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ARTICLE

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

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Abstract

Biological soil crusts (biocrusts) cover about 12% of the Earth's land masses, thereby providing ecosystem services and affecting biogeochemical fluxes on a global scale. They comprise photoautotrophic cyanobacteria, algae, lichens and mosses, which grow together with heterotrophic microorganisms, forming a model system to study facilitative interactions and assembly principles in natural communities. Biocrusts can be classified into cyanobacteria-, lichen-, and bryophyte-dominated types, which reflect stages of ecological succession. In this study, we examined whether these categories include a shift in heterotrophic communities and whether this may be linked to altered physiological properties. We analyzed the microbial community composition by means of qPCR and high-throughput amplicon sequencing and utilized flux measurements to investigate their physiological properties. Our results revealed that once 16S and 18S rRNA gene copy numbers increase, fungi become more predominant and alpha diversity increases with progressing succession. Bacterial communities differed significantly between biocrust types with a shift from more generalized to specialized organisms along succession. CO_2 gas exchange measurements revealed large respiration rates of late successional crusts being significantly higher than those of initial biocrusts, and different successional stages showed distinct NO and HONO emission patterns. Thus, our study suggests that the photoautotrophic organisms facilitate specific microbial communities, which themselves strongly influence the overall physiological properties of biocrusts and hence local to global nutrient cycles.

Introduction

In dryland regions, where vascular vegetation is usually quite sparse, the uppermost millimeters of the soil develop biological soil crusts (biocrusts), which comprise bryophytes, algae, fungi (including lichens), and bacteria (including cyanobacteria) in varying proportions [1, 2]. Multicellular organisms in these communities are poikilohydric [3, 4], being rapidly reactivated once water is available [5]. These characteristics allow them to outlast periods of drought and colonize extreme environments [6].

According to the dominating photoautotrophic organism, biocrusts are classified into cyanobacteria-, lichen-, and moss-dominated types [1, 7, 8]. These types generally reflect different successional stages [9], with the course and speed of succession being influenced by climate, soil and the preceding disturbance event [10–14]. During the early stages of biocrust formation, when cyanobacteria are few in number, heterotrophic diazotrophs may contribute to N_2-fixation [15]. Under favorable environmental conditions cyanobacteria-dominated biocrusts may develop into lichen- and bryophyte-dominated biocrusts [2]. It is well known that facilitation plays a major role in community succession [16, 17]. Early stages in the succession of biocrusts can suffer from nutrient limitations or flourish from supplementary nutrients, which may kick-start the process of development [17–19].

Stefanie Maier and Alexandra Tamm contributed equally to this work.

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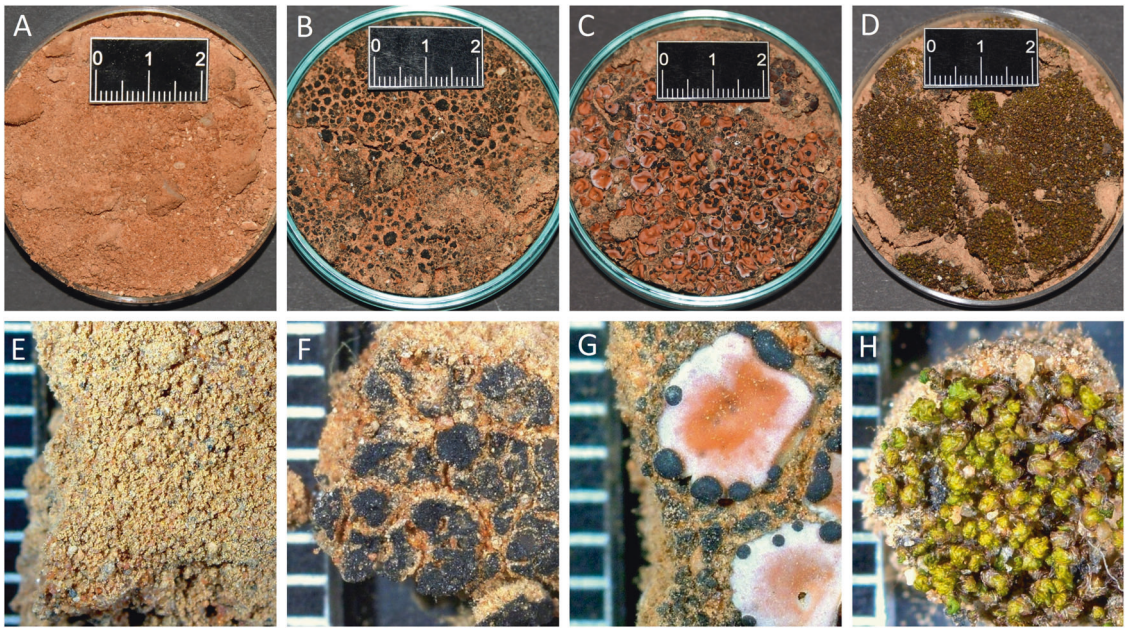

Fig. 1 Different types of biocrusts. **(a–d)** Present the different biocrust types and bare soil in an overview (scale = 2 cm), **(e–h)** present close-up views of the respective biocrust types and bare soil, taken with a stereomicroscope (scale = 7 mm). **(a, e)** Bare soil; **(b, f)** cyanobacteria-dominated biocrust plus cyanolichens with *Collema coccophorum* Tuck. as dominating species; **(c, g)** chlorolichen-dominated biocrust with *Psora decipiens* (Hedwig) Hoffm. as dominating species; **(d, h)** moss-dominated biocrust with *Ceratodon purpureus* (Hedw.) Brid as dominating species

Biocrusts have been demonstrated to play a central role in many dryland ecosystem processes, as they form the zone of nutrient accumulation and transformation [2]. By photosynthetic uptake of atmospheric CO_2 and fixation of atmospheric N_2 [20], biocrusts serve as elemental sinks in the environment. These nutrients serve as food source for plants, animals and other organisms in often strongly depleted dryland ecosystems [21–23]. The contributions of biocrusts to carbon and nitrogen cycles may be significant, as cryptogamic ground covers, including biocrusts, take up 590 Tg a^{-1} of carbon in steppes and deserts, while the biological nitrogen fixation adds up to 25.7 Tg a^{-1} of nitrogen [22]. Biocrusts are known to affect soil carbon cycling, as Castillo-Monroy et al. [24] found that 42% of the annual soil respiration of a dryland ecosystem was attributable to biocrust-dominated areas. In Kalahari soils, CO_2 effluxes resulted from cyanobacteria and heterotrophic microorganisms in the below-crust soil, which were activated by rainfall [25, 26]. The amount of carbon mineralized and hence the quantity of CO_2 emissions is also dependent on the amount of available carbon [27–32]. Thus, the question arises if an alteration of the microbial community will affect the respiration rate and physiological properties of different types of biocrusts.

Most studies are focused on the interaction between a few pairs of species, and neither the complexity of natural communities, such as biocrusts, nor the potential roles of biotic interactions as drivers of ecosystem functioning are being considered [33]. The objective of this study was to investigate the relevance of the dominating photoautotrophic organisms for the overall biocrust microbial composition. We hypothesize that the photoautotrophic organisms affect the composition of the heterotrophic microbial community, thus also the physiological properties of different biocrust types. To study these issues, we analyzed the microbial composition depending on surface cover type, i.e., bare soil and different biocrust types by means of qPCR and high-throughput 16S rRNA gene and fungal internal transcribed spacer (ITS) region sequencing. Secondly, we assessed the physiological response of different biocrust types to varying temperature conditions by conducting CO_2 gas exchange measurements and investigated the effects of nitrogen cycling on their gaseous reactive N emissions during wetting and drying cycles.

Material and methods

Sampling area

Samples were collected near the village Soebatsfontein next to BIOTA observatory No. 22, in the Northern Cape Province, South Africa. The area is located within the Succulent Karoo biome known for its unique flora of succulent plants, high plant diversity and biocrust cover [34, 35].

Sampling and storage

Moss-dominated biocrusts comprised *Ceradoton purpureus* (Hedw.) Brid [8] (Fig. 1d, h). Lichen-dominated types were characterized by the chlorolichen *Psora decipiens* (Hedwig) Hoffm. (Fig. 1c, g). Cyanobacteria-dominated biocrusts contained the genera *Chroococcidiopsis*, *Pseudoanabaena*, *Phormidium*, *Leptolyngbya, Microcoleus*, and *Nostoc*, sometimes growing together with lichens of the species *Collema coccophorum* Tuck. [1, 36] (Fig. 1b, f). Bare soil samples (Fig. 1a, e) used for physiological and pH-measurements mainly occurred in spots after recent disturbance. The detailed collection procedure is described in the Supplementary Methods.

DNA extraction, 16S rRNA gene PCR amplification and sequencing

The biocrust samples were separated into two fractions using a sterile laboratory spatula, i.e., dominating photoautotrophic organisms and the remaining biocrust, and only the second fraction was used for DNA extraction. Homogenization was performed with liquid N_2 using a mortar and pestle [37]. DNA extraction was done with the PowerSoil® DNA Isolation Kit (MO BIO, Carlsbad, California) according to the manufacturer's protocol. An incubation step at 70 °C for 7 min was included, followed by 2 × 1 min of bead beating. Additionally, a negative extraction control reaction was performed. 16S sequence-based amplicon generation, indexing and sequencing of amplicon libraries on a MiSeq platform (Illumina, Eindhoven, Netherlands) with v3 600 cycles chemistry was performed as described in Kozich et al. [38].

The raw NGS data have been deposited in the European Nucleotide Archive at EMBL-EBI (Study accession number PRJEB22584). Merged paired-end data, 2,971,627 reads from 28 samples, were quality-trimmed, leaving 2,608,992 sequences. After removal of mitochondria, chloroplast and control sample sequences 2,454,673 remained (min: 48,009, max: 124,674). For further details, also on quantitative PCR analyses and fungal ITS amplification, refer to the description in the Supplementary Methods and Supplementary Table S1.

Biomass and soil parameters

After measurements, the samples were dried in an oven at 60 °C and the dry weight was gravimetrically determined. Surface area was determined by means of image analysis of digital photos of the samples using the free software ImageJ (National Institutes of Health, Bethesda, MD, USA).

Chlorophyll was extracted according to the method established by Ronen & Galun [39] using DMSO (Dimethylsulfoxide). The total nitrogen and carbon content was determined by CHN analysis (Elementar Vario Micro Cube, 2016 Elementar Analysensysteme GmbH, Hanau, Germany). pH values of each biocrust type and bare soil (n = 4) were analyzed electrometrically [40]. A detailed description of the biomass and soil parameter determination can be found in the Supplementary Methods.

CO_2 gas exchange measurements

Before measurements, the samples were unfrozen and acclimated for at least two days to be fully reactivated again. During that time, they were kept in a climate chamber at 17–22 °C and 50–100 µmol PPFD m^{-2} s^{-1} (photosynthetic photon flux density) under a light-dark regime of 14:10 h (moss-dominated crusts) and 12:12 h (cyanobacteria- and lichen-dominated crusts). Once a day, the samples were sprayed with distilled water. These conditions have been shown to be well suited for acclimation of freezer-stored biocrust samples [8, 41].

CO_2 gas exchange measurements of three replicates of each biocrust type were performed with a portable photosynthesis system (GFS 3000, Walz GmbH, Effeltrich, Germany) under controlled laboratory conditions. Combined light and water response curves were measured at seven different temperatures (7–37 °C). For that, the samples were wetted until complete saturation and light cycles (0–1500 µmol PPFD m^{-2} s^{-1}) were repeated until full dehydration. The samples were weighed before and after each light cycle to determine the water content. At the beginning and the end of each measurement period, a water curve at 17 °C was performed to verify the stability of the net photosynthesis (NP) rates of each replicate. Samples of chlorolichen- and cyanobacteria-dominated biocrusts were physiologically stable throughout the measurement period, whereas those of moss-dominated biocrusts were corrected for a decline of physiological properties (see [8]). After measurement of the complete biocrusts, the samples were separated into the dominating photoautotrophic organisms and the heterotrophic biocrust part. Subsequently, CO_2 gas exchange measurements were repeated using the photoautotrophic compounds of cyanobacteria- and chlorolichen-dominated biocrusts, for moss-dominated biocrusts the remaining biocrust without moss stems was analyzed [8].

Dynamic chamber measurements

Nitrous acid (HONO) and nitric oxide (NO) emissions of biocrusts were measured with a laboratory dynamic chamber system, described in detail by Wu et al. [42] and Weber et al. [43]. To characterize emissions of different types of biocrusts, samples were wetted with distilled water to water-holding capacity and placed in a ~47 L Teflon chamber,

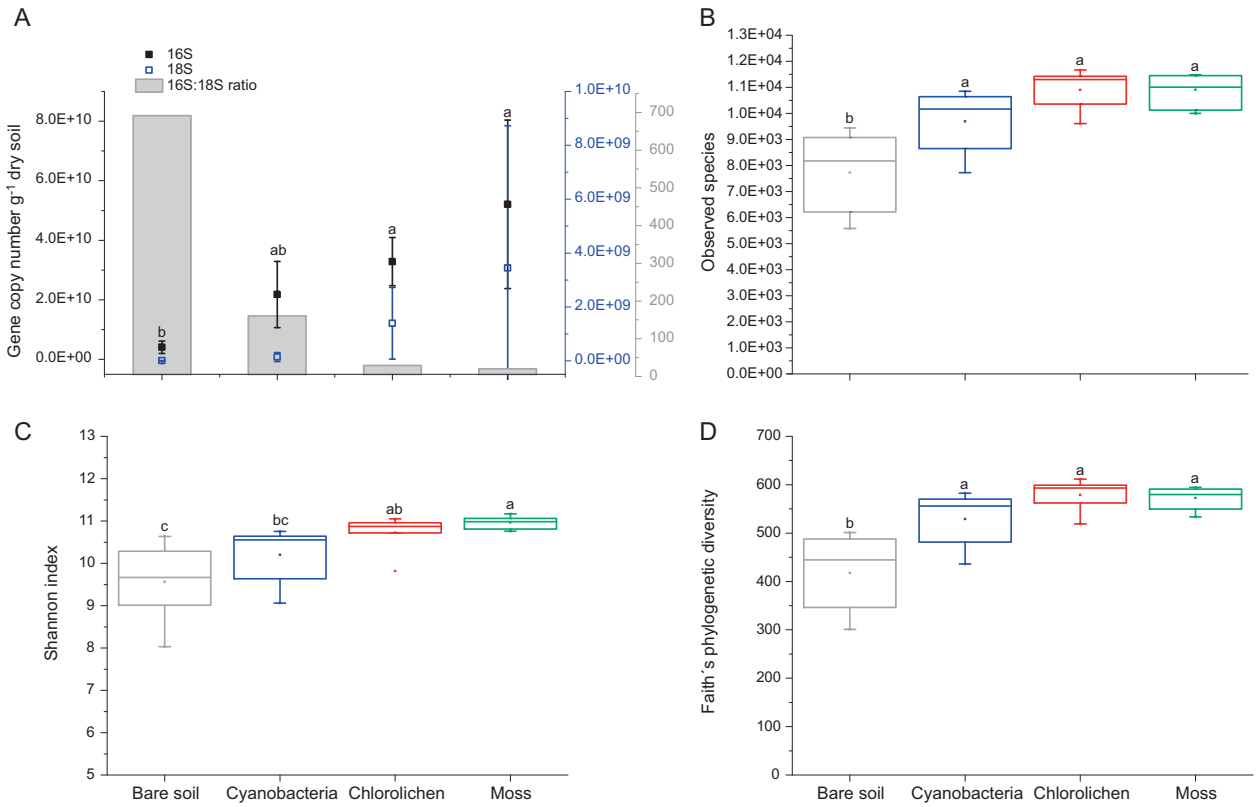

Fig. 2 Abundance and diversity of bacteria and fungi in different types of biocrusts and bare soil. Quantitative real time PCR estimates of bacterial and fungal abundance and its ratio in bare soil and biocrusts (**a**). Comparison of alpha diversity of cyanobacteria-, chlorolichen-, and moss-dominated biocrusts and bare soil; Observed species (**b**), Shannon diversity index (**c**), and Faith's phylogenetic diversity index (**d**). Based on amplicon 16 S rRNA gene data; rarefaction with a depth of 48 009 reads per sample. Boxes limit the 25th- and 7th-percentile with the median presented as line and mean values marked as point inside. Error bars present the 1st- and 99th-percentile and outliers are shown as dots below and above. Significant differences ($P < 0.05$) are marked by different letters

which was located in a temperature controlled cabinet ($T =$ 25 °C). The chamber was continuously flushed with purified gas without HONO, NO_x, O_3, C_xH_y and H_2O at a flow rate of 8 L min^{-1}. HONO, NO_x, O_3, CO_2 and H_2O concentrations in the headspace were monitored during drying of the samples. NO and NO_2 were measured with a gas chemiluminescence detector (Model 42 C, Thermo Electron Corporation, USA; limit of detection (LOD) ~120 ppt), whereas HONO emissions were detected with a long path absorption photometer (LOPAP; QUMA Elektronik & Analytik GmbH, Wuppertal, Germany; LOD ~ 5 ppt). Fluxes of the reactive nitrogen gases were calculated based on the surface area of the samples, inlet and outlet gas concentrations and flushing flow rate.

Results

Abundance and diversity

The bacterial and fungal gene copy numbers were highest in moss-dominated biocrusts (Fig. 2a). There was a statistically significant difference in bacterial and fungal gene copy numbers between the different soil/biocrust types (Kruskal–Wallis H test; $x^2(3) = 19.12$, $P = 0.0003$ and $x^2(3) = 18.60$, $P = 0.0003$, respectively). The abundance of bacteria and fungi in bare soil was significantly lower than in chlorolichen- and moss-dominated biocrusts. (Nemenyi post hoc test, Fig. 2a). The ratio of bacterial and fungal gene copy numbers decreased with succession.

Alpha diversity values increased with the succession, regardless of sequencing depth and diversity measure used (Fig. 2b–d, Supplementary Figure S1). Observed species numbers (i.e. number of OTUs) ranged between 5 586.3 and 11 668.7 and differed significantly depending on soil/biocrust type ($F\ (3,\ 24) = 14.48$, $P = 1.36 \times 10^{-5}$). Post hoc comparison identified bare soil to have significantly lower bacterial diversity compared to biocrusts (Fig. 2b). The Shannon index of species diversity ranged between 8.0 and 11.2, increasing in a stepwise manner from bare soil along succession. It varied significantly depending on crust type (Kruskal–Wallis H test; $x^2(3) = 18.77$, $P = 0.0003$; Fig. 2c) with a significantly higher value for moss- as compared to cyanobacteria-dominated biocrusts and bare soil. Also phylogenetic diversity values differed significantly as a

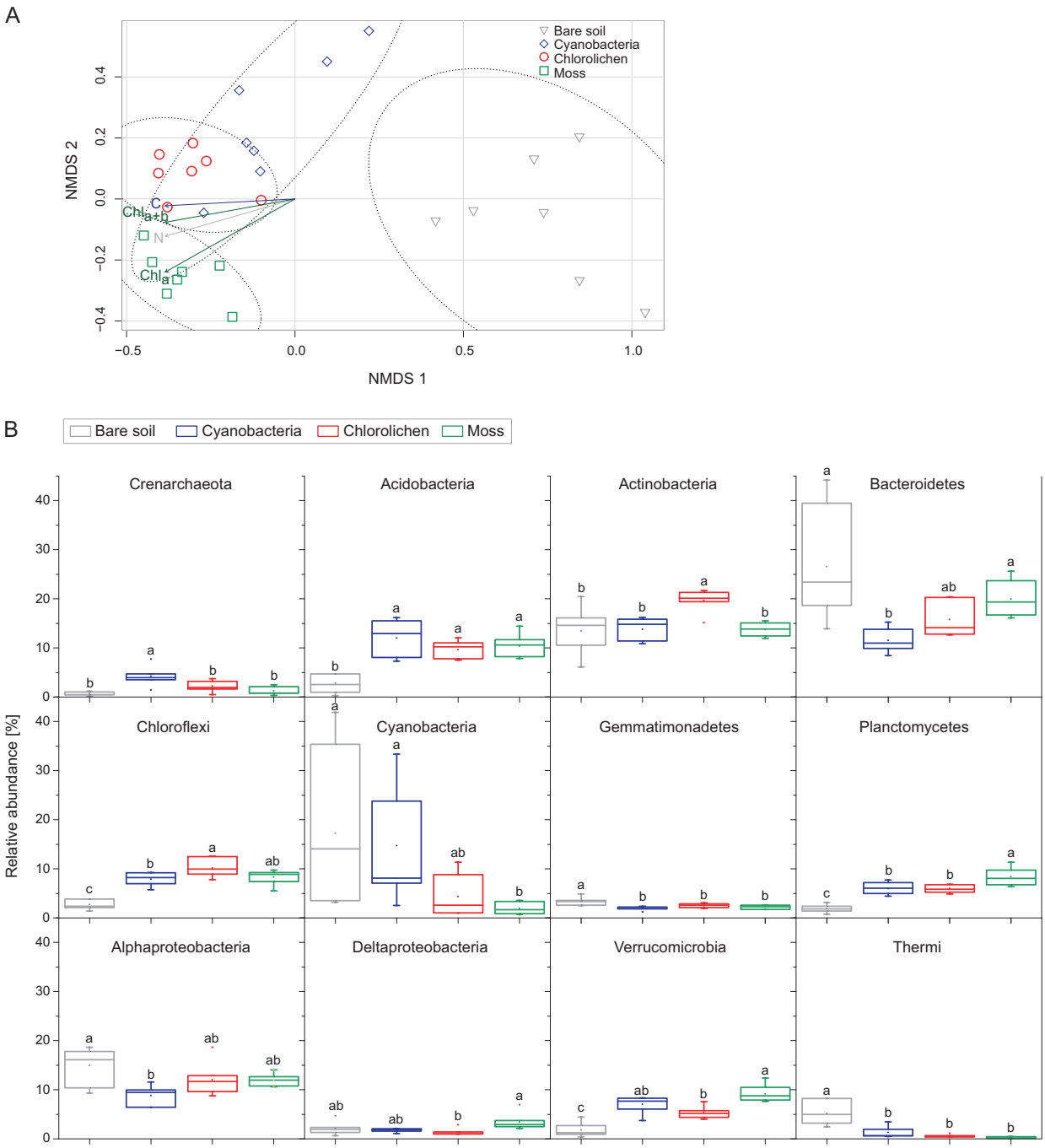

Fig. 3 Dissimilarity of bacterial community composition in bare soil as compared to different biocrust types. Ordination using Non-Metric Multidimensional Scaling derived from Bray–Curtis dissimilarity; based on amplicon 16S rRNA gene data, rarefaction with a depth of 48 009 reads per sample; symbols coded by category. 95% confidence ellipses are shown. The function envfit from the R vegan package was used to fit environmental vectors onto the ordination (**a**). Relative abundance of bacterial and archaeal taxa in crust habitats and bare soil. Based on amplicon 16S rRNA gene data; rarefaction with a depth of 48 009 reads per sample. Boxes limit the 25th- and 75th percentile with the median presented as line and mean values marked as point inside. Error bars present the 1st and 99th percentile and outliers are shown as dots below and above. Significant differences ($P < 0.05$) are marked by different letters. $n = 7$ per category (**b**)

function of surface cover type ($F(3,24) = 15.77$, $P = 7.05 \times 10^{-6}$) with significantly higher phylogenetic diversity values of biocrusts as compared to soil samples not covered by crust (Fig. 2d).

The samples of biocrust types and bare soil formed clearly delimited groups using OTU-based metrics (Bray–Curtis dissimilarity; Fig. 3a) and also clustered separately when community differences were measured

using phylogenetic metrics. Whereas samples of bare soil plotted separately and at some distance, samples of cyanobacteria-dominated crusts overlapped with those of chlorolichen- and moss-dominated crust samples, retracing the development from cyanobacteria- towards moss-dominated biocrusts. Soil/biocrust type was a good predictor of community composition based on Bray–Curtis dissimilarity (ANOSIM $R = 0.76$, $P < 0.001$). Furthermore, the divergence in community composition measured by phylogenetic metrics was associated with surface cover type (ANOSIM analyses of unweighted UniFrac distances $R = 0.82$, $P < 0.001$, ANOSIM of weighted UniFrac $R = 0.56$, $P < 0.001$).

The development from cyanobacteria- towards moss-dominated biocrusts can partly be seen in fungal community composition (Supplementary figure 2A). Samples of bare soil plotted separately, samples of cyanobacteria-dominated crusts overlapped with those of moss-dominated crust samples (Bray–Curtis dissimilarity; ANOSIM $R = 0.66$, $P < 0.001$).

The correlations between soil parameters and NMDS axes of Fig. 3a and S2A are listed in Supplementary Table S2.

Microbial composition

Across all samples, the most abundant phyla were Bacteroidetes (average: 18.5% across all samples), Proteobacteria (15.8%), and Actinobacteria (15.2%), with smaller contributions of Cyanobacteria (9.5%), Acidobacteria (8.7%), Chloroflexi (7.3%), Verrucomicrobia (5.8%) and Planctomycetes (5.6%). Furthermore, the phyla Gemmatimonadetes, Thermi and Armatimonadetes were present. At class level, Alphaproteobacteria (11.9%), Saprospirae (9.2%), Cytophagia (7.6%), Actinobacteria (5.5%), Chloracidobacteria (5.5%), Oscillatoriophycideae (5.9%) and Spartobacteria (5.3%) mainly contributed to the bacterial community. The Alphaproteobacteria were primarily represented by the order Sphingomonadales (4.7%) and Rhizobiales (3.9%) and the class Saprospirae by the order Saprospirales (9.2%). Among the Cytophagia, the order Cytophagales (7.6%) was predominantly detected.

Comparing the samples of the four soil/biocrust types, significant taxonomic differences were observed between all of them (Table 1). We also observed statistically significant differences in the relative abundance between the soil/biocrust types for all bacterial phyla (relative abundance >1%) as well as for Crenarchaeota, (ANOVA, $P < 0.01$; Fig. 3b; Supplementary Table S3). The relative abundance of Acidobacteria, Chloroflexi, Planctomycetes and Verrucomicrobia was significantly higher in biocrusts compared to bare soil, whereas the relative abundance of Gemmatimonadetes and Thermi was significantly higher in the bare soil as compared to biocrusts.

Table 1 Comparison of the bacterial taxonomic composition at phylum level across the four categories: cyanobacteria-, chlorolichen-, moss-dominated biocrust associated soil and bare soil

		Bare	Moss	Cyanobacteria
Chlorolichen	U	$P < 0.00001$	$P < 0.00001$	$P < 0.00001$
	B	$P < 0.00001$	$P < 0.00005$	$P < 0.00001$
Cyanobacteria	U	$P < 0.00027$	$P < 0.00001$	
	B	$P < 0.0016$	$P < 0.00001$	
Moss	U	$P < 0.00001$		
	B	$P < 0.00001$		

Unadjusted (U) and Bonferroni (B)-adjusted p values for all pairwise comparisons between chlorolichen, cyanobacteria, moss, and bare soil samples. This suggests that all four sample sets are statistically different at phylum level (Generalized Wald-type test statistic; taxa composition data analysis approach introduced by La Rosa et al. (2012)) [79]

Also on OTU-level, we observed significant differences between surface cover types. Comparing bare soil bacterial communities with those of biocrusts, the number of OTUs that were differentially abundant increased with succession (737/1699/1647; DESeq2, Benjamini–Hochberg-adjusted P value < 0.01, $n = 7$). The relative abundance of OTUs in bare soil showed most similarities to cyanobacteria-dominated biocrusts, but OTUs related to 17 taxa also had higher relative abundances in the cyanobacteria-dominated biocrusts compared to bare soil. Microbial communities of chlorolichen- and moss-dominated biocrusts were less similar to bare soil, even if these still shared fractions. OTUs related to 32 taxa in chlorolichen- and 27 in moss-dominated crusts were significantly more abundant in biocrusts than in bare soil.

Largest changes were observed for OTUs related to the family Phormidiaceae in cyanobacteria-dominated biocrusts compared to bare soil, and for Chitinophagaceae (Bacteroidetes) in chlorolichen- and moss-dominated biocrusts compared to bare soil. A notable decrease in relative abundance was observed for OTUs classified as Sporichthyaceae, Euzebyaceae, Geodermatophilaceae, Micrococcaceae (Actinobacteria), Flavobacteriaceae, Flammeovirgaceae, Rhodothermaceae (Bacteroidetes), Erythrobacteraceae, Rhodobacteraceae (Alphaproteobacteria), Comamonadaceae (Betaproteobacteria), Bacteriovoracaceae (Deltaproteobacteria), Pseudanabaenaceae (Cyanobacteria), Bacillaceae, Planococcaceae (Firmicutes) and, Trueperaceae (Thermi) for all biocrust types compared to bare soil (Supplementary Figure S3).

Cyanobacteria-dominated biocrusts had a significantly increased relative abundance of OTUs related to the family Phormidiaceae and Trueperaceae compared to moss-covered soil, whereas both Chitinophagaceae and Cytophagaceae had a reduced relative abundance in cyanobacteria-dominated biocrusts (DESeq2, Benjamini–Hochberg-adjusted P value <0.01, $n = 7$, Fig. 4a).

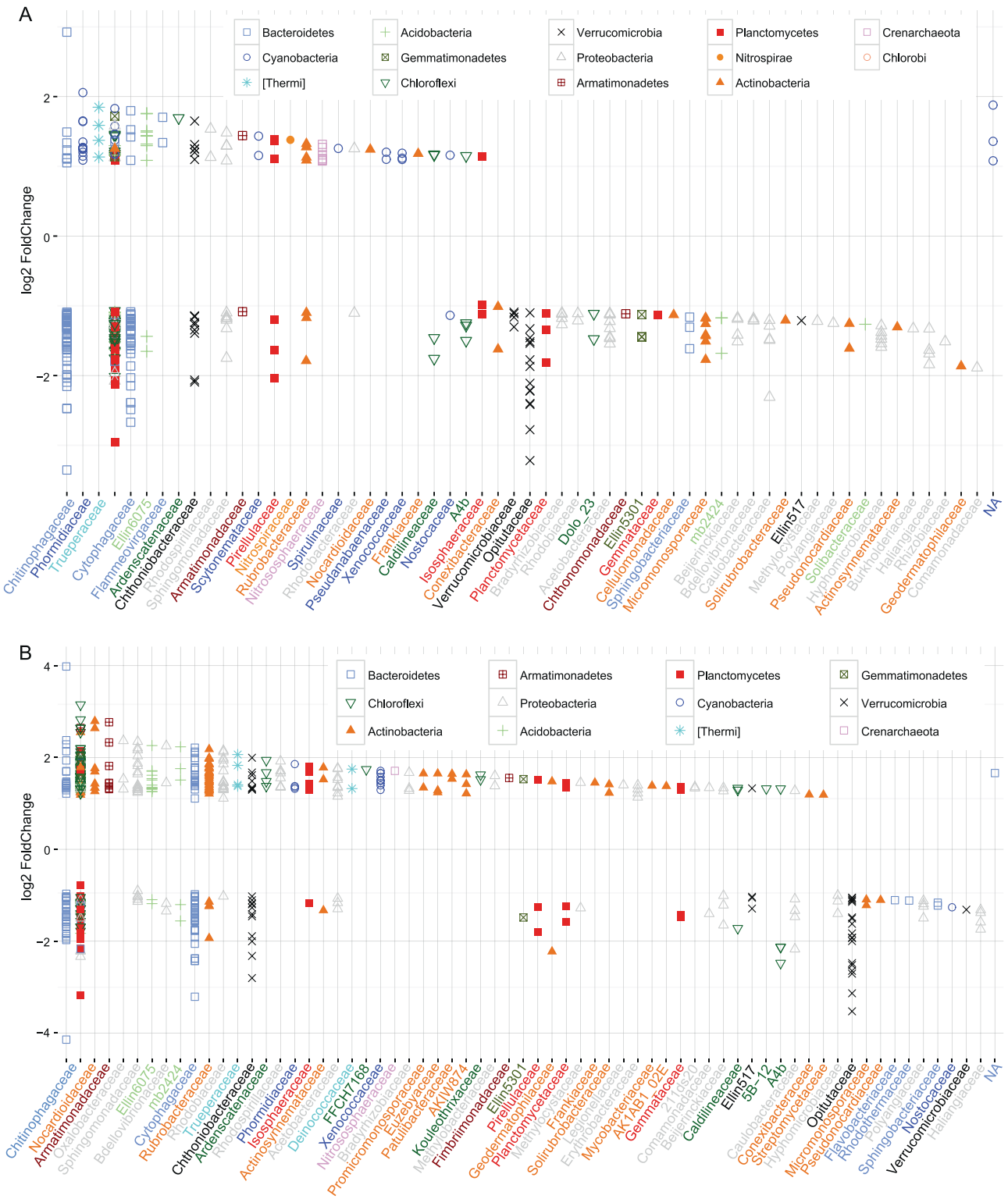

Fig. 4 Relative abundance of bacterial OTUs at family-level in cyanobacteria-dominated (**a**) and chlorolichen-dominated (**b**) compared to moss-dominated biocrusts. Each symbol represents an OTU and is colored according to phylum. The family level is plotted on the *x*-axis. Data shown as log2 Fold Change, positive values indicate an increased presence in cyanobacteria-/chlorolichen-dominated as compared to moss-dominated biocrusts (DESeq2, Benjamini–Hochberg adjusted $P < 0.01$, $n = 7$)

Nocardioidaceae, Armatiomonadaceae and Oxalobacteraceae were elevated in chlorolichen- compared to moss-dominated biocrusts (DESeq2, Benjamini–Hochberg-adjusted P value < 0.01, $n = 7$, Fig. 4b). A notable decrease in relative abundance was observed for OTUs classified as Lecanorales and Pleosporales for cyanobacteria-, chlorolichen- and moss-dominated biocrusts compared to bare soil. (Supplementary Figure S2B–D).

Abundant and ubiquitous members of the microbial community across all soil and biocrust samples were identified according to the two-parameter model introduced by Li et al. [44]. Using this measure, the following eight core taxa were detected at family level (ubiquity cutoff: 80%, abundance cutoff: 1%): Bradyrhizobiaceae, Rhodobiaceae, Sphingomonadaceae, Ellin6075, Chthoniobacteraceae, Rubrobacteraceae, Cytophagaceae, and Chitinophagaceae. Furthermore, we identified 59 out of 500 OTUs (11.8%) as generalists, including taxa, which were identified according to the approach by Li et al. [44] (Fig. 5a, b). 134 OTUs were defined as specialists for the four habitats (26.8%) (Fig. 5c and Supplementary Figure S4). The majority of the specialists, 95 OTUs, could be ascribed to the habitat soil.

Soil parameters

Both total carbon and nitrogen contents increased with biocrust succession (Fig. 6a, b; Supplementary Table S4). Total carbon contents were significantly higher in moss-dominated compared to the other biocrust types and furthermore all crust types showed significant differences to bare soil. Nitrogen contents were significantly higher in moss-dominated biocrusts compared to bare soil and the other types of biocrusts. pH values of biocrusts and bare soil were in a neutral to weakly alkaline range (7.4–8) with moss-dominated reaching significantly higher values than chlorolichen-dominated biocrusts, whereas cyanobacteria-dominated biocrusts and bare soil did not differ significantly from them (Fig. 6c, Supplementary Table S4).

Photosynthetically active biomass measured as chlorophyll$_a$ (chl$_a$) and chl$_{a+b}$ contents showed similar patterns of increasing contents with progressing biocrust succession (Fig. 6d, e). Whereas bare soil had only small amounts of chl$_a$ and chl$_{a+b}$, cyanobacteria- and chlorolichen-dominated biocrusts showed significantly higher chlorophyll contents in a medium range. Moss-dominated biocrusts had the highest contents with 84.9 ± 18.4 mg chl$_a$ m^{-2} and 140.6 ± 25.6 mg chl$_{a+b}$ m^{-2}, being significantly higher than in all other biocrust types and in bare soil samples.

Soil respiration

Respiration of all biocrust types (complete biocrusts) increased with ascending temperature and reached highest values between 3.3 µmol CO_2 m^{-2} s^{-1} for moss-dominated, 3.2 µmol CO_2 m^{-2} s^{-1} for chlorolichen-dominated and 2.2 µmol CO_2 m^{-2} s^{-1} for cyanobacteria-dominated biocrusts at 37 °C (Fig. 7a). Soil respiration of the heterotrophic fraction of cyanobacteria- and chlorolichen-dominated biocrusts was similar (fluctuating around zero; maximum values of 0.27 and 0.16 µmol CO_2 m^{-2} s^{-1}), whereas in moss-dominated biocrusts significantly higher values were reached at 37 °C (1.94 µmol CO_2 m^{-2} s^{-1}; Fig. 7, Supplementary Table S5).

Reactive nitrogen emissions

Characteristic HONO and NO emission patterns were observed for all types of biocrusts, whereas for bare soil no significant amounts of reactive nitrogen emissions were measured (Fig. 7b, c). Cyanobacteria-dominated biocrusts showed highest HONO and NO emissions (208 ± 15 ng m^{-2} s^{-1} of NO–N and 173 ± 18 ng m^{-2} s^{-1} of HONO–N) at a range of 0–10% of soil water content (SWC), which equals ~20–25% water-holding capacity (WHC). Smaller HONO and NO emission were released from chlorolichen- and moss-dominated biocrusts and extended over a wider range of 0–30% SWC (~20–80% WHC), where the highest fluxes amounted to 94.85 and 47.61 ng m^{-2} s^{-1} of NO–N and 61.86 and 46.14 ng m^{-2} s^{-1} of HONO–N, respectively. Opposed to this, the average fluxes of bare soil samples were lower by more than a factor of 20 (9 ± 3 ng m^{-2} s^{-1} for NO–N and 5 ± 2 ng m^{-2} s^{-1} for HONO–N).

Discussion

Our investigations revealed a clear shift of the heterotrophic community composition along biocrust succession, which apparently causes altered physiological properties. Also, nutrient and chlorophyll contents varied between the successional stages. Thus, we could verify our hypothesis, i.e., that the photoautotrophic organisms affect the composition of the heterotrophic community, which influences the physiological properties of the biocrusts.

Shift in diversity and relative abundance along the successional stages

We observed that 16S and 18S rRNA gene copy numbers as well as alpha diversity values were highest in late successional biocrust stages, which is in line with the results of a study conducted at the Colorado Plateau [45]. The Shannon index ranged from 8.0 to 11.2, being quite high in comparison to forests soils at Shennongjia Mountain in China, where values of 7.0 for coniferous forests up to 8.1 for evergreen broadleaved forests were determined [46].

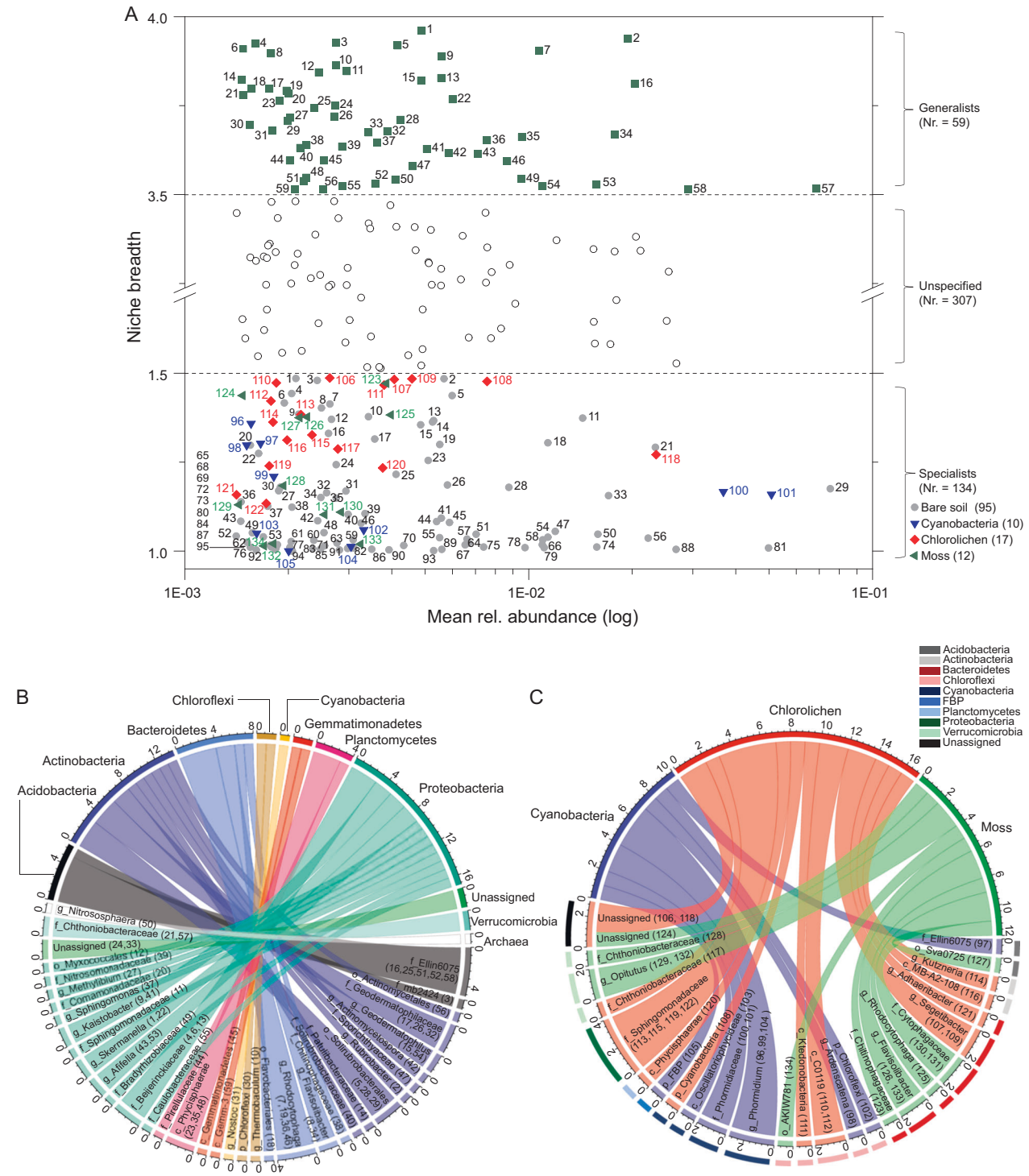

Fig. 5 Analysis of habitat specialists and generalists. Niche breadth (*B*) of OTUs identified in bare soil and different biocrust types. Each symbol represents an OTU. OTUs that are present along a wider range of habitats have a higher *B* value and are considered habitat generalists (numbered from 1-59), while OTUs with a *B* value < 1.5 are considered habitat specialists (numbered from 1-134). Numbers adjacent to points refer to numbers in brackets in B, C and S4. Open circles show OTUs that could not be defined as generalists or specialists (**a**). Circos plot showing taxonomic assignment for OTUs identified as generalists (**b**) and specialists in cyanobacteria-, chlorolichen-, and moss-dominated biocrusts (**c**). Specialists in bare soil are shown in Supplementary Figure S4. Numbers outside of the circles indicate the number of OTUs

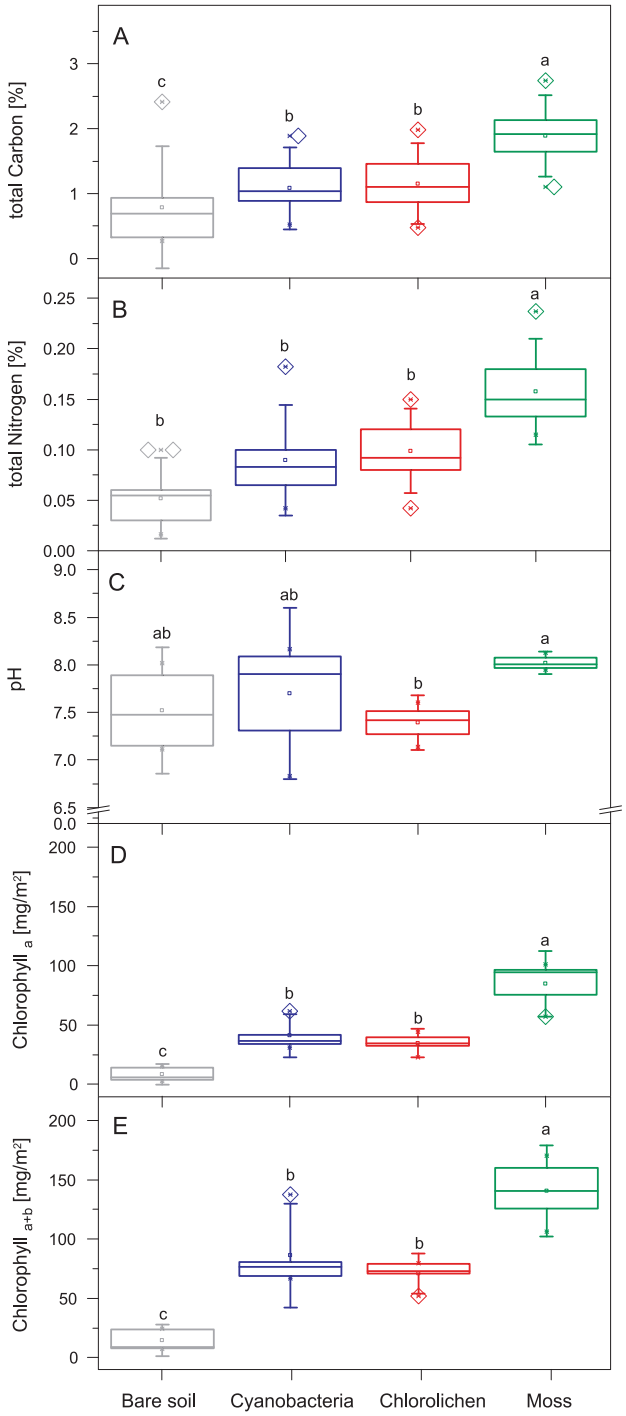

Fig. 6 Total carbon (**a**), total nitrogen (**b**), pH (**c**) chlorophyll$_a$ (**d**), and chlorophyll$_{a+b}$ contents (**e**) of different types of biocrusts plus bare soil. Boxes limit the 25th- and 75th percentile with the median presented as line and mean values marked as point inside. Error bars present the 1st and 99th percentile and outliers are shown as dots below and above. Significant differences ($P < 0.05$) are marked by different letters

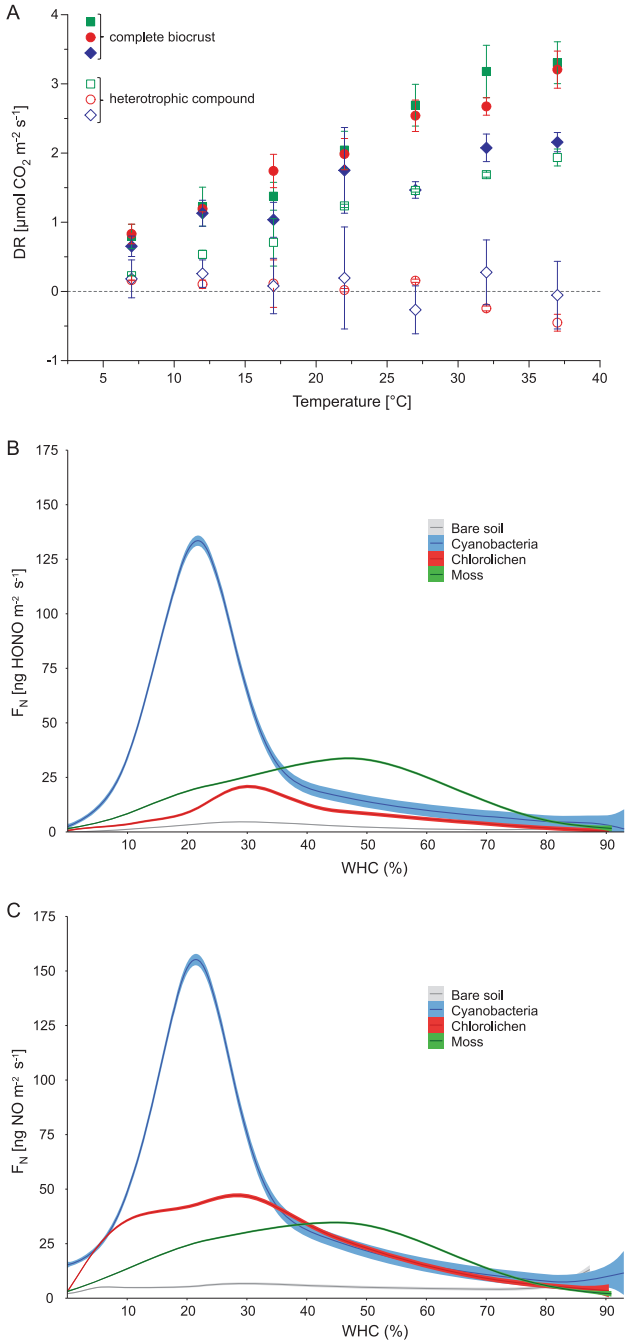

Fig. 7 Respiration (DR) of different biocrust types and their heterotrophic fraction at varying temperatures (**a**). Differently colored symbols represent mean DR values of cyanobacteria- (closed rhombus, blue), chlorolichen- (closed triangle, red), and moss-dominated biocrusts (closed square, green). DR values of the heterotrophic fraction of different biocrust types are shown as open symbols. Measurements were conducted under optimum water conditions at 380 ppm CO_2 ($n = 3$). Characteristic emission patterns of HONO (**b**) and NO (**c**) from different biocrust types and bare soil. The flux was calculated based on four replicates of each type of biocrust by geom_smooth model in ggplot2 of R software. The upper and lower bands show the pointwise 95% confidence interval around the mean

However, it is comparable to temperate grassland and forest soils in Germany, where values of 10.1 and 9.5 were obtained, respectively [47]. The bacterial community of

bare soil was different from biocrust communities and successional stage determined the assembly of heterotrophic soil communities. Besides heterotrophic organisms, cyanobacteria were also present, with the highest relative abundance in bare soil. This is consistent with the view that cyanobacteria act as pioneers in the stabilization process of soils [48, 49]. The data suggest that fractions of the bacterial community in cyanobacteria-dominated biocrusts are retained during the development of chlorolichen- and moss-dominated biocrusts. Changes of bacterial communities at different ages of biocrusts have also been reported for the Tengger Desert, China [50].

During successional dynamics in plants, key processes such as facilitation between community members, resulting in reduced environmental stress, take place [51]. Similar mechanisms probably also occur in biocrusts, e.g., via substrate stabilization and nutrient input by early colonizers, thus driving microbial succession. Our data show an increased relative abundance of members of the families Geodermatophilaceae, Bacillaceae, and Trueperaceae (Supplementary Figure S1) in early biocrust stages. Geodermatophilaceae are spore-forming and capable to grow in nutrient-poor biotopes such as dry soils or mineral rock. They have been reported to resist oxidative stress, heavy metals, exposure to high gamma ionizing radiation, UV light, as well as desiccation, and were shown to survive in the atmosphere [52]. *Modestobacter versicolor* for instance has been isolated from biocrusts from the Colorado Plateau in USA [53]. Similarly resistant are Trueperaceae [54] and endospores of Bacillaceae, which potentially survive unfavorable conditions for thousands of years as well as intercontinental dispersal [55]. The predominance of bacterial taxa, which are known to be highly tolerant to environmental stresses, in early biocrust stages could be linked to the successional development of biocrusts. During development, biocrusts frequently form a layered structure characterized by an upper layer colonized by heavily pigmented fungi and cyanobacteria and an underlying layer with less-UV-tolerant organisms [4]. This might create niches for less robust bacterial taxa. Analyses revealed that the relative abundance of some bacterial taxa with metabolic versatility and broadly adapted genera, decreased along succession. Examples are the family Flavobacteriaceae [56], Sporichthyaceae and Comamonadaceae [57].

Our data suggest that the dominating photoautotrophic organism may facilitate the persistence of a specific microbial community, potentially induced by locally modified environmental conditions, as e.g., light, temperature, and nutrient conditions. A previous study has shown that the composition of microbial metacommunities at lichen-rock interfaces was strongly influenced by the lichen species [58]. Similarly, research on the rhizosphere has shown that the assembly of microbiota is influenced by plant species among other factors [59]. Bare soil, but also the atmosphere can be regarded as reservoir of microorganisms and the physicochemical properties and biogeographical processes affect the microbial population of varying habitats [60, 61].

Altered microbial composition reflected by shifts in nutrient composition

Our study revealed increasing carbon and nitrogen contents from bare soil via initial to developed biocrusts (Fig. 6a, b) and statistically significant correlations between community composition and soil parameters (Supplementary Table S2). Li et al. [62] found the same trend of nutrient-level enhancement along successional stages in biocrusts from Delate Country, Inner Mongolia. Moreover, it was shown that crust bryophytes influence soil fertility not only by intercepting dust particles, but also by facilitating development of microorganism communities that increase the nutrient status of biocrusts [63]. Such an improved nutrient status within well-developed biocrusts may be linked to the abundance of biomass-degrading microorganisms in the substrate. Our data revealed that Chitinophagaceae and Cytophagaceae, cellulose and chitin-degrading taxa, are more abundant in the biocrusts compared to soil without cryptogams (Supplementary Figure S1).

Bacteria have also been shown to respond individually to different carbon sources. Cleveland et al. [64] demonstrated a shift in bacterial composition combined with increased CO_2 fluxes as a result of the addition of low-molecular-weight organic carbon. Recent studies have shown that rhizodeposits such as low-molecular-mass compounds (sugars, amino acids, organic acids) and polymerized sugar are partly responsible for variations in rhizospere-associated bacteria and bulk soil microbial communities [59].

In our study, OTUs classified as Chloroflexi occurred more frequently in biocrusts as compared to bare soil. Chloroflexi have been observed to occur in close contact with Cyanobacteria in microbial mats [65]. Burow et al. [66] proposed a metabolic pathway between *Microcoleus* spp. fermenting photosynthates to organic acids, and Chloroflexi spp., taking these up to be stored as polyhydroxyalkanoates. This metabolic link might be a more general phenomenon which could explain the increased occurrence of OTUs classified as Chloroflexi in biocrust associated soil.

Effects of altered microbial community composition on soil respiration and reactive nitrogen gas emissions

Our results provide evidence that altered microbial communities may affect soil respiration, which is higher in

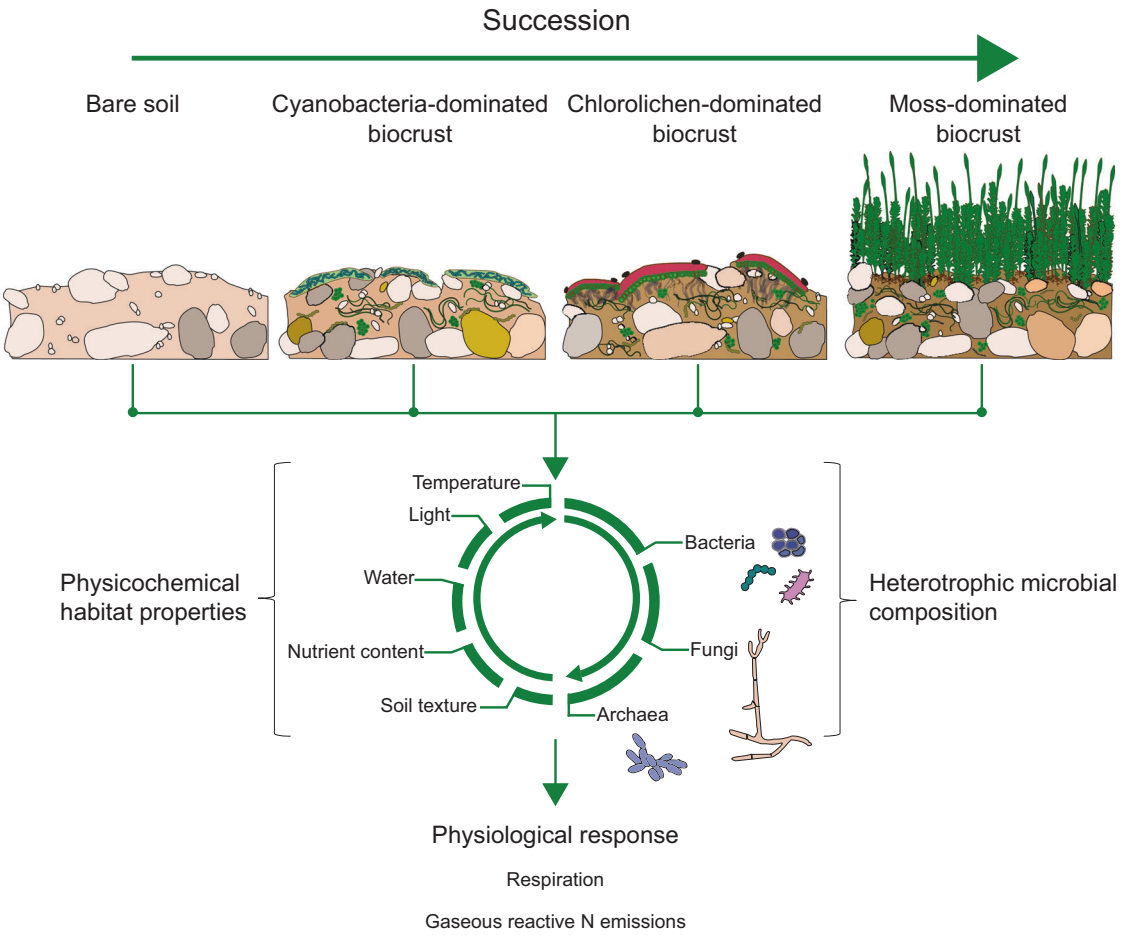

Fig. 8 Schematic diagram showing the successional development of biocrusts. Along succession, the heterotrophic community as well as physicochemical properties within biocrusts change, causing altered physiological properties

moss-dominated as compared to earlier biocrust stages and bare soil (Fig. 7a). Plant cover has been shown to affect soil respiration and microbial enzyme activity in arid ecosystems [67]. Furthermore, our results are in line with findings of previous studies, where soil respiration rates have been shown to be twice as high in well-developed late compared to early successional biocrusts, and to be significantly higher in biocrust-dominated compared to open microsites with low biocrust cover [24, 68]. High respiration values of moss-dominated biocrusts may be favored by microbial decomposition of carbohydrates, as moss-dominated biocrusts comprise a lot of dead plant material. These results are in line with a previous study, which demonstrated a shift in the bacterial composition and an increase in CO_2 fluxes upon the addition of low-molecular-weight organic carbon [64].

Recent studies showed that reactive nitrogen gases, such as HONO, NO and the greenhouse gas N_2O (nitrous oxide) are emitted by biocrusts [43, 69, 70]. Moreover, gas fluxes from soils are affected by soil pH, nitrite concentration, and also the presence of ammonia-oxidizing bacteria [71–73]. In our laboratory study, the HONO and NO emission patterns changed with the successional stages of biocrusts, resulting in a sharp peak for cyanobacteria-dominated biocrusts and a wider range of moderately increased emissions for chlorolichen- and moss-dominated biocrusts (Fig. 7). The sharp peak of cyanobacteria-dominated biocrusts at low soil water contents may indicate that NO and HONO are emitted under oxic conditions during nitrification. Our results suggest an increased relative abundance of the family Nitrospiraceae in cyanobacteria- compared to moss-dominated biocrusts (Fig. 4a). *Nitrospira* has recently been described as the first bacterial genus observed to conduct complete nitrification, i.e., ammonium and nitrite oxidation [74]. Also Nitrososphaeraceae revealed an increased relative abundance in cyanobacteria- compared to moss-dominated biocrusts and bare soil (Fig. 4a and Supplementary Figure S3A). Archaeal populations have previously been observed in biocrusts [75, 76] and archaeal *amoA* genes (Nitrososphaera) are widely present in biocrusts in desert regions across the United States, indicating that archaea are likely involved in ammonia oxidation in biocrusts [77]. Thus,

peaking emissions of HONO and NO in cyanobacteria-dominated biocrusts could be explained by increased abundance of nitrifying bacteria and archaea.

Conclusions

Our study, to our knowledge, is one of the first investigating the microbial community composition within biocrusts along different successional stages and linking these with their physiological properties. While the succession of photoautotrophs, used for classification of crust types, has been known and modeled [1, 9, 78], we show here that they strongly affect the heterotrophic microbial composition and the physiological properties, probably via impacting the physicochemical habitat properties (Fig. 8). The community composition combined with particular habitat conditions likely determines the physiological properties of different successional stages of biocrusts. These aspects need to be incorporated in future ecological models, which help to understand the functioning and vulnerability of biocrusts towards disturbance such as land use alterations and climate change.

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Compliance with Ethical Standards

Conflict of interest :The authors declare that they have no conflict of interest.

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