

## Structure and function of biological soil crusts from Antarctica with a special respect to their microtopography and UV-B sensitivity

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### Abstract

Although an extensive professional literature exists on biological soil crusts (BSCs), especially on the species composition of hetero- and autotrophs forming the micro-biological community, micromorphological information on BSCs is extremely scarce. In our study, we focused on microstructure of the BSCs from the James Ross Island (Antarctica). We combined the approach of digital microscopy to study surface roughness of the BSCs with taxonomy of BSC-forming autotrophs and chlorophyll fluorescence study focused on the photosynthetic functioning of BSCs when exposed to controlled UV-B stress. Microprofiling of BSCs resulted in the finding that the examined BSCs might be classified as fine-grained surface with roughness characteristics: Ra (37.9 µm) and Rz (136.9 µm). The BSCs were rich in microautotrophs, both algae and cyanobacteria, however, *Microcoleus* sp. was found dominating species. It formed multifilament ropes on and inside the BSCs. Under UV-B stress, *Microcoleus*- and *Nostoc*-dominated BSC parts showed similar sensitivity and acclimatory response so long-term UV-B treatment, however, *Microcoleus* seemed to be slightly more sensitive to UV-B. *Microcoleus*-dominated parts of BSCs showed less pronounced acclimation to UV-B treatment than *Nostoc*-dominated parts. It was reflected in lower values of maximum ( $F_v/F_m$ ) and effective ( $\Phi_{PSII}$ ) quantum yields recorded after 6 d exposition.

**Key words:** surface roughness, 3-D digital microscopy, cyanobacteria, ecophysiology, UV-B

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### Introduction

Biological soil crusts (BSCs) encompassing green algae, cyanobacteria, lichens, bryophytes, heterotrophic bacteria and mi-

crofungi are keystone species in arid environments because of their role in nitrogen- and carbon-fixation, weathering and soil

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stabilization, all depending on the photosynthesis of the BSCs. Despite their importance, little is known about the BSCs eco-physiological functioning in desert and semidesert Earth locations (including polar deserts) under a wide variety of climatic conditions. It is well established that especially crustose chlorolichens (BSCs constituents) account for a large proportion of biomass in the arid zones, where photosynthesis is mainly limited due to low water availability. Moreover, BSCs can be the only vegetation cover in arid and semi-arid regions such as hot and cold deserts or xerothermic steppe vegetation (Belnap and Lange 2001).

BSCs are complex communities of diminutive organisms integrated with particles of the soil surface. Potential of integration of cyanobacteria, green algae, lichens, mosses and other organisms is high. Therefore, these communities form a cohesive, thin horizontal layer over surface which integrates with the fine soil particles (Belnap and Lange 2001). The diversity and abundance of soil cyanobacterial and microalgal communities as first colonizers of high mountain soils may profoundly affect nutrient availability for pioneer vascular plants. In the less-studied hyperarid deserts of the world (see *e.g.* Büdel et al. 2013 for Sonoran desert), terricolous lichens are often the most widespread and conspicuous components of biological soil crusts. The success of lichen growth in hyperarid deserts is attributed to their poikilohydric nature, whereby they are metabolically active only when exposed to moisture (Green et al. 2018).

BSCs have been extensively studied in arid lands of temperate regions, particularly semi-arid steppes and warm hyperarid deserts (see *e.g.* Büdel et al. 2013 for Sonoran desert). In last decades, Arctic soil crusts (Pushkareva et al. 2017) and Antarctic soil crusts (Colesie et al. 2014), as well as Himalayan ones (Čápková et al.

2016) have received some attention. However, studies are still missing that include the spatial distribution of the photosynthetic activities within BSCs, which is of great importance to assess heterogeneity of photosynthesis and production of different parts of BSCs. Such heterogeneity could be studied by several chlorophyll fluorescence imaging as shown for *e.g.* lichens (Barták et al. 2000) and soil crusts (Kleeefeld et al. 2018). In polar regions (field studies), structure of BSCs including their carbon contents was studied by Jung et al. (2018), Colesie et al. (2014, 2016). Photosynthetic performance of BSCs based on chlorophyll fluorescence in relation to spectral reflectance data was studied by Yamano et al. (2006).

Within last decades, several aspects of BSCs functioning have been investigated such as *e.g.* UV-B radiation effects on primary photosynthetic processes monitored by chlorophyll fluorescence (Hui et al. 2015), water relations (Colesie et al. 2017) and the role of winter snow precipitations on their photosynthetic characteristics (Hui et al. 2018). Some studies focused on the photoinhibition of photosynthesis in BSCs dominating components and recovery (Wu et al. 2017) and involvement of photoprotective mechanism activated in BSC-forming cyanobacteria (Ohad et al. 2010, for *Microcoleus vaginatus*).

Community forming biological soil crusts are typically resistant against UV radiation, however the extent of resistance is species-specific. Cyanobacteria *per se* have a high potential to resist high doses of UV radiation, and temperature extremes. Lichens are resistant against high UV doses, especially when in dehydrated and thus physiologically inactive state. Some algal lichens, however, showed limited resistance to UV (UV-B, Ůnal and Uyanikgil 2011). In our study, sensitivity of BSCs from the James Ross Island (Antarctica) was studied.

## Material and Methods

### *BSCs sampling*

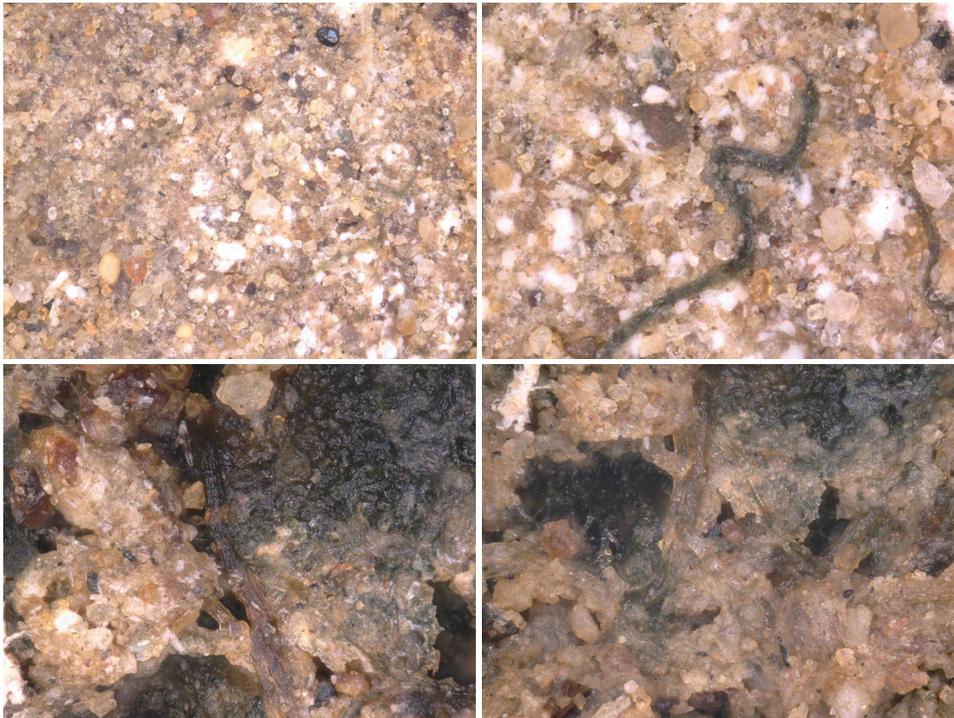
Samples of BSCs were collected at the James Ross Island, Antarctica in the locality dominated by soil crusts and lichen-dominated small-area vegetation oases (63°48'18''S, 57°54'43''W). The collection site is located 1 km W of the Mendel

station. The BSCs were collected in semi-dry state, placed into petri dishes and dried under natural conditions. In dry state, they were transported to the laboratories in the Czech Republic in a portable fridge at controlled temperature of 5°C.

### *BSCs microprofiling*

Topography of the BSC surface and microprofile of the BSCs was evaluated in dry state using a digital VHX-5000 microscope (Keyence, Japan) with a maximum resolution of 18 megapixels and the magnification of 1000. The BSC samples were in-

vestigated both in dry and wet state. The images (120) of the upper surface of BSCs were stored in a 3-D format with a maximum resolution of 18 megapixels. The 3-D images allowed to analyze BSC microrelief with the resolution of 1 µm.



**Fig. 1.** Surface of the biological soil crusts (BSCs) from the James Ross Island (Antarctica) with low (the upper panels) and high abundance of microautotrophs located on the BSCs surface. Multifilament ropes are distinguished on the surface. Photo by N. Tschense.

The differences in vertical arrangement of the surface of BSCs were evaluated as well as the proportion of BSCs-forming components such as the clusters of algae/

cyanobacteria, microlichens located on the surface of BSCs. Surface roughness was evaluated by Ra, Rz parameters (*see* Results and Discussion).

### *Species forming BSC-community*

Autotrophic microorganisms were determined by optical microscopy. Small pieces of the BSC were taken by a pinsetter. Then the pieces were desintegrated

in a drop of water and BSC fragments were studied under the Olympus microscope. Species were identified into genera according to their morphological features.

### *Sensitivity to UV-B stress*

Pieces of BSCs (area of about 8.0 cm<sup>2</sup>) were re-wetted for 72 h by regular spraying by demineralized water. During re-wetting period, the BSCs were kept on light at the temperature of 10°C. Then, the BSCs were exposed to 1.4 W m<sup>-2</sup> of UV-B radiation combined with 50 μmol m<sup>-2</sup> s<sup>-1</sup> of photosynthetically active radiation (PAR). The exposition lasted 6 days. During the exposition period, photosynthetic performance of the BSCs was monitored repeatedly (mostly in 24 h interval) using slow chlorophyll fluorescence kinetics supplemented

by saturation pulse method measured by a FluorCam device (PSI, Drásov). For measuring procedure, see *e.g.* Hájek et al. (2009). After UV-B treatment, the measurement was repeated 20 h after the exposition to determine the early phase of recovery of the community. Time courses of potential (F<sub>v</sub>/F<sub>M</sub>) and effective quantum yield of photochemical processes of photosynthesis (Φ<sub>PSII</sub>) were evaluated for *Nostoc*- and *Microcoleus*-dominated parts so that the species-specific effect of UV-B treatment could be distinguished.

## **Results and Discussion**

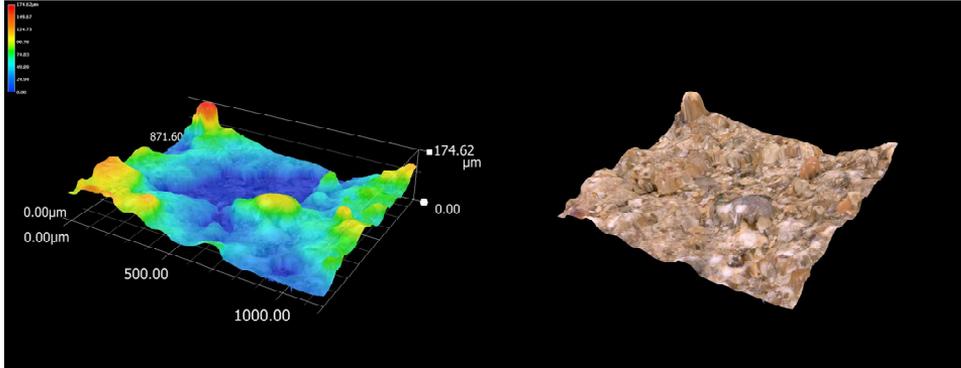
The surface of BSCs was formed by mineral grains bound into aggregates by fungi hyphae and clusters of microautotrophs (Fig. 1). In the studied samples, a wide range of surface cover by autotrophic microorganisms was apparent, ranging from almost no autotrophs on the BSC surface to more than 75% cover. The size of mineral grain clusters and the abundance of big grains (about 1 mm) differed between the samples, however, majority of the investigated BSCs were fine-grained with low appearance of surface autotrophs. For the analysis of BSC surface profile, the lichen-free BSC samples were selected. Therefore, the results presented in Fig. 3, Fig. 4 are valid only for fine-grained BSCs. Typically, the difference between minima and maxima of the profile

reached the range of 150–200 μm (Fig. 2). Surface roughness of the investigated fine-grained BSCs was evaluated by two amplitude parameters: (1) mean arithmetic deviation of the primary profile (Ra), and (2) maximum height of the profile (Rz). Ra was calculated as mean value of filtered roughness profile determined from deviations about the center line within the evaluation length of 1500 μm.

Mean Ra reached 37.9 ± 19.4 μm which means that the surface might be classified as the first class that includes microrelief variations from individual soil grains to aggregates on the order of 0.053–2.0 mm. In this respect, the BSCs from the James Ross Island belonged to those having a fine microstructure. Rz value was found 136.9 μm. It has been shown for cyano-

bacteria-dominated BSCs, that cyanobacteria may alter soil surface roughness and decrease soil loss after precipitation (Bullard et al. 2018). The authors conclude that

regardless of the rainfall amount and intensity, soil erosion is limited in cyanobacteria-dominated BSCs which highlights their importance for soil stabilization.



**Fig. 2.** Visualization of microtopography of the surface of a BSC with low cover of microbiological items. View on 3-D structure of the BSC surface (right) and false-colour 3-D image of BSC microstructure (left).

Examined BSCs were formed by several microautotrophs, however, *Microcoleus* sp. was dominant species. Characteristics of the isolated and determined microalgae and cyanobacteria is given in Table 1. The dominant role of *Microcoleus* sp. corresponds well to the categorization of BSCs in cold deserts overviewed by Jung et al. (2018) who attributes the role to the fact that filamentous cyanobacteria stabilize soils due to the presence of extracellular polysaccharidic matrix. These cyanobacteria, such as *Microcoleus* or *Leptolyngbya*

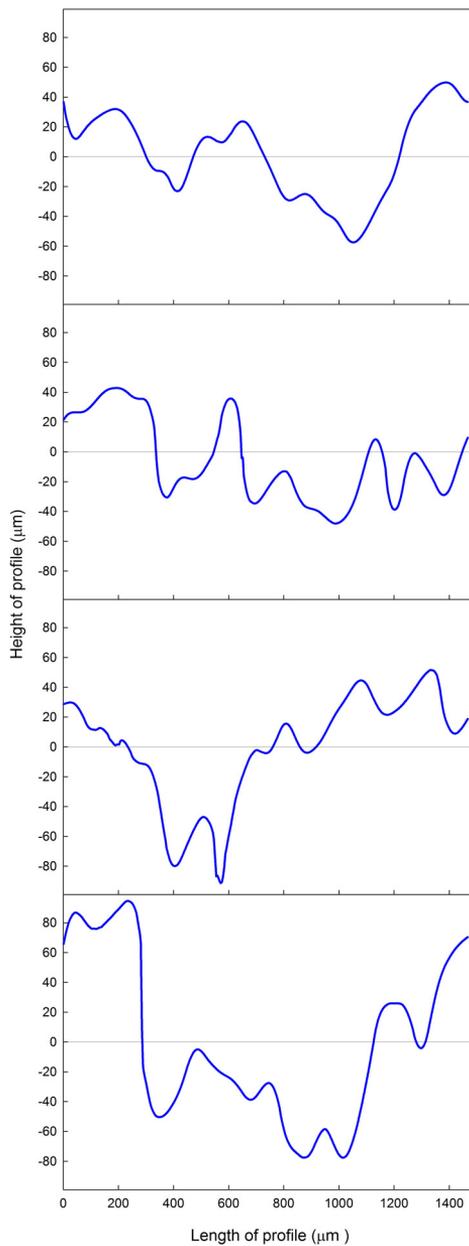
cause crust formation and are also the most abundant cyanobacteria species in BSCs. Building filaments is an essential feature that enables cyanobacteria to colonize physically unstable environments, such as e.g. cryoturbated soils, and they act as successful pioneers in the bio-stabilization process against erosion (Garcia-Pichel and Wojciechowski 2009). *Microcoleus* sp. is, together with other cyanobacteria, reported from the James Ross Island (see e.g. Strunecký et al. 2012).

Species	Description
<i>Microcoleus</i> sp.	filamentous cyanobacterium without heterocysts, motile, forms most of the biomass
<i>Nostoc</i> sp.	filamentous cyanobacterium with heterocysts, non-motile
<i>Leptolyngbya</i> sp.	filamentous cyanobacterium without heterocysts, thin filaments
<i>Calothrix</i> sp.	filamentous cyanobacterium with terminal heterocyst
<i>Gloeocapsa</i> sp.	unicellular cyanobacterium with thick EPS
<i>Hantzschia</i> sp.	diatom
coccoid algae	3 undetermined species

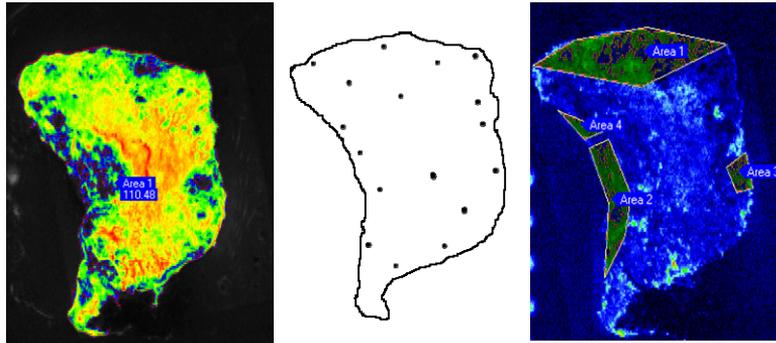
**Table 1.** Characteristics of the microautotrophs genera found in the BSCs from the James Ross Island, Antarctica.

Chlorophyll fluorescence imaging revealed heterogeneity of chlorophyll fluorescence signal over the selected BSCs (see Fig. 4 – left), as well as chlorophyll fluorescence parameters ( $F_V/F_M$ , and  $\Phi_{PSII}$  in particular). To find out, which part of this heterogeneity relates to the differences in species composition, microscopic analysis of several spots of the BSC were done (Fig. 4, center). Areas with clear dominance of *Microcoleus* and *Nostoc* were distinguished and evaluated separately (Fig. 4, left). Time courses of  $F_V/F_M$ , and  $\Phi_{PSII}$  revealed that the two species exhibited UV-B induced decline in both parameters during first hours of exposition. Then, the  $F_V/F_M$ , and  $\Phi_{PSII}$  values gradually increased indicating a positive adjustment of the parameters to UV-B (Fig. 5, right, after day 1). *Microcoleus*-dominated areas of the crust showed more pronounced decrease in the two parameters, but the recovery after the end of exposition was faster than in *Nostoc*-dominated areas. The effects of UV-B on cyanobacteria were even visible – after rehydration, the BSCs were brightly green, but turned to brownish grey during exposition to UV-B. Moreover, UV-B treatment caused significant changes of slow fluorescence kinetic time course taken after 1 h of exposition (see Fig. 5 – left). These changes suggest UV-B induced changes in PS II functioning and activation of photoprotective mechanisms such as e.g. detachment of light-harvesting complexes from core PS II and the change in redox state of plastoquinone pool.

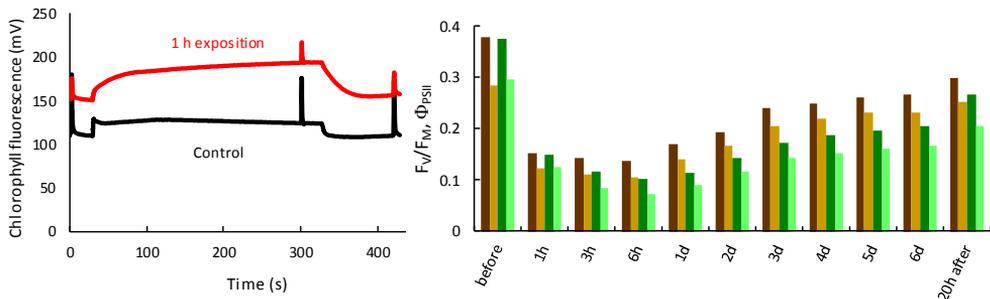
Faster positive adjustment could be explained by motility of *Microcoleus* sp. which could move deeper into the uppermost layer of the crusts during the UV-B treatment. Therefore, *Microcoleus*-dominated areas may be capable to adjust faster than *Nostoc*-dominated areas. It is well documented, that when wet, mucilage of cyanobacteria swell and filaments of cyanobacteria move up toward the BSCs (Belnap et al. 2001).



**Fig. 3.** Selected (typical) micro profiles of fine-grained biological soil crusts expressed in relation to medium (central) line. The differences are given in  $\mu\text{m}$  along the measuring distance 1 500  $\mu\text{m}$ .



**Fig. 4.** Chlorophyll fluorescence imaging of BSC (left – distribution of steady-state chlorophyll fluorescence), indication of the spots from which the samples for species determination were taken (centre), and indication of the areas with clear dominance of either *Nostoc* or *Microcoleus* (right).



**Fig. 5.** Chl. fluorescence curves recorded before (black) and after 1 h UV-B treatment (red) – left. Timecourses of  $F_v/F_m$  and  $\Phi_{PSII}$  recorded for *Nostoc*- (deep and light brown) and *Microcoleus*-dominated (deep and light green) BSCs during UV-B treatment – right.

## Concluding remarks

In this paper, we presented a small study testing microstructure of BSC surface and composition of microautotrophs. Due to limited number replicates, the study can be considered preliminary. The interpretation of the results is largely descriptive rather than statistical. Nevertheless, a comparison

of this study with other research done in BSC from polar (*e.g.* Sehnal et al. 2014) and alpine regions suggests the importance of integrated research in the follow-up studies focuses on BSC functioning in polar regions under ongoing global climate change.

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