) metabolites were prepared using *Pseudomonas stutzeri* RCH2 as described in Swenson et al. 2015b. The final metabolite lysate was resuspended in LC/MS-grade water then added (500 uL) to each soil (1 g) or EPS (extracted from 1 g biocrust) in 15 mL polystyrene tubes and shaken (Orbital-genie, Scientific Industries, Bohemia, NY) at 200 rpm at 4 °C for 15 min. Soluble (non-sorbed) metabolites were extracted with LC/MS-grade water (4 mL) by shaking at 200 rpm at 4 °C for 60 min and centrifuged at 3220 x g for 15 min. Supernatants were filtered through 0.45 um syringe filters (Pall Acrodisc Supor membrane) into 5 mL Eppendorf tubes and lyophilized for 12 h. Five replicates for biocrust and subcrust (each taken from their pool of homogenized and autoclaved soils) and three replicates for EPS were analyzed. Controls (each with 3–5 replicates) included water (no soil or EPS and no lysates), no-soil or EPS (^{13}C lysates only) and no-lysates (soil or EPS only), which underwent the same extraction conditions as the experimental samples. Unlabeled (^{12}C) *P. stutzeri* lysates were prepared for metabolite identification. To account for pH affects that occur during soil incubation (raising the pH to 9.3), the sorption experiment was also performed by incubating ^{13}C lysates with neutral water and pH 9.3 water ($n = 3$).

LC/MS

Lyophilized samples from the sorption experiments were resuspended in methanol (150 uL) containing internal standards (each at 5 μg/mL), vortexed then filtered through 0.22 um centrifugal membranes (Nanosep MF, Pall Corporation, Port Washington, NY). Metabolites were analyzed using normal-phase LC/MS with a ZIC-HILIC column (100 mm × 2.1 mm, 3.5 μm, 200 Å, Millipore) for the biocrust and subcrust experiments and a 150 mm × 2.1 mm, 3.5 μm, 200 Å column (Merck Sequant, Darmstadt, Germany) for the EPS experiment. An Agilent 1290 series UHPLC (Agilent Technologies, Santa Clara, California, USA) was used for metabolite separation using two mobile phases: 5 mM ammonium acetate in water (A) and 95% acetonitrile, 5% 100 mM ammonium acetate in water (B). Chromatography was performed using the following gradient: 100% B for 1.5 min (at 0.45 mL/min), linear decrease to 65% B by 15 min (at 0.45 mL/min), then decrease to 0% B by 18 min (at 0.6 mL/min), held until 23 min (at 0.6 mL/min) then returned to initial conditions by 25 min (at 0.45 mL/min) with a total runtime of 30 min. Column temperature was maintained at 40 °C. Negative and positive mode data were collected at a mass range of 70–1050 *m/z* in centroid data mode on a Thermo QExactive (Thermo Fisher Scientific, Waltham, MA). Fragmentation spectra (MS/MS) were acquired for some metabolites using collision energies of 10–40 eV.

Data analysis

Metabolomics data were analyzed using Metabolite Atlas with the Python programming language (Yao et al. 2015). Instrument performance was monitored throughout the sequences by analyzing sample internal standards and quality control mixtures that were run at the beginning (in triplicate), end (in triplicate) and individually interspersed every 15 samples. Metabolite identifications were based on unlabeled ^{12}C–lysates and confirmed by the *m/z* shift in ^{13}C–lysates corresponding to the number of carbons for each metabolite. Identifications were verified using two orthogonal data relative to authentic standards including retention time, *m/z* and MS/MS fragmentation patterns as provided in Supplementary Table 1. Some metabolites were unavailable as authentic standards and were putatively identified based on theoretical retention time, *m/z* and corresponding ^{13}C shift in *m/z*. Metabolites that were detected in the water controls and those that were affected by increased pH were excluded from sorption analyses.

Percent sorption and statistical analyses

For each metabolite, percent sorption was calculated by subtracting the ^{13}C–*m/z* peak height contribution from the soil background (no-lysate controls) and dividing by the no-soil control peak height:

$$^{13}\mathrm{C}\ m/z\ \text{peak area}_{(\text{soil+lysates})} - {}^{13}\mathrm{C}\ m/z\ \text{peak area}_{(\text{soil})} = \text{recovered}\ {}^{13}\mathrm{C}\ m/z_{(\text{lysates})}$$

$$100-\left[\left(\text{recovered}\ {}^{13}\mathrm{C}\ m/z_{(\text{lysates})}/\text{added}\ {}^{13}\mathrm{C}\ m/z_{(\text{no-soil control})}\right)\ \mathrm{x}\ 100\right] = \%\ \text{sorbed}$$

Since the focus of this test was to assess sorption effects, any negative values were set equal to zero. The degree of variation in sorption profiles were visualized by PCA ordination on percent sorption values using ggbiplot in R. To examine specific metabolites, a metabolite was considered "sorbed" if the recovered ^{13}C $m/z_{\text{(lysates)}}$ was significantly less than ^{13}C $m/z_{\text{(no-soil control)}}$ with a p-value <0.01. Specifically, significance was determined based on the one-sided Dunnet's test (using the multcomp package in R) on recovered ^{13}C $m/z_{\text{(lysates)}}$ values compared to ^{13}C $m/z_{\text{(no-soil control)}}$ values, all normalized to the average of ^{13}C $m/z_{\text{(no-soil control)}}$ peak areas within each dataset (Supplementary Table 2A). To assess whether sorption dynamics were a result of matrix effects or ion suppression from soil or EPS background metabolites, internal standards were also analyzed by the Dunnet's test except using a two-sided analysis with a $p < 0.01$ considered significant (Supplementary Table 2A). Additional analyses used to exclude substantial matrix effects included comparing peak heights of the ^{12}C metabolite from the soil background with the ^{13}C metabolites from the added lysates (Supplementary Table 2B).

Results

Biocrust and subcrust soil properties

To explore the impact of biocrust organic matrix constituents (i.e. the EPM and other cellular components) on metabolite sorption, two biocrust fractions were initially compared to examine the effects of EPM abundance, biocrust versus subcrust (Fig. 2b). XRPD was used to characterize the mineral composition of the biocrust and subcrust, both of which were found to be composed primarily of quartz (at least 84%) (Fig. 2c). Although $n = 1$ for the XRPD analyses, our results suggest that the subcrust contained higher levels of minerals (Fig. 2c) including potassium-feldspar (K-spar), calcite, albite low, smectite and dolomite (Supplementary Table 3). Chlorophyll *a*, a common proxy for biocrust biomass (Yu et al. 2012), was found to be highest in the biocrust indicating that this sample had a higher abundance of phototrophic organisms than the subcrust (Fig. 2d). As expected, the sunscreen pigment, scytonemin, a proxy for biocrust maturity level, was also higher in the biocrust (Fig. 2d) and total carbon was approximately two times higher in the biocrust than the subcrust (Fig. 2e).

To understand the types of physiochemical interactions that may occur during metabolite sorption, the pH of the soils and of the reactions were measured. Soils were alkaline with pH values ranging from 9.3 to 9.4. The sorption conditions did not alter the pH and remained the same from the beginning to the end of the 1 h water extraction (ranging from 9.1 to 9.4). Furthermore, the metabolites analyzed for this study were not affected by this increased pH (Supplementary Table 4).

Metabolite identification

A ^{13}C isotopically-labeled lysate prepared from the soil bacterium, *Pseudomonas stutzerii,* was used to differentiate added metabolites from the diverse endogenous biocrust, subcrust and EPS metabolites. This organism was selected since the metabolite composition is well-characterized (Swenson et al. 2015a, 2015b) and has much in common with biocrust porewater metabolite composition (Baran et al. 2015). Normal-phase LC/MS analysis led to the annotation of 76 small polar metabolites (Supplementary Table 1), most verified by authentic standards as indicated in the methods. Of these, 9 metabolites were not verified by standards and are considered putative. Metabolites spanned many classes including amino acids, organic acids, nucleosides, nucleotides, nucleobases, carbohydrates, cofactors and vitamins.

Overall sorption dynamics

Metabolite sorption patterns were visualized by PCA ordination, which displayed a clear separation of the biocrust from the subcrust along the first principal component which accounted for 63.8% of the variance (Fig. 3). The EPS (consisting of pooled loosely bound and bound fractions) samples clustered with the biocrust along the first principal component, but separated along the second, presumably because there was a core set of sorbed metabolites common to both biocrust and EPS, but EPS also had some unique sorptive properties. Furthermore, the distributions of percent sorption demonstrate that the subcrust overall sorbed less than the biocrust and EPS (Fig. 3). While instrument artifacts (e.g. ion suppression due to competing ions from the soil background) may skew results, this was unlikely the

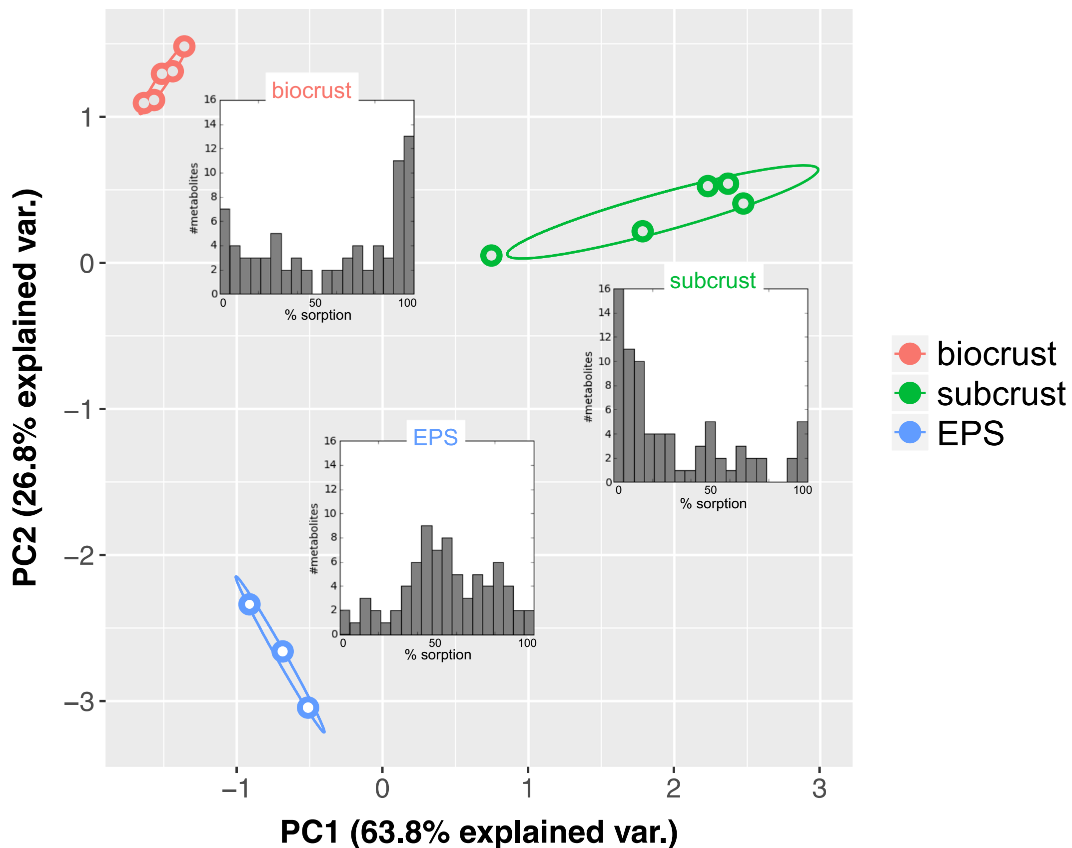

Fig. 3 PCA ordination and distribution of metabolite sorption on the biocrust versus the subcrust. Sorption data for each soil sample and EPS were clustered and the percent variance explained is indicated on each axis. The average distributions of sorption for each sample are displayed in the inset histograms

case in our study. This assertion is supported by the observation that the internal standards within the retention time window of most metabolites (0–12 min) were not significantly different from the non-soil controls and experimental samples (Supplementary Table 2A). Further, the ratios of soil background ^{12}C metabolite to added ^{13}C metabolites did not display any trends with sorption (Supplementary Table 2B).

For this study, a metabolite was considered "sorbed" when there was a significant ($p < 0.01$) decrease of metabolites relative to the non-soil controls. Of the 76 metabolites detected from the bacterial lysate, 67 displayed sorption in at least one condition (Fig. 4a and b): 57 on biocrust, 33 on subcrust and 61 on EPS. Of this pool of metabolites, 30 sorbed on all three samples and represented a relatively even distribution of metabolite classes, however, compared to the biocrust/ EPS-specific metabolites, there was a slight enrichment of nucleotides and/or phosphate-containing metabolites (e.g. FAD, NADH, adenosine monophosphate, deoxyadensoine monophosphate, 5'-thymidylic acid, uridine diphosphate glucose, uridine 5'-monophosphate), some nucleosides (e.g. 5-methyluridine, S-adenosyl-homocysteine, S-adenosyl-methionine and methylthioadenosine) and nucleobases (e.g. urate, uracil, 3-methyladenine, adenine, cytosine, dihydrouracil, thymine and xanthine) (Fig. 4c).

Metabolite sorption on biocrust and EPS versus subcrust

While field replicates were not utilized (to avoid spatial soil heterogeneity), results were obtained by comparing biocrust and subcrust replicates from homogenized soil samples. Comparing metabolite sorption on subcrust versus biocrust and the biocrust-extracted EPS yields important information regarding metabolite retention by the EPM within biocrust. Interestingly, the subcrust did not uniquely sorb any metabolites. On the other hand, more than 25% of metabolites analyzed in this study displayed preferential sorption on both the biocrust

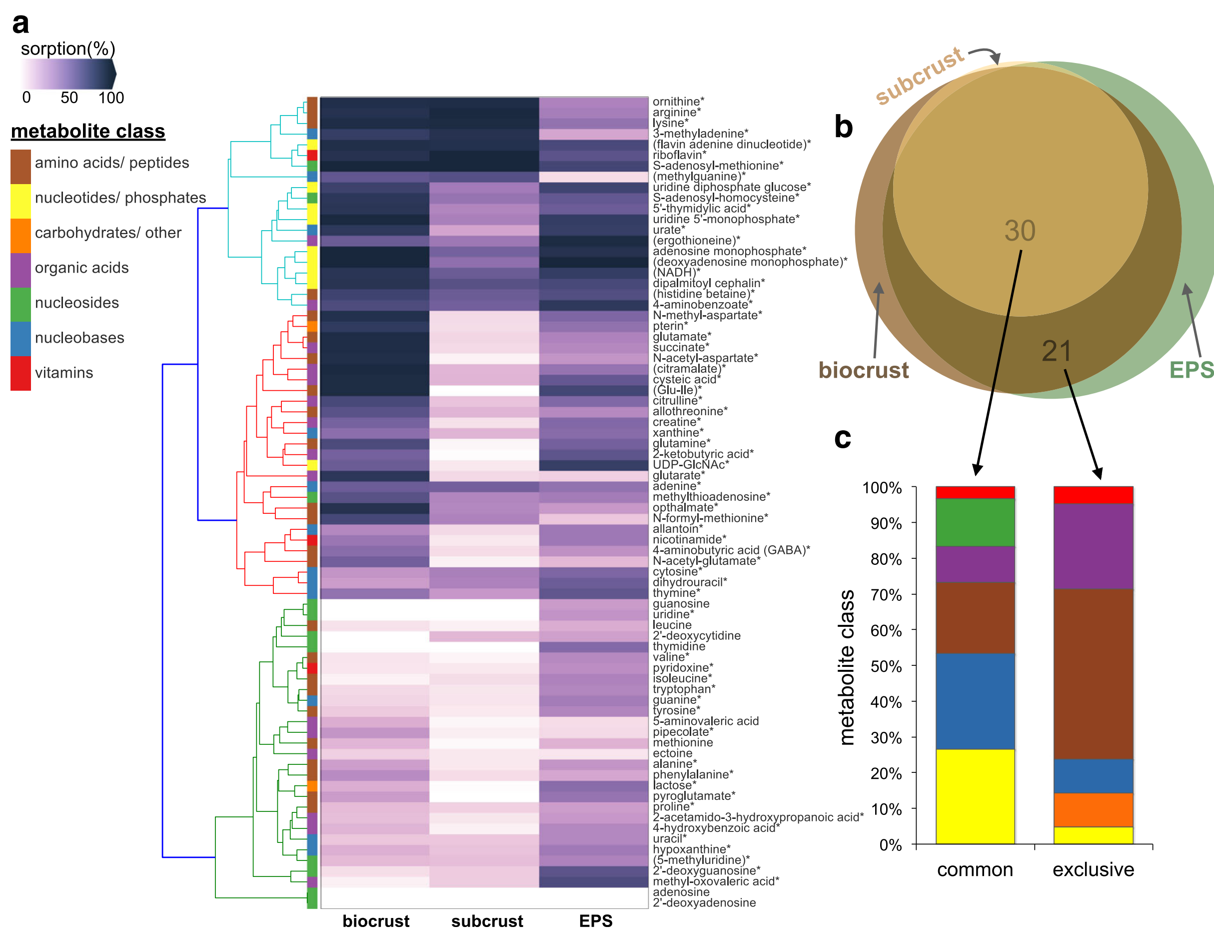

Fig. 4 Bacterial metabolite sorption on biocrusts, subcrusts and EPS. For each metabolite, the percent sorption relative to the non-soil control is displayed for the biocrust, the underlying subcrust and EPS extracted from the biocrust. Putative metabolite identifications are indicated in parentheses (**a**). Out of the 76 metabolites analyzed, 30 were significantly sorbed ($p < 0.01$) on both the biocrust and subcrust and 21 metabolites sorbed exclusively on both biocrust and EPS (but not the subcrust) (**b**). Compared to the fraction of metabolites that sorbed on both the biocrust and subcrust ("common"), biocrust- and EPS-specific ("exclusive") metabolites were enriched in amino acids and organic acids (**c**). $n = 5$ for each soil and non-soil control. $n = 3$ for the EPS experimental set. *These metabolites were significantly sorbed ($p < 0.01$) in at least one condition (see Supplementary Table 1)

and EPS (rather than on subcrust). These metabolites were highly enriched in generally anionic organic acids (e.g. citrulline, creatine and succinate) and amino acids (e.g. alanine, glutamate, glutamine, N-acetyl- and N-methyl-aspartate, proline and tyrosine) (Fig. 4c). This substantial sorption of metabolites on EPS is consistent with its known function in biocrust, and as further evidence of this, EPS sorbed more metabolites than biocrust (Fig. 4a and b). These nine EPS-exclusive metabolites were from a variety of metabolite classes and include 2-acetamido-3-hydroxypropanoic acid, 4-hydroxybenzoic acid, guanine, isoleucine, methyl-oxovaleric acid, pyridoxine, tryptophan, uridine and valine.

Discussion

The central focus of this study was to demonstrate a readily accessible method used to assess metabolite sorption on the inactive chemical constituents present in biocrust and subcrust. Overall, our results support what would be expected based on general biocrust characteristics: biocrust sorbed more metabolites than subcrust, which could be attributed to passive interactions with non-bioactive components (particularly EPS). This method avoids costs associated with purchasing ^{13}C–labeled metabolites by using bacteria to produce a relevant metabolite mixture and is rapid with only a

15 min sorption incubation. While this approach yielded some important insights into these chemical interactions, one limitation is the disruption of the soil structure induced by homogenizing and autoclaving. Thus, it is important to keep in mind that with intact biocrust, structural interactions are likely to result in altered metabolic sorption dynamics. Additionally, effects due to soil heterogeneity were limited by using replicates from homogenized soil rather than using spaced-out field replicates. This approach limits the general conclusions that can be drawn for biocrust and our observed results only demonstrate differences for biocrust and subcrust from these specific samples.

Metabolite sorption on biocrust and subcrust

Among the most overall sorptive bacterial metabolite classes were phosphate-containing metabolites, organic acids and amino acids (e.g. uridine 5'-monophosphate, adenosine monophosphate and citramalate) which were also the most sorptive organic matter components on an iron oxide mineral, ferrihydrite (Swenson et al. 2015a) and on a sandy loam soil (Swenson et al. 2015b). Many of these metabolites also sorbed to all three samples tested here, all of which presumably contain varying concentrations of EPS. However, the mineral composition undoubtably contributes to the sorption effects observed, especially with the subcrust where higher mineral content was observed relative to the biocrust (Supplementary Table 3). Many of these minerals are known to form physiochemical interactions with organic material (e.g. direct bond formation with metal cations or electrostatic interactions) (Kleber et al. 2007), especially nucleic acids (Cleaves II et al. 2011; Feuillie et al. 2013). Phosphate-containing metabolites (including nucleotides), which sorbed to both the subcrust and biocrust, may be bound to surface metals (K+, Al + or Na+) present on the surface of many of these minerals or possibly be sorbed by a ligand exchange mechanism (Feuillie et al. 2013). Despite these known roles of minerals, of the metabolites that sorbed to the subcrust, only two (methylguanine and N-formyl-methionine) did not sorb on EPS.

Increased nutrient retention with biocrust (EPS)

Biocrusts, in many cases, have significantly more biomass than subcrusts and typically have unique microbial communities (Garcia-Pichel et al. 2003; Steven et al. 2013). As biocrusts age, the soil surface becomes colonized with more microbial species, increasing the overall biomass, microbial diversity and concentration and diversity of EPS that they produce (Garcia-Pichel et al. 2003; Bowker et al. 2008). This aligns well with our observation that the biocrust was the most sorptive, sorbing 57 metabolites compared to the subcrust (33 metabolites) (Fig. 4, Supplementary Figure 1). Presumably, since the EPM constitutes a large component of the organic fraction of biocrusts (Belnap and Gardner 1993), sorption mechanisms are likely dominated by the EPM. This was further validated with our sorption experiment with EPS extracted from the biocrust.

The isolated EPS was surprisingly more sorptive than the biocrust it was extracted from. Perhaps this is due to the increased purity and accessibility of 'sticky' functional groups following EPS extraction. Because natural biocrust EPS is always surrounded by a variety of other soil components (e.g. minerals), our results are likely over-representative of what would be observed in the environment. At the very least, here we demonstrate specific metabolites that can be sorbed on this particular pool of EPS. These data also confirm the long-known role of EPS acting as glue (De Philippis and Vincenzini 1998), but has never been explored from an untargeted metabolomics (sorption) perspective.

The exclusive biocrust-sorptive (and EPS-sorptive) metabolites were highly enriched in amino acids and organic acids. It is difficult to determine the exact mechanism of how these metabolites sorb on EPM components since the complete biochemical composition of biocrust EPM (and specifically the EPS) is not yet fully understood. Further, it is unclear how the biocrust EPM may have been altered during our experimental processing and how much (and specifically which fractions) of EPS were extracted from biocrust. Typically, Na_2EDTA is used for EPS extractions, but it was avoided here to prevent LC/MS contamination. Regardless, our approach likely extracted loosely-bound EPS and more tightly-bound sheaths and capsules ('glycocalix' EPS), all of complex composition (Rossi et al. 2017). A large fraction of the EPM is known to consist of saccharidic components (Pereira et al. 2009; Chen et al. 2014) with a small fraction of peptides, lipids and nucleic acids. Many of these components present negatively charged sulfate and pyruvyl groups on the surface of EPS (Rossi and De Philippis 2015) and this would especially be true under the alkaline conditions of our biocrust sorption experiments. This chemically diverse composition of

EPS might support a range of physiochemical interactions (hydrogen bonding, van der Waals interactions, ionic attractive forces, electrostatic attractive forces, Flemming and Wingender 2010) and likely explains the sorption of metabolites observed in this study. Future studies are needed to further characterize EPS composition and especially how it varies across different environments, laboratory conditions and extraction methods. Such information will facilitate our understanding of chemical interactions within the EPM and the role EPS components may have in microbial ecology. Especially in biocrusts, where water availability is limited, the EPM likely plays a major role in limiting nutrient loss and supporting growth of metabolically diverse microbial communities, also owing to its capacity to trap and retain water in these soils (Fischer et al. 2010; Colica et al. 2014).

Microbial processing of biocrust-sorbed metabolites

We previously investigated how biocrust soil water metabolites change after a wetting event, providing a snapshot of the in situ dynamics of nutrients (Baran et al. 2015). In the present study, we remove the potential for microbial processing by autoclaving biocrusts and subcrusts, revealing the role of passive processes in metabolite sorption. Many of the most sorptive metabolites on biocrust (e.g. succinate, lysine, glutamate, arginine, urate, glutamine, citrulline, ergothioneine, N-acetyl-glutamate, adenine) were also found to decrease in biocrust soil water over time in biologically active light and dark biocrusts (Supplementary Figure 1). Interestingly, all but one of these metabolites is an organic nitrogen source, further highlighting the important contribution of the organic fraction of biocrusts to passively collect biologically relevant and critical nutrients.

Several studies have shown that microbial production of EPS supplies microbial communities with essential C-, N- and P-containing nutrients (Wolfaardt et al. 1999; Flemming and Wingender 2010; Mager and Thomas 2011) and our observations suggest that this pool of nutrients may include metabolites that are produced outside of the EPM and are trapped by sorption mechanisms. We previously performed exometabolomics in order to provide insights into metabolite targeting by biocrust bacterial isolates (Baran et al. 2015). Twenty-two of the metabolites assessed for biocrust sorption were previously evaluated for consumption (or release) by *M. vaginatus* and heterotrophs physically associated with *M. vaginatus*. We found that for the highly sorbed metabolites on biocrust, the top two are released by *M. vaginatus* and all (except methylguanine) are consumed by the heterotrophs (Baran et al. 2015) (Supplementary Figure 2). These include citramalate, succinate, glutamate, glutamine, citrulline, adenine, xanthine, and cytosine, all important C and N sources for microbes.

This process of biocrust retention of critical nutrients may enable microbes to recover from the shock of wetting and regain precious metabolites. Additionally, microbes may 'mine' the EPM for these trapped nutrients. This hypothesis stems from the observation that microbes can indeed degrade biocrust EPS (using sucrases and dehydrogenases) into monomers and feed from the released sugars (Chen et al. 2014). Furthermore, Cyanobacteria have been shown to consume EPS components (Stuart et al. 2016) and specifically, *M. vaginatus*, the primary producer of the biocrust in our study, expresses polysaccharide degrading-enzymes (e.g. glycosyl hydrolases) during wetting (Rajeev et al. 2013). Fungi, which are commonly present in biocrusts, possess glucanases, that degrade (1-3)-ß- and (1-6)-ß-glucans (Seviour et al. 1992). Overall, the metabolite retention capacity of the EPS fraction of biocrusts may be beneficial to sustain the diverse biocrust microbial community by preventing energy rich molecules from leaching down to the subcrust layer after a rain event. This mechanism may not only hold true for biocrust, but plant root tips also secrete EPS-like mucilage (Sasse et al. 2017) which may also function to retain nutrients that are essential to plant-microbe interactions.

Conclusion

Expanding our readily accessible ^{13}C–bacterial metabolite sorption method to biocrust revealed that, for these particular samples, the upper biocrust layer is almost two times more sorptive than the underlying subcrust. After further examination of the metabolic components that are exclusively retained by the biocrust, we found selectivity for anionic organic acids and amino acids. These specific interactions were confirmed to be due to the distinctive presence of EPS (or more generally, the EPM) in biocrusts where most of the EPS polymers are primarily derived from microbial exudates (Rossi and De Philippis 2015). However, our approach demonstrates that many important EPS-bound metabolites may be derived by selective sorption from incoming

metabolites (especially during rainfall). This indicates that in addition to the variety of other critical roles in terrestrial ecosystems, during limited rainfall events, the EPM may serve as a selective filter (Flemming and Wingender 2010) by trapping energy rich nutrients that can be further used in situ in the biocrust layer. This function may be especially critical in supporting diverse microbial communities within nutrient-deprived arid land soils, promoting the survival of these globally important ecosystems.

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