Supplementary Material

Titel: Photoautotrophic organisms control microbial abundance, diversity, and physiology in different types of biological soil crusts

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Supplementary Methods

Sampling area

All samples were collected in the vicinity of the village Soebatsfontein next to the BIOTA observatory No. 22 (http://www.biota-africa.org, 30.1865°S, 17.5434°E, 392 m a.s.l.), which is situated approximately 60 km south of Springbok, Northern Cape Province, South Africa. The sampling area is located within the Succulent Karoo biome, which is known for its unique flora of succulent plants and very high plant diversity (Van Jaarsveld, 1987). At our study site, the dominant soil types are Leptosol, Durisol and Cambisol, where the texture of the soil ranges from sandy to silty loam, representing pure sands in the topsoils (Haarmeyer *et al.*, 2010).

The Succulent Karoo is characterized by a semi-arid climate, where most of the precipitation occurs during the winter months (July and August) and the annual precipitation averages about 131 mm. The annual mean air temperature is 19.4°C (Average values from

2001-2009; Haarmeyer *et al*., 2010), varying between maximum values of 44°C being reached in February and 2°C occurring in July (http://www.biota-africa.org).
Biocrusts in the area regularly cover the interspaces between plants as well as underneath shrubs. Whereas cyanobacteria- and lichen-dominated biocrusts occur both in interspaces and beneath vegetation, moss-dominated crusts are mainly restricted to the shaded areas beneath shrubs (Dojani *et al.*, 2011; Weber *et al.*, 2012).

Sampling and storage

Moss-dominated biocrusts contained the species *Ceradoton purpureus* (Hedw.) Brid as dominating photobiont (Weber *et al.*, 2012) (Figure 1D, H). The genus *Ceratodon* is known to occur in hot arid regions throughout the world, as the western US drylands (Rosentreter *et al.*, 2007) and the semi-deserts of Australia (Eldridge and Tozer, 1997). In lichen-dominated biocrusts the chlorolichen *Psora decipiens* (Hedwig) Hoffm. was dominating (Figure 1C, G). The lichen *Psora decipiens* is found in soil crusts in North America, Mexico, Africa, around the Mediterranean Sea, in Europe, throughout the Middle East, in India, in many areas of Asia, in Australia, New Zealand, and even in the high Arctic and the nival belt of the Alps (Belnap *et al.*, 2003). Cyanobacteria-dominated biocrusts mainly comprised cyanobacteria, with genera such as *Chroococcidiopsis*, *Pseudoanabaena*, *Phormidium*, *Leptolyngbya, Microcoleus*, and *Nostoc*, but also lichens of the species *Collema coccophorum* Tuck. with cyanobacteria as photobionts were sometimes present (Büdel *et al.*, 2009) (Figure 1B, F). The moss and lichen taxa have previously been identified based on morphological parameters (Büdel *et al*., 2009; Weber *et al*., 2012), whereas the dominating cyanobacteria have been identified by means of both morphological and genetic identification techniques (biphasic approach; Büdel *et al*., 2009 ; Dojani *et al.*, 2014).

Biocrust and bare soil samples (Figure 1A, E) for physiological experiments and pH-measurements were collected in petri dishes (5.5 cm diameter) in October 2008 and October 2009 (moss-dominated biocrusts) and October 2010 (cyanobacteria- and green-algal lichen-dominated biocrusts). The samples for CO_2 gas exchange measurements were collected in petri dishes (5.5 cm diameter) in October 2008 and October 2009 (moss-dominated biocrusts) and October 2010 (cyanobacteria- and green-algal lichen-dominated biocrusts). During sampling, the base of a petri dish (lined with some pieces of soft cellulose tissue) was pressed about one centimeter deep into the soil before the sample could be carefully removed with the help of a trowel. After drying under ambient dry conditions, the samples were closed and sealed with Parafilm (Brand GmbH, Wertheim, Germany) and transported to Germany. Until measurements at the University of Kaiserslautern in winter 2010/2011 (moss-dominated crusts), winter 2011/2012 (green-algal lichen-dominated crusts) and in spring 2013 (cyanobacterial lichen-dominated crusts) the samples were stored in a freezer at -20°C.

In previous measurements, it had been shown that dry biocrusts could outlast under these conditions without harm (Weber *et al.*, 2012). The different biocrust and bare soil samples for the NO- and HONO- measurements were collected in November 2013 in stainless steel cylinders (5.0 cm diameter, 2.6 cm high; Eijkelkamp Agrisearch Equipment, Netherlands) as described in (Weber *et al.*, 2015). To avoid cracking of the biocrust surface, the latter was covered with a piece of cloth and cautiously watered. After that the steel cylinders were put on the biocrust surface, covered with a wooden board and, using a hammer, the cylinders were driven into the soil. The samples were removed from the ground similarly to those collected for the gas exchange measurements. To minimize metabolic activity, the samples were air-dried in the camp, then closed with special plastic lids and transported to the Max Planck Institute for Chemistry in Mainz. Until analysis they were stored in the fridge at 5°C.

For DNA extraction, seven replicates of the three types of biocrusts and seven bare soil samples without a visible crust were sampled in October 2013. The soil samples were collected using sterile equipment and stored in petri dishes (5.5 cm diameter). After collection, the samples were immediately placed in a portable freezer and kept at -20°C during the trip to the University of Cape Town, where they were stored at -80°C until processing in the laboratory.

Quantitative PCR analyses to estimate bacterial and fungal small-subunit rRNA gene abundances

DNA standards were produced from *Escherichia coli (*DSM 1116) 16S rRNA gene and the *Saccharomyces cerevisiae* (ATCC 9763) 18S rRNA gene. The amplified products were run on a 1% 1xTAE agarose gel to confirm specificity of the amplification and were cloned using the pGEM®-T Easy Vector System kit (Promega, Madison, WI, USA). Plasmids were isolated using the illustra plasmidPrep Mini Spin kit (GE Healthcare, Buckinghamshire, UK). Plasmid DNA was linearized by digestion with PstI (New England BioLabs, Frankfurt am Main, Germany) at 37°C for 2 h followed by enzyme inactivation at 80°C for 20 min. Standard curves were generated using 10-fold serial dilution of the plasmid containing a full-length copy of *Escherichia coli* 16S rRNA gene or the *Saccharomyces cerevisiae* 18S rRNA gene. Target genes were amplified using KAPA SYBR® FAST qPCR Kit Master Mix Universal (Kapa Biosystems Ltd., London, UK). The qPCR reactions contained 5 µl 2x KAPA SYBR® FAST qPCR Master Mix Universal, 0.2 µl of each 10 µM forward and reverse primers, 0.2 µl 50x ROX Low and 1.4 µl PCR-grade water. Standard and environmental DNA samples were added at 3.0 µl per reaction. The reaction was carried out on an Applied Biosystems® 7500 instrument (Applied Biosystems, California, USA) using a program of 95°C for 3 min followed by 40 cycles of 95°C for 3 s, 51°C for 20 s and 72°C for 32 s (bacteria). For the 18S gene essays, thermal cycling conditions were as follows: one cycle of 95°C for 3 min; 40 cycles of

95°C for 3 s, 50°C for 20 s, 72°C for 32 s. For fungi, the primer used were FR1 (5´- AICCATTCAATCGGTAIT- 3´) and FF390 (5´- CGATAACGAACGAGACCT – 3´), while 338f (5´- ACTCCTACGGGAGGCAGCAG – 3´) and 518r (5´- ATTACCGCGGCTGCTGG) were used for bacteria. The specificity of the amplification was verified by a melting curve analysis after each qPCR run as well as with agarose gel electrophoresis. Gene copy numbers were calculated using a regression equation relating the cycle threshold value to the known number of copies in the standards. All qPCR reactions were run in duplicate.

16S rRNA gene PCR amplification and sequencing

16S rRNA gene amplification and sequencing on an Illumina MiSeq platform to assess bacterial community structure, 16S sequence based amplicon generation, purification and indexing as well as sequencing of amplicon libraries on a MiSeq (Illumina, Eindhoven, Netherlands) with v3 600 cycles chemistry was performed as described in Kozich *et al*. (2013). Briefly, 25 ng of genomic DNA were used in a 25 µl PCR reaction performed in triplicates. For amplification, HPLC purified primers (Eurofins MWG, Ebersberg, Germany) targeting the hypervariable region V4 from Bacteria and Archaea (F515 – 5´GTGCCAGCMGCCGCGGTAA 3´, R806 – 5´GGACTACHVGGGTWTCTAAT 3´) were used (Bates *et al.*, 2011). A PCR mix of 25 µl was prepared in triplicates containing 1 x Fast Start High Fidelity Buffer (Roche Diagnostics, Mannheim, Germany), 1.25 U High Fidelity Enzyme (Roche Diagnostics, Mannheim, Germany), 200 µM dNTPs (Roche Diagnostics, Mannheim, Germany), 0.4 µM primers and PCR-grade water (Roche Diagnostics, Mannheim, Germany). A no-template control was included. Thermal cycling conditions were for initial denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 95°C for 45 s, annealing at 55°C for 45 s and extension at 72°C for 1 min with a final extension of 7 min at 72°C. PCR products were checked on an agarose gel, the triplicates were pooled and normalized with a SequalPrep Normalization Plate (Life Technologies) according to suppliers instructions. 15 µl of normalized PCR product were used for Indexing PCR according to Kozich et al. (2013). 5 µl of each sample were pooled to a final library that was purified by standard procedures, quantified by Picro Green (Life Technolgies) and diluted to 4 nM to run on a MiSeq. Version 3 600 cycle chemistry (Illumina, Eindhoven, Netherlands) was used according to manufacturer‘s instructions to run the 6 pM library with 10% PhiX.

Processing of Illumina amplicon sequence data

Library adapter trimmed overlapping paired-end read data from the Illumina platform were merged using the software FLASH (Fast Length Adjustment of Short reads) (Magoč and Salzberg, 2011). Sequence data, 2 971 627 reads from 28 samples, were pre-processed

using the Quantitative Insights into Microbial Ecology (QIIME) pipeline v. 1.8.0. (Caporaso *et al.*, 2011). Briefly, for quality-filtering of the high-throughput sequencing data default settings in QIIME were used, yielding 2 608 992 sequence reads (min average quality score allowed: 25). An open-reference operational taxonomic unit (OTU) picking protocol on the demultiplexed Illumina data was used, where usearch61 (Edgar, 2010) was applied to compare sequences to an OTUs reference data set (Greengenes v. 13_8) and any reads which did not match a reference sequence at greater or equal to 97 % sequence identity were subsequently clustered de novo. For performing sequence alignment PyNAST was chosen (Caporaso *et al.*, 2010). The method for inferring the phylogenetic tree was FastTree (Price *et al.*, 2009). For downstream analyses chloroplast and mitochondria sequences as well as OTUs present in control reactions (negative extraction control and no-template PCR control) were excluded, leaving 2 454 673 sequences. Furthermore, samples were rarefied to 48 009 sequences per sample except when we performed tests for detecting taxa that are differentially abundant across habitats using DESeq2 package (Love *et al.*, 2014).

Several metrics were used to estimate within-sample diversity (alpha diversity), including Shannon index, which measures both richness and evenness, and Faith´s phylogenetic diversity (Faith, 1992). Non-Metric Multidimensional Scaling was performed on Bray-Curtis dissimilarity and phylogenetic metrics, weighted and unweighted UniFrac distances, to determine the influence of microhabitat using the R phyloseq package (McMurdie and Holmes, 2013; R Core Team, 2013). UniFrac allows the comparison of microbial communities using phylogenetic information. The weighted version takes into account differences in relative abundances (Lozupone and Knight 2005; Lozupone *et al*. 2011). The function envfit from the R vegan package was used to relate patterns in bacterial communities with environmental parameters (Oksanen *et al.*, 2015). For the comparison of the samples between the four microhabitats we applied a taxa composition data analysis approach at the phylum level introduced by La Rosa *et al*. (2012). The shared taxa (core members) across biocrust types were identified using a two-parameter model and visualized with a ubiquity vs. abundance plot (taxonomic ubiquity cut-off = 0.8, abundance = 0.01). The term “abundance” is defined as the proportion of a taxon of interest in a sample and “ubiquity” describes the proportion of samples where a taxon is detected (Li *et al.*, 2013). We identified the ‘niche breadth’ using the formula

$$Bj = \frac{1}{\sum_{i=1}^{N} P^2 ij}$$

where B_j indicates niche breadth and P_{ij} is the proportion of individuals belonging to species *j* present in a given habitat *i*. OTUs that were more evenly distributed along a wider range of habitats were considered as habitat generalists, whereas OTUs with a lower *B*-value were regarded as habitat specialists. Niche breadth was calculated for the 500 OTUs with highest mean relative abundance. The values for niche breadth ranged from 1 to 3.96. OTUs with *B*

> 3.5 were regarded as generalists, OTUs with *B* < 1.5 were considered as specialists (Levins, 1968; Logares *et al.*, 2012).

Fungal ribosomal internal transcribed spacer (ITS) amplification and pyrosequencing

We used FLX 454 one way read (Lib-L kit, Primer A, Primer B, Roche 454 Life Science, Branford, CT, USA) fusion primers (Table S1). The template specific sequence was ITS1F (5´- CTTGGTCATTTAGAGGAAGTAA – 3´) and ITS2R (5´- GCTGCGTTCTTCATCGATGC – 3´) targeting the ITS1 region. For amplification a 25 µl PCR master mix containing 1 x Fast Start High Fidelity Buffer (Roche Diagnostics, Mannheim, Germany), 1.25 U High Fidelity Enzyme (Roche Diagnostics, Mannheim, Germany), 200 µM dNTPs (Roche Diagnostics, Mannheim, Germany), 0.4 µM barcoded primers (Eurofins MWG, Ebersberg, Germany), PCR-grade water (Roche Diagnostics, Mannheim, Germany) and 20 ng total genomic DNA was prepared in triplicates. Denaturation of double stranded DNA was performed at 95°C for 3 min followed by 32 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 45 s, extension at 72°C for 1 min and a final extension of 7 min at 72°C. Amplicons were loaded to a 1% agarose gel and purified with a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Determination of dsDNA concentration after amplicon purification was performed using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, CA, USA) according to manufacturer's instructions. After quantification, 30 barcode labeled amplicons were pooled equimolar, again size selected by gel purification, and analyzed on a 2100 Bio Analyzer (Agilent Technologies, Waldbronn, Germany) using a DNA 7500 kit. With the pooled samples emulsion PCR was performed using the GS Titanium MV emPCR Kit and method (Lib-L) (Roche 454 Life Science, Branford, CT, USA) according to manufacturers' instructions. Sequencing of an equimolar pool of 30 samples on a 1/4 PTP was performed using the GS FLX Titanium Sequencing Kit XLR70 (Roche 454 Life Science, Branford, CT, USA) according to manufacturer's instructions.

Raw sequence data were processed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline v. 1.8.0. (Caporaso *et al.*, 2011). Fungal ITS amplicon data were analyzed using the QIIME/UNITE reference OTUs according to the Fungal ITS QIIME analysis tutorial. A quality control of the reads and denoising was performed to reduce the amount of sequencing errors, characteristic for pyrosequencing technology employed by 454 sequencing machines. Each 454 run was preprocessed with the QIIME script split_libraries.py, to assign barcodes to samples and filter low quality reads (min average quality score allowed: 25). Denoising was performed with denoise_wrapper. Another cleanup step performed was to detect and remove chimeras using USEARCH (usearch61 v6.1.544). The fungal ITS1 region was extracted using ITSx (Bengtsson-Palme *et al.*, 2013). An open-reference operational taxonomic unit (OTU) picking approach was used to compare

sequences to an OTUs reference data set (UNITE fungal database QIIME release v. 12_11; reference database called: 97_otus.fasta and taxonomy database called 97_otu_taxonomy.txt) and any reads which did not match a reference sequence at greater or equal to 97 % sequence identity were subsequently clustered de novo. Non-Metric Multidimensional Scaling was performed on Bray-Curtis dissimilarity using the R phyloseq package (McMurdie and Holmes, 2013; R Core Team, 2013). The function envfit from the R vegan package was used to relate patterns in fungal communities with environmental parameters (Oksanen *et al*. 2015; R Core Team, 2013). The R DESeq2 package was used to detect taxa that are differentially abundant across habitats (Love *et al.*, 2014).

Biomass and soil parameters

Chlorophyll was extracted according to the method established by Ronen and Galun (1984) using DMSO (Dimethylsulfoxide) with a spatula tip of $CaCO_3$ to avoid acidification and concomitant phaeophytinization of chlorophyll. After spectrophotometry, the chlorophyll$_a$ (Chl_a) content was calculated according to Arnon *et al.* (1974), whereas chlorophyll$_{a+b}$ (Chl_{a+b}) was determined according to Lange (pers. comm.), as described in Weber *et al.* (2013). A detailed description of the chlorophyll determination can be found in the Supplementary Methods.

For determination of the total nitrogen and carbon content, thirteen samples of each biocrust type and of bare soil were dried for 1h at 105°C and subsequently fine ground and homogenized using an agate mortar. The CHN analysis (Elementar Vario Micro Cube, 2016 Elementar Analysensysteme GmbH, Hanau, Germany) was conducted at the Department of Chemistry, division Chemical Analytics, of the University of Kaiserslautern. PH values of each biocrust type and bare soil (n = 4) were analyzed electrometrically (Streubing and Fangmeier, 1992). For more detailed information see the Supplementary Methods.

Analysis of chlorophyll content

Chlorophyll (Chl) was extracted in 100% DMSO (dimethylsulfoxide) for 90 min (two times) at 65°C (Ronen and Galun, 1984). $CaCO_3$ was added to avoid acidification and concomitant phaophytinization of chlorophyll. After spectrophotometric analysis the Chl_a content was on the one side calculated according to Arnon *et al.* (1974) whereas the Chl_{a+b} content was calculated according to Lange, Bilger, and Pfanz (pers. comm.) described in (Weber *et al.*, 2013) as follows:

Chl_a [µg] = [12.19 · (OD_{665} - OD_{700})] · a

Chl_a [µg] = [20.2 · (OD_{648} - OD_{700}) + 8.02 · (OD_{665} - OD_{700})] · a [ml]

Where Chl_{a+b}[µg], Chl_a[µg]: chlorophyll content of the sample in micrograms
E_{648}, E_{665}, E_{700}: absorption at the given wavelengths,
a: amount of DMSO [ml].
The chlorophyll content per surface area [mg/m^{-2}] was calculated by dividing the Chl value by the surface area of the sample (Weber *et al.*, 2013).

Analysis of pH

For analysis of pH-values, 0.5 g of substrate (upper 5 mm of the crust/soil) of each type of biocrust and bare soil (n = 4), were mixed with 1.25 ml of distilled water and shaken for 15 min. After 45 minutes of sedimentation the pH-values were determined electrometrically with the help of a glass electrode (Minitrode, Hamilton Messtechnik GmbH, Höchst, Germany).

CO_2 gas exchange measurements – Calculation of soil respiration rates

Soil respiration rates of cyanobacteria- and lichen-dominated biocrusts were calculated by subtracting the dark respiration (DR) values of the photoautotrophic organisms from those of the complete biocrust. As the present study is focused on the effect of biocrust-defining autotrophic organisms on the heterotrophic community, we investigate the respiratory properties of the heterotrophs, whereas the NP of complete crusts is analyzed in separate publications (Weber *et al.* 2012; Tamm *et al.* in prep.).

Statistical Analyses

Molecular analyses

A one-way between subjects ANOVA and post hoc comparison using the Tukey´s honestly significant difference (HSD) test was conducted to compare alpha diversity-related values (Observed species number, Shannon index of species diversity, Faith´s Phylogenetic Diversity) and gene copy numbers between bare soil and different biocrust types (R´s stats package). Where assumptions for parametric tests were not met, a Kruskal-Wallis test for independent samples and a Tukey and Kramer (Nemenyi) post hoc test were performed (R´s stats package). ANOSIM was applied using the QIIME script compare_categories.py (R´s vegan package) to test whether the grouping of samples by a given category (bare soil and biocrust type) was statistically significant. In addition, we used DESeq2, an alternative method for normalization, based on a model using negative binomial distribution, to perform analyses of differential abundance of taxa across sample types (test=”wald” and fitType =”parametric”). It was assumed that the abundance of an OTU differed if its mean proportion

was significantly different between two sample classes (biocrust type and/or bare soil) (Love *et al.*, 2014).

Biomass, soil parameters and respiration

All acquired data were first tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). For parametric data (i.e., Chl_a, pH and total nitrogen), a one-way ANOVA with a Fisher LSD post hoc test was conducted using OriginPro b9.2.214 (OriginLab Corporation, Northampton, Massachusetts, USA; Supplementary Table S4). For non-parametric data (i.e., Chl_{a+b} and total C), a Kruskal-Wallis test for independent samples with Fisher LSD post hoc test was applied using Statistica12 (StatSoft, Oklahoma, USA; Supplementary Table S4). Effects of temperature and biocrust type on soil respiration, transformed into the natural logarithm, were analyzed using a two way ANOVA with a Fisher LSD post hoc test (OriginLab Corporation, Northampton, Massachusetts, USA; Supplementary Table S5). A significance level of $p \leq 0.05$ was chosen in all cas

Supplementary Results

Figures

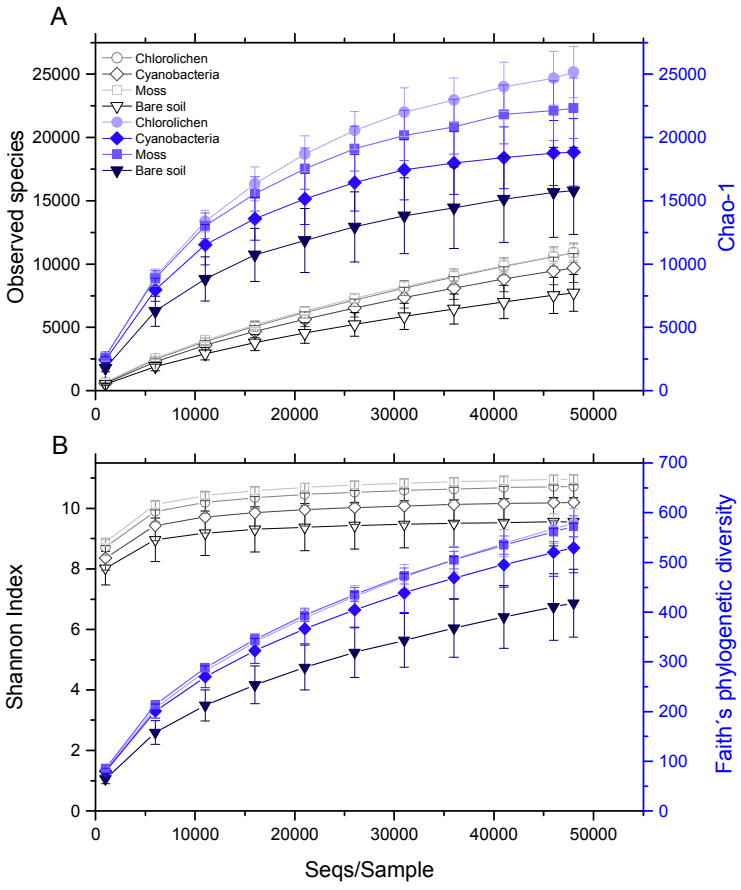

Fig. S1 Rarefaction curves of the bacterial 16S rRNA gene data calculated at multiple sequence depths and measures of alpha diversity.

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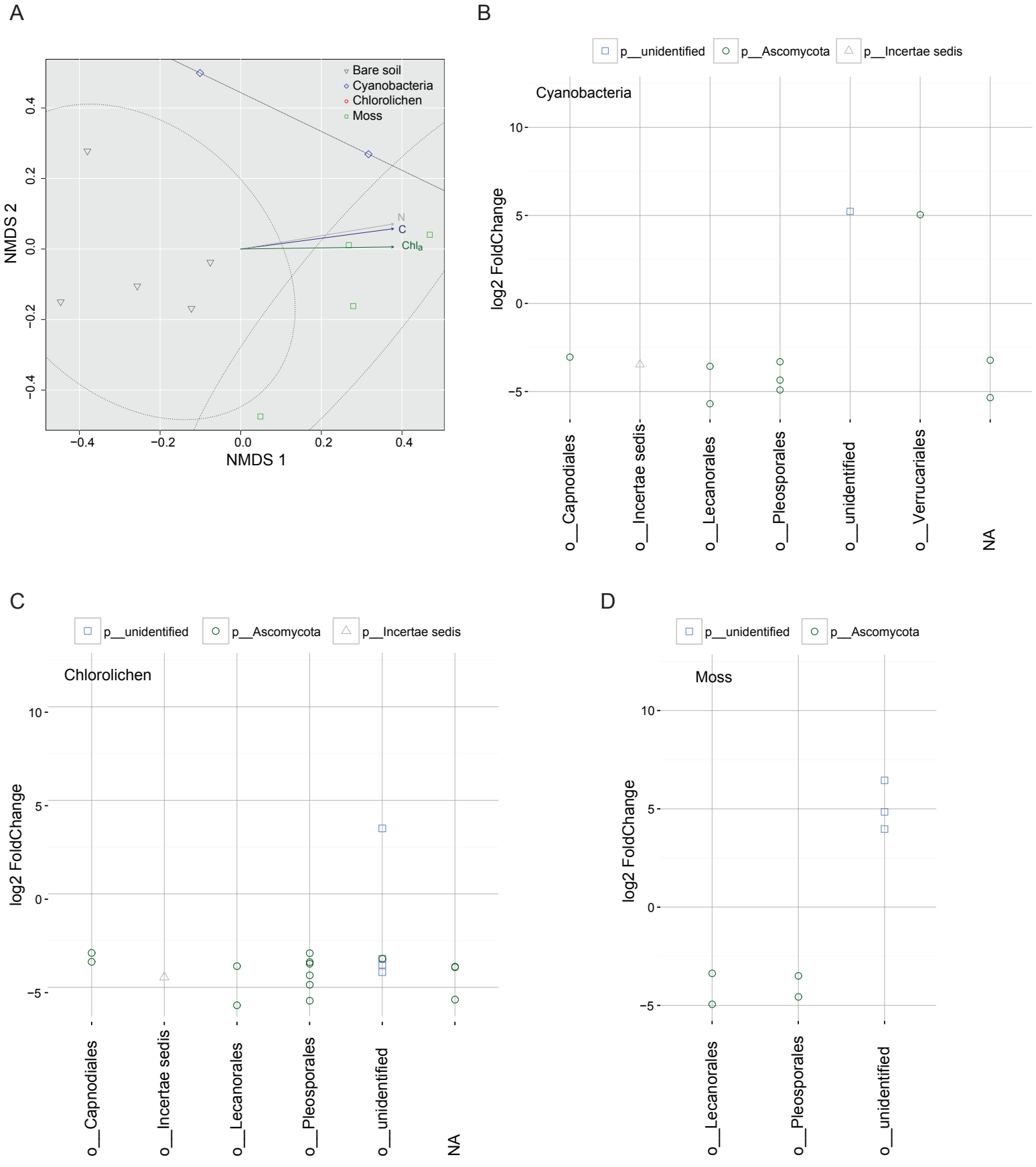

Fig. S2 Fungal community analysis. Ordination using Non-Metric Multidimensional Scaling (NMDS) derived from Bray-Curtis dissimilarity; based on amplicon ITS data. Samples with insufficient number of quality sequencing reads were removed. 95% confidence ellipses are shown (A). Log2 Fold Change of OTUs proportions for the three biocrust types versus the bare soil (DESeq2, Benjamini-Hochberg adjusted P-value < 0.01, n=5) (B-D).

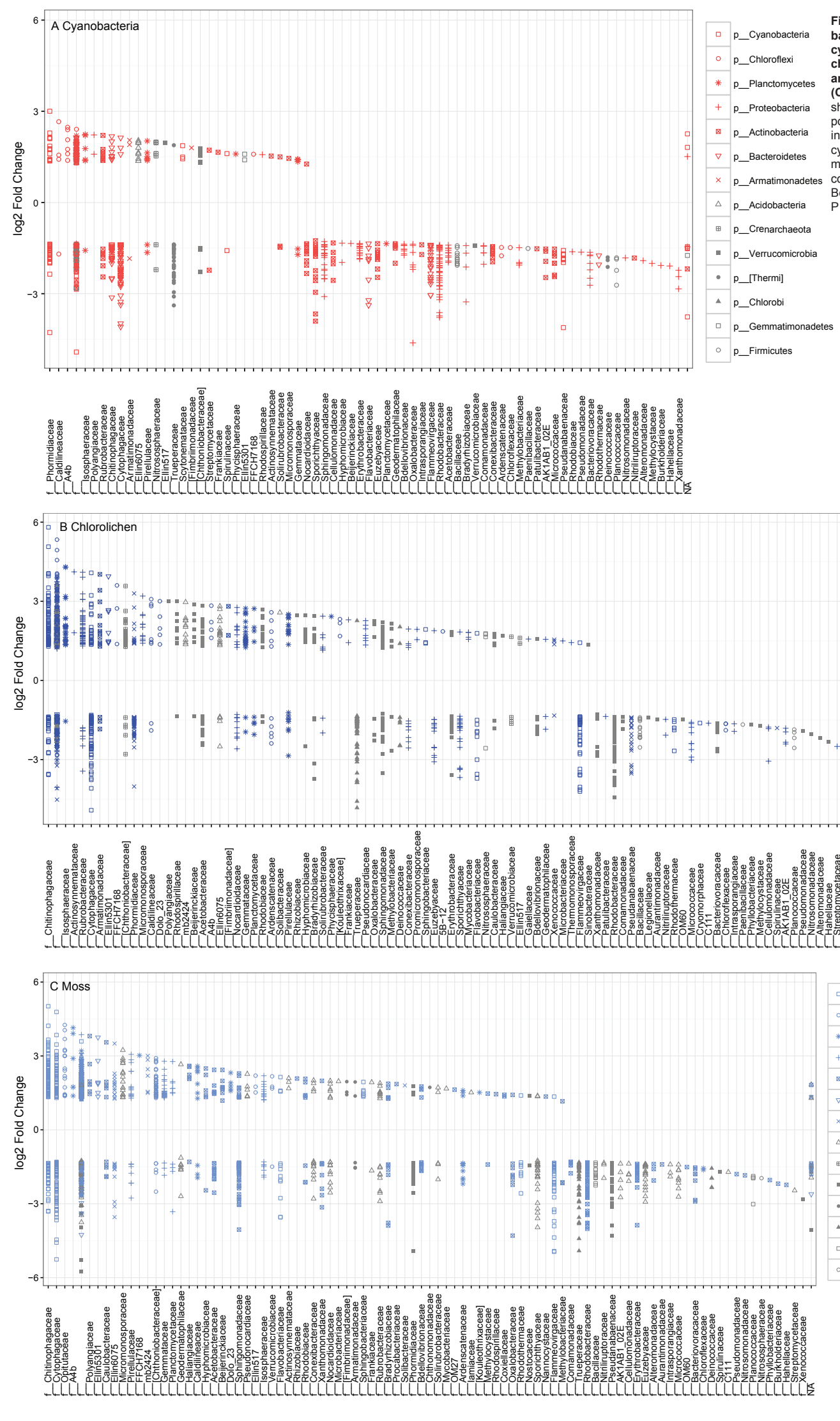

Fig. S3 Relative abundance of bacterial OTUs at family-level in cyanobacteria-dominated (A), chlorolichen-dominated (B) and moss-dominated biocrusts (C) compared to bare soil. Data shown as log2 fold change, positive values indicate an increased presence in cyanobacteria-/chlorolichen-/moss-dominated biocrust as compared to bare soil (DESeq2, Benjamini-Hochberg adjusted P value < 0.01, n=7)

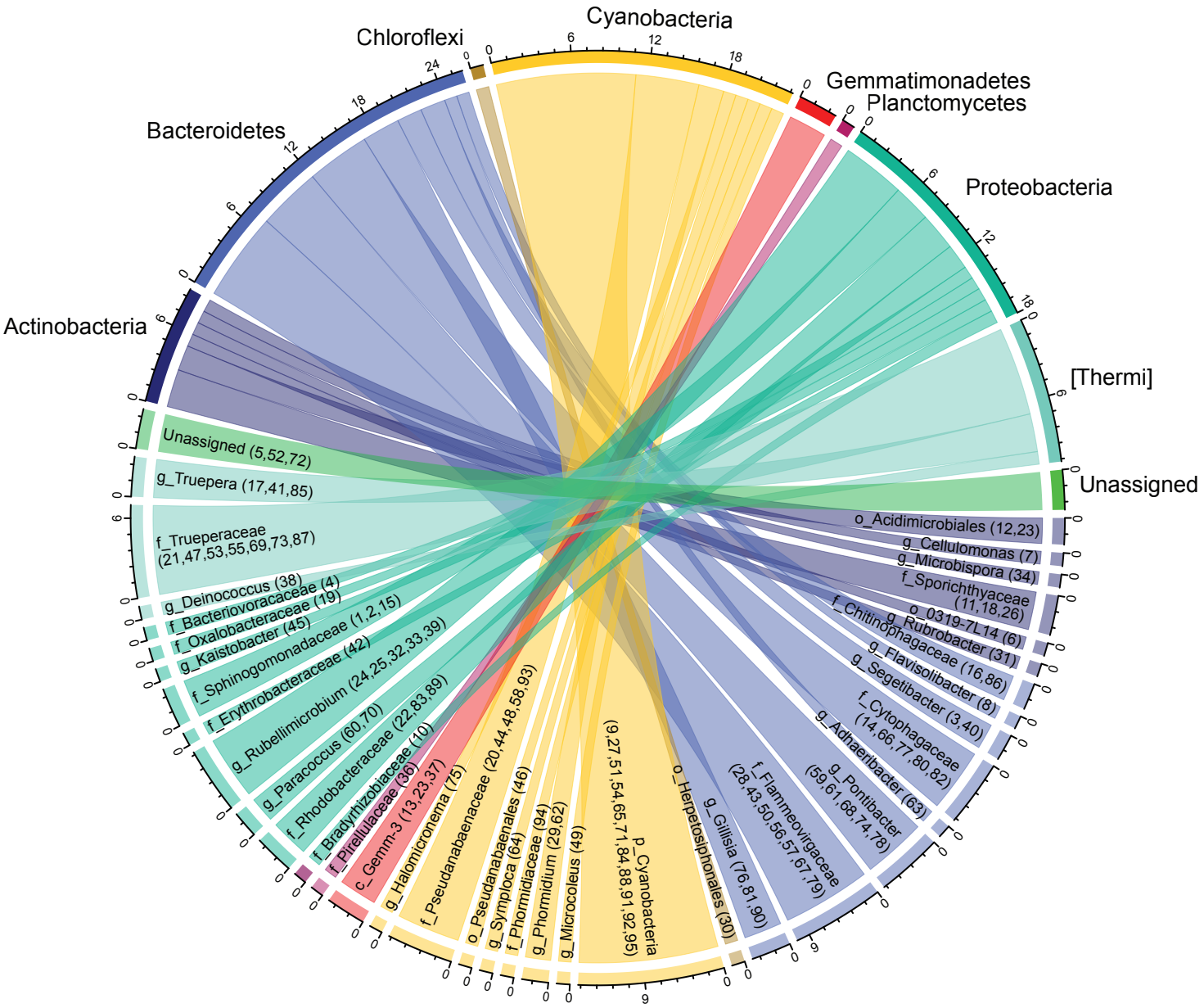

Fig. S4 Analysis of habitat specialists and generalists. Niche breadth (*B*) of OTUs was calculated according to Levins (Levins, 1968; Logares et al., 2012). OTUs with a *B*-value < 1.5 were considered as habitat specialists. Specialists of bare soil are shown. Numbers in brackets refer to figure 5A. Numbers outside of circles indicate the number of OTUs.

Supplementary Tables

Table S1 Barcode primer sequences used in this study: amplicons were sequenced from the Titanium A adaptor (CCATCTCATCCCTGCGTGTCTCCGAC), followed by a 4 bases key sequence (TCAG), the 8 bases barcode and the template specific sequence. The reverse primer was used with the Titanium B adaptor (CCTATCCCCTGTGTGCCTTGGCAGTC) and the target specific sequence but without barcode sequence (CCTATCCCCTGTGTGCCTTGGCAGTC GCTGCGTTCTTCATCGATGC).

Barcode ID	Barcode (5‘ - 3‘)	target specific sequence
1	ACGAGTGCGT	CTTGGTCATTTAGAGGAAGTAA
2	ACGCTCGACA	CTTGGTCATTTAGAGGAAGTAA
3	AGACGCACTC	CTTGGTCATTTAGAGGAAGTAA
4	AGCACTGTAG	CTTGGTCATTTAGAGGAAGTAA
5	ATCAGACACG	CTTGGTCATTTAGAGGAAGTAA
6	ATATCGCGAG	CTTGGTCATTTAGAGGAAGTAA
7	CGTGTCTCTA	CTTGGTCATTTAGAGGAAGTAA
8	CTCGCGTGTC	CTTGGTCATTTAGAGGAAGTAA
10	TCTCTATGCG	CTTGGTCATTTAGAGGAAGTAA
11	TGATACGTCT	CTTGGTCATTTAGAGGAAGTAA
13	CATAGTAGTG	CTTGGTCATTTAGAGGAAGTAA
14	CGAGAGATAC	CTTGGTCATTTAGAGGAAGTAA
15	ATACGACGTA	CTTGGTCATTTAGAGGAAGTAA
16	TCACGTACTA	CTTGGTCATTTAGAGGAAGTAA
17	CGTCTAGTAC	CTTGGTCATTTAGAGGAAGTAA
18	TCTACGTAGC	CTTGGTCATTTAGAGGAAGTAA
19	TGTACTACTC	CTTGGTCATTTAGAGGAAGTAA
20	ACGACTACAG	CTTGGTCATTTAGAGGAAGTAA
21	CGTAGACTAG	CTTGGTCATTTAGAGGAAGTAA
22	TACGAGTATG	CTTGGTCATTTAGAGGAAGTAA
23	TACTCTCGTG	CTTGGTCATTTAGAGGAAGTAA
24	TAGAGACGAG	CTTGGTCATTTAGAGGAAGTAA
25	TCGTCGCTCG	CTTGGTCATTTAGAGGAAGTAA
26	ACATACGCGT	CTTGGTCATTTAGAGGAAGTAA
27	ACGCGAGTAT	CTTGGTCATTTAGAGGAAGTAA
28	ACTACTATGT	CTTGGTCATTTAGAGGAAGTAA
29	ACTGTACAGT	CTTGGTCATTTAGAGGAAGTAA
30	AGACTATACT	CTTGGTCATTTAGAGGAAGTAA
31	AGCGTCGTCT	CTTGGTCATTTAGAGGAAGTAA
32	AGTACGCTAT	CTTGGTCATTTAGAGGAAGTAA

Table S2 Correlation between soil parameters and NMDS axes

Bacteria	r(Axis 1)	r(Axis 2)	R^2	P
total Carbon	-0.998	-0.058	0.349	0.004
total Nitrogen	-0.953	-0.303	0.356	0.001
pH	-0.456	0.890	0.021	0.886
Chl_{a+b}	-0.981	-0.195	0.578	0.001
Chl_a	-0.849	-0.528	0.5575	0.003
Fungi	**r(Axis 1)**	**r(Axis 2)**	**R^2**	**P**
total Carbon	0.989	0.150	0.623	0.023
total Nitrogen	0.983	0.185	0.556	0.031
pH	0.983	-0.181	0.028	0.912
Chl_{a+b}	0.987	0.163	0.548	0.060
Chl_a	1.00	0.016	0.571	0.042

Table S3 Comparison of relative abundances of bacterial taxa in chlorolichen-, cyanobacteria-, moss-dominated biocrust and bare soil.

Crenarchaeota	Moss	Chlorolichen	Cyanobacteria
Bare soil	P=8.10e-1 (Tukey´s HSD test)	P=8.72e-2 (Tukey´s HSD test)	P=4.00e-5 (Tukey´s HSD test)
Cyanobacteria	P=4.10 e-4 (Tukey´s HSD test)	P=2.01e-2 (Tukey´s HSD test)	
Chlorolichen	P=3.98e-1 (Tukey´s HSD test)		
Acidobacteria	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P =3.00e-5 (Tukey´s HSD test)	P= 1.40e-4 (Tukey´s HSD test)	P=1.70e-6 (Tukey´s HSD test)
Cyanobacteria	P=6.16e-1 (Tukey´s HSD test)	P=2.94e-1 (Tukey´s HSD test)	
Chlorolichen	P=9.37e-1 (Tukey´s HSD test)		
Actinobacteria	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=1 (Nemenyi-Test)	P=2.90e-2 (Nemenyi-Test)	P=9.99e-1 (Nemenyi-Test)
Cyanobacteria	P=9.96e-1 (Nemenyi-Test)	P=4.20e-2 (Nemenyi-Test)	
Chlorolichen	P= 2.20e-2 (Nemenyi-Test)		
Armatimonadetes	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=1.17e-1 (Nemenyi-Test)	P=2.00e-3 (Nemenyi-Test)	P=5.70e-3 (Nemenyi-Test)
Cyanobacteria	P=6.05e-1 (Nemenyi-Test)	P=9.91e-1 (Nemenyi-Test)	
Chlorolichen	P=4.21e-1 (Nemenyi-Test)		
Bacteroidetes	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=9.37e-1 (Nemenyi-Test)	P=1.95e-1 (Nemenyi-Test)	P=1.10e-3 (Nemenyi-Test)
Cyanobacteria	P=8.80e-3 (Nemenyi-Test)	P=2.96e-1 (Nemenyi-Test)	
Chlorolichen	P=5.01e-1 (Nemenyi-Test)		
Chloroflexi	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P<1.00e-5 (Tukey´s HSD test)	P<1.00e-5 (Tukey´s HSD test)	P<1.00e-5 (Tukey´s HSD test)
Cyanobacteria	P=9.46e-1 (Tukey´s HSD test	P=2.77e-2 (Tukey´s HSD test)	
Chlorolichen	P=8.92e-1 (Tukey´s HSD test)		
Cyanobacteria	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=1.60e-2 (Nemenyi-Test)	P=1.95e-1 (Nemenyi-Test)	P=1.00e+0 (Nemenyi-Test)
Cyanobacteria	P=2.00e-2 (Nemenyi-Test)	P=2.21e-1 (Nemenyi-Test)	
Chlorolichen	P=7.64e-1 (Nemenyi-Test)		

Table S3 Continued

Gemmatimonadetes	Moss	Chlorolichen	Cyanobacteria
Bare soil	P=1.60e-3 (Tukey´s HSD test)	P=1.89e-2 (Tukey´s HSD test)	P=1.20e-3 (Tukey´s HSD test)
Cyanobacteria	P=7.27e-1 (Tukey´s HSD test)	P=1.92e-1 (Tukey´s HSD test)	
Chlorolichen	P=7.36e-1 (Tukey´s HSD test)		
Planctomycetes	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P<1.00e-5 (Tukey´s HSD test)	P=1.00e-5 (Tukey´s HSD test)	P=1.00e-4 (Tukey´s HSD test)
Cyanobacteria	P=4.35e-3 (Tukey´s HSD test)	P=1.00e-1 (Tukey´s HSD test)	
Chlorolichen	P=4.52e-3 (Tukey´s HSD test)		
Alphaproteobacteria	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=1.74e-1 (Tukey´s HSD test)	P=2.11e-1 (Tukey´s HSD test)	P=1.61e-3 (Tukey´s HSD test)
Cyanobacteria	P=1.84e-1 (Tukey´s HSD test)	P=1.15e-1 (Tukey´s HSD test)	
Chlorolichen	P=1.00e+0 (Tukey´s HSD test)		
Deltaproteobacteria	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=1.60e-1 (Nemenyi-Test)	P=4.41e-1 (Nemenyi-Test)	P=9.80e-1 (Nemenyi-Test)
Cyanobacteria	P=6.50e-2 (Nemenyi-Test)	P=6.87e-1 (Nemenyi-Test)	
Chlorolichen	P=2.00e-3 (Nemenyi-Test)		
Verrucomicrobia	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P<1.00e-5 (Tukey´s HSD test)	P=8.60e-4 (Tukey´s HSD test)	P<1.00e-5 (Tukey´s HSD test)
Cyanobacteria	P=5.67e-2 (Tukey´s HSD test)	P=1.67e-1 (Tukey´s HSD test)	
Chlorolichen	P=3.40e-4 (Tukey´s HSD test)		
[Thermi]	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P<1.00e-5 (Tukey´s HSD test)	P<1.00e-5 (Tukey´s HSD test)	P=5.00e-4 (Tukey´s HSD test)
Cyanobacteria	P=3.98e-1 (Tukey´s HSD test)	P=7.13e-1 (Tukey´s HSD test)	
Chlorolichen	P=9.50e-1 (Tukey´s HSD test)		

Table S4 Comparison of soil parameters across chlorolichen-, cyanobacteria-, moss-dominated biocrust and bare soil. Fisher Test (Fisher LSD) adjusted P-values for all pairwise comparisons between chlorolichen, cyanobacteria, moss and bare soil samples.

Total carbon	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=4.18e-7 (Fisher LSD test)	P=5.81e-2 (Fisher LSD test)	P=1.24e-1 (Fisher LSD test)
Cyanobacteria	P=8.60e-5 (Fisher LSD test)	P=7.08e-1 (Fisher LSD test)	
Chlorolichen	P=2.87e-4 (Fisher LSD test)		
Total nitrogen	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P =4.28e-11 (Fisher LSD test)	P= 4.66e-4 (Fisher LSD test)	P=4.08e-3 (Fisher LSD test)
Cyanobacteria	P= 1.68e-6 (Fisher LSD test)	P=4.63e-1 (Fisher LSD test)	
Chlorolichen	P= 2.10e-5 (Fisher LSD test)		
pH	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=9.12e-2 (Fisher LSD test)	P=6.53e-1 (Fisher LSD test)	P=5.20e-1 (Fisher LSD test)
Cyanobacteria	P=2.63e-1 (Fisher LSD test)	P=2.83e-1 (Fisher LSD test)	
Chlorolichen	P=4.04e-2 (Fisher LSD test)		
Chl_{a+b}	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=4.76e-8 (Fisher LSD test)	P= 5.50e-4 (Fisher LSD test)	P= 5.24e-5 (Fisher LSD test)
Cyanobacteria	P= 7.54e-4 (Fisher LSD test)	P=2.63e-1 (Fisher LSD test)	
Chlorolichen	P= 7.05e-5 (Fisher LSD test)		
Chl_a	**Moss**	**Chlorolichen**	**Cyanobacteria**
Bare soil	P=2.62e-8 (Fisher LSD test)	P=3.26e-3 (Fisher LSD test)	P= 5.49e-4 (Fisher LSD test)
Cyanobacteria	P=3.08e-5 (Fisher LSD test)	P=4.18e-1 (Fisher LSD test)	
Chlorolichen	P=6.52e-6 (Fisher LSD test)		

Table S5 Comparison of soil respiration across chlorolichen-, cyanobacteria-, and moss-dominated biocrust. Fisher Test (Fisher LSD) adjusted *p*-values for all pairwise comparisons between chlorolichen, cyanobacteria, moss and bare soil samples.

Soil respiration	**Moss**	**Chlorolichen**
Cyanobacteria	P=8.00 e-6 (Fisher LSD test)	P=4.90e-1 (Fisher LSD test)
Chlorolichen	P=8.53 e-7 (Fisher LSD test)	

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