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Biogeosciences Discussions

# *Interactive comment on* "Uncovering biological soil crusts: Carbon content and structure of intact Arctic, Antarctic and alpine biological soil crusts" *by* Patrick Jung et al.

**Patrick Jung et al.**

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Statement: The present manuscript has the value of providing information regarding biological soil crust communities from poor studied locations. At the same time, the manuscript introduces new methodologies that can be used to further understand biocrust structure and organization. Although I like the approach the authors used, I believe that a further effort in identifying cyanobacterial and microalgae species from the studied samples (by light microscopy or molecular survey) would have provided more insights and would have been helpful in supporting some of the points the authors make in the discussion and conclusion. Overall, there is a need to improve redaction, gram-



mar and the flow of the manuscript (mostly in the discussion). This can be achieved by removing no relevant information (discussion), splitting and shortening sentences, and using connectors and punctuation (overall).

Response to reviewer: We are pleased to inform you, that a second study regarding cyanobacterial diversity of the same habitats is already in process.

Abstract: Line 12: main primary producers instead of main producers The section has been redrafted as suggested at page 1 lines 12.

Introduction: Page1 Line 2: Change by cyanobacteria, bacteria, microalgae. Page 2 Line 3: missing a connector between (BCS), dominate these ecosystems.

The section has been changed as follows at page 2 line 1 to 2: Conglomerations of soil particles, cyanobacteria, bacteria, green algae, microfungi, lichens and bryophytes create a skin known as biological soil crusts (BSC) that dominate these ecosystems (Belnap et al., 2001; Williams et al., 2017).

Page 2 Line 13: I do not think that Johansen 1993 is the most relevant/precise reference for the two previous sentences, mostly when referring to hot and cold deserts worldwide. It should include other citations as well.

The following references were added: -Lacap-Bugler, D. C., Lee, K. K., Archer, S., Gillman, L. N., Lau, M. C., Leuzinger, S., de los Rios-Murillo, A. (2017). Global diversity of desert hypolithic cyanobacteria. Frontiers in microbiology, 8. -Jungblut, Anne D., and Warwick F. Vincent. "Cyanobacteria in Polar and Alpine Ecosystems." Psychrophiles: From Biodiversity to Biotechnology. Springer, Cham, 2017. 181-206. Johansen 1993 was removed.

Page 2 Lines 15-19 Citations are missing. Reference added: Potts, Malcolm. "Desiccation tolerance: a simple process?." Trends in microbiology 9.11 (2001): 553-559.

Methods: Page 3 Line 27 Can you clarify, within the given T/ frost and ice days, when biological activity is expected/have been predicted? Can you provide for all locations

an approximation of current expected biological activity?

Response to referee: We support the idea of adding activity periods and provide appropriate references for each section / habitat as far as possible.

Addition of page 4 line 17: Lichens assigned to BSC from Antarctica are known to show photosynthetic activity patterns after snow melt and during fog but only low rates of photosynthetic productivity and growth rates are expected throughout the year (Colesie et al., 2016).

Addition of page 3 line 30: Cyanobacteria dominated BSCs and lichens of Hochtor are known to be continuously active in terms of photosynthesis throughout the year, activated by fog, dew, rainfall and after snow melt (Colesie et al., 2016; Büdel et al., 2014)

Addition of page 4 line 9: At least for lichens of Spitsbergen it is known that they seem solely active during ice and snow free times where they are activated by rainfall and snow melt with low contributions to carbon fixation throughout the year. (Uchida, Masaki, et al. "Estimation of the annual primary production of the lichen Cetrariella delisei in a glacier foreland in the High Arctic, NyâĂŘÅlesund, Svalbard." Polar Research 25.1 (2006): 39-49)

Page 4 Lines 19-15 Please provide number of samples collected/analyzed per location. Were samples collected randomly or within a given transect?

Additional information was inserted in this section of the manuscript: 20 samples were randomly selected from areas where BSC dominated, a 9 cm petri dish was pressed 1 cm into the BSC surface and excess soil was removed with the petri dish lid. However, due to the heterogeneous nature of BSCs the thickness of the BSC itself varied from 1-2 mm (in Geopol) to up to 1 cm (in Hochtor).

Page 5 Line 2 What type of Chlorophyll was targeted? Added at page 5 line 2: chlorophyll-a



Page 5 Line 5-6 You are saying that chlorophyll a from green algae was excited using a 555nm beam and that chlorophyll a from cyanobacteria was excited by using a 639nm beam. It is not clear to me, how an excitation at 55nm will have no effect on chlorophyll a from cyanobacteria and vice versa.

Response to referee: For sure, there are other wavelengths that are also suitable but they often excite minerals or particles in the soil which create background noise. For this reason, we tried a variety of possible wavelengths (for chlorophyll a and phycobilli proteins) and found this to be the most adequate for the different soil types of all four locations. A high proportion of quartz in the soil for example, can make it difficult to apply CLSM because quartz interferes with the excitation wavelengths. To demonstrate that the chosen wavelengths are sufficient to discriminate chlorophyll a and the phycobilli proteins (cyanobacteria versus green algae) we included figure 1 and especially figure 2.

Page 5 Line 18 Explain what do you mean by cyanobacteria and green algae were isolated. Also, from which solution?

Paragraph was added at page 5 lines 18-20 as follows: Macroscopic cyanobacterial thalli and green algae mats were picked from the surface of BSC samples from Hochtor and transferred to a drop of water at an objective slide.

Page 5 Lines 24-25 Sentence difficult to follow Sentence at page 5 line 24 to 25 was redrafted as follows: Thallus structures and excreted EPS by cyanobacteria and green algae were also taken into consideration. This was possible because at least the periphery of the EPS reflects the fluorescence signal that is coming from the cells.

Page 5 Line 25-28 Does it mean that cyanobacteria associated to lichens were also neglected? How did you discriminate chlorophyll a fluorescence from mosses?

Response to referee: For this measurements lichens and their photobionts as well as bryophytes were neglected. It was not discriminated between fluorescence of chloro-

phyll a from bryophytes and chlorophyll a from green algae / cyanobacteria. The applied technique does also visualize bryophytes. Their rectangular cells form a pattern that is visible and makes it possible to identify them.

Page 6 Line 2 move meaning for the abbreviation for voxels from line 6 to line 2 Page 6 Line 11 Use past tense

Tenses have been changed, sentences redrafted and all corrections were inserted. Please see page 6 lines 1 to 11. The explanation of voxel (value on a regular grid in three-dimensional space) was added in brackets at page 6 line 2 and was removed from page 6 line 6.

Page 6 Line 11-13 Sentence difficult to follow/understand The paragraph has been changed as follows: The EPS and dead cells created a dense matrix together with the soil. This texture changed where the BSC structure ended and the pure soil started. This point could be estimated by the scale bar and is therefore indicated as the end of the graphs.

Page 6 Line 23 Normally distributed data. Correction was included at page 6 line 23.

Results Page 6 Lines 27-30 Define a tense (past or present). Recurrent change in tense in the manuscript.

Punctuation and grammar was carefully checked and corrected throughout the manuscript.

Page 7 Lines 18-21 Improve sentences flow.

Paragraph at page 7 line 18 to 21 was redrafted as follows: The total organic carbon content of soils varies between 7 % and 17 % (Fig. 6), with Hochtor and Geopol being significantly different to Ny-Ålesund ($p \leq 0.05$). Based on CLSM–IA This total organic C can be divided into carbon evaluated from active photosynthetic organisms (apC), and non-photosynthetic carbon (npC). The latter includes dead organic material and remaining EPS.



Page 7 Line 22 How did you measure bryophytes contribution to apC and npC. Can you please explain how this differentiation was made? I also do not see them (bryophytes) marked in any of your biomaps, neither in figure 6. Being this the case, please use arrows to show them in your biomaps.

Response to referee: To estimate total organic carbon, your sample (including soil, cyanobacteria, lichens, bryophytes, green algae, fungi, heterotrophic bacteria etc.) gets burned in a muffle oven at 500 °C. During that process all biomass gets lost. The difference in weight in percent is the total organic carbon content. This total organic carbon can be split into carbon coming from dead organisms and carbon coming from living organisms. The fraction of carbon coming from active organisms was measured by CLSM, based on fluorescence (apC). This includes cyanobacteria and green algae (as well as lichens and bryophytes <200 $\mu$m) without any discrimination between the organisms. Subtracting apC from total organic carbon reveals the amount of carbon coming from dead proportions of the BSC (npC).

Page 7 Line 24 Please clarify what you mean by cyanobacteria occupy between 7 and 23 %. Is there any difference among locations?

Response to reviewer: With this technique, the total cross section area (from the top of the BSC up to 1 cm depth) was estimated that is occupied by green algae or cyanobacteria within the BSC. Across all four sites only cyanobacteria occupied between 7 and 23 percent of the total area.

Sentence at page 7 lines 23 to 24 was changed as follows: Cyanobacteria with their EPS and thallus structure occupy between 7 (Livingston) and 23 % (Ny-Ålesund) of the total area of BSC that was visualized in the 2D biomaps. In comparison, green algae contribute with 0.5 to 2 % as a minor group (Fig. 6).

Discussion: Page 7 Line 28 -30 Revise sentences. Either add punctuation/connectors or split into more sentences. Revise this throughout the manuscript. Page 8 Line 8 named instead of called

Corrections are inserted and sentences have been redrafted at page 7 line 27 to 30 and page 8 line 1 and 9 to 13.

Page 8 Line 9-10 I do not understand this sentence/what you are aiming to communicate Response to reviewer: The group of Ranaaan (2016) also used a technique to visualize BSC. This study is discussed here because it is recent, only a few studies provide visualization techniques that are applicable to BSC and they found similar structures. Additionally, it shows the need for such techniques and demonstrates the benefits of our study.

Page 8 Line 18 “hard to detect” by what means? Please explain Response to reviewer: Cryptic stages of green algae and cyanobacteria are often impossible to detect by light microscopy because they occur in low abundances, have atypical morphologies or can be very small.

Page 8 Line 19-20 What and how is supported by Budel et al., 2014 and Peer et al., 2013? Response to reviewer: Eukaryotic green algae are rarely dominant in BSC and none were found exclusively in BSC. We could detect only a minor proportion of eukaryotic green algae within BSC. This is confirmed by the named references which stated the same for Hochtor.

Page 8 Lines 22-24 Which technique was used in Budel et al., 2014, was it the same time of the year? These sentences need a better flow to communicate better the point the authors are trying to make. A take home message from this finding is missing.

Sentence was changed at page 8 lines 22 to 24 as follows: Interestingly, Büdel et al., (2014) showed by Illumina sequencing that within the microbiome of BSC from Hochtor, cyanobacteria contributed only 1.6 % to the total bacterial diversity, whereas we show that cyanobacteria occupy 20 % of space within the crust. This shows that the role of cyanobacteria within microbiome studies that are based on DNA proportions might be underestimated.



Page 8 Line 28 Which literature? Add references and some comparisons Light regime parameters were added to the description of the sampling sites and highlighted again at page 8 line 28: Hochtor: 600-1500 PAR ($\mu$mol m-2 s-1) with strong fluctuations (Büdel, Burkhard, et al. "Improved appreciation of the functioning and importance of biological soil crusts in Europe: the Soil Crust International Project (SCIN)." Biodiversity and conservation 23.7 (2014): 1639-1658.); (Colesie, Claudia, et al. "Summer activity patterns of Antarctic and high alpine lichen-dominated biological soil crustsâĂŤSimilar but different?." Arctic, Antarctic, and Alpine Research 48.3 (2016): 449-460.) Antarctica: 1200 PAR ($\mu$mol m-2 s-1) (Xiong, Fusheng S., and Thomas A. Day. "Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of Antarctic vascular plants." Plant Physiology 125.2 (2001): 738-751.) Svalbard: 1200 PAR ($\mu$mol m-2 s-1) (Barták, Milos, Peter Váczi, and Josef Hájek. "Photosynthetic activity in three vascular species of Spitsbergen vegetation during summer season in response to microclimate." Polish Polar Research 33.4 (2012): 443.)

Page 8 Lines 28-30. These sentences are hard to follow, please re-write. How different in thickness were your biocrusts at the studied locations? Do your results agree with your light regime explanation?

The sentences at page 8 line 28 to 30 have been changed as follows: Light regime could be a responsible factor, because all four sites share similar daylight times with PAR exceeding 1200 $\mu$mol m-2 s-1 (Colesie et al. 2016; Xiong et al. 2001; Barták et al., 2012), but with the strongest fluctuations at Hochtor (Büdel et al, 2014). The appearance of photoautotrophic organisms up to these depths may be possible due to a diverse community composition of organisms with different adaptions regarding light regime. This idea supports a previous study that states a continuous year around photosynthetic activity of the cyanobacteria dominated BSC of Hochtor (Büdel et al., 2014).

Page 8 Line34 Page 9 Line 4 I do not see the point of adding the dark and light crust classification.

Sentence at page 8 line 34 and page 9 line 4 have been removed.

Page 8 Line34 Page 9 Line 16 Therefore is missing the last e Correction has been included at page 8 line 34 and page 9 line 16.

Page 9 Lines 20-41 Please provide references Reference added: (CANNONE, N.; GUGLIELMIN, M. Relationships between periglacial features and vegetation development in Victoria Land, continental Antarctica. Antarctic Science, 2010, 22. Jg., Nr. 6, S. 703-713).

Page 10 Line 5 demonstrated instead of could demonstrate Correction has been included at page 9 line 5.

Figures: Figures 1 Add used wavelength for each channel. Add arrows to show fluorescence from EPS. Include either here or as a supplementary a similar panel showing a filamentous cyanobacteria. Figure 2 Add used wavelength for each channel. I am not sure you need to show figures 2 a and c.

Used wavelengths were added to the figure caption, as well as arrows indicating the EPS in figure 1. We agreed to remove figure 2a and 2c and combined figure 1a-d with figure 2b and 2 d to a new figure 1a-f.

Figure 3 Add arrows to indicate filamentous and single coccoidal organisms. Also show differentiation between cyanobacteria and green algae. Indicate profile depth for each panel. Indicate biocrust position in the profile. In the results, you mentioned that Nostoc is on top as well as within the biocrust (Page 7 Line 3). I only see what you identified as Nostoc within but not on top of the biocrust. Clarifying the biocrust position in the profile may help with this. I also do not clearly understand how you concluded that what white triangles are showing is Nostoc. Maybe a zoom in will help. Also, Nostoc from figure 3b looks different from Nostoc in figure 3a, especially color wise. Add PAL, PIL layers to figure 3 and provide measurements (profile depth).

We added a zoom to the figure 1 as 1e. In this new image bryophytes, Nostoc, coc-



coidal and filamentous cyanobacteria, as well as green algae are highlighted. We also indicated where PIL and PAL is placed for each panel and added a scale to clarify where the top is.

Response to referee: Nostoc was identified by picking it from the BSC surface and light microscopy investigations. Based on a second study that is in preparation, we know that different Nostoc species are present. The arrow in figure 1b for example shows Nostoc gelatinosus.

Figure 4. Provide layers measurements (profile depth). Optional since already asked in figure 3. Although I acknowledge the effort and recognized its beauty, I do not see the need to include Figure 4 in the main text. It could be supplementary. I leave it to the authors to decide.

Figure 4 represents a schematic illustration, to demonstrate and simplify BSC structures and the PIL-PAL proportion in general. With this study possibly being a part of a special issue of biogeoscience we want to introduce BSC related content to a broad audience. Figure 4 makes it possible to understand complex relationships between different types of cyanobacteria and green algae with soil and their role as ecosystem engineers in extreme habitats. For this reason we would be pleased to keep this figure.

Figure 6. I do not see bryophytes represented in the figure, however, their contribution to the apC and npC was mentioned in the results. Response to referee: No discrimination between different organism took place during the estimation of the total organic carbon content by loss on ignition, because the whole sample with all biomass is burned. The CLSM technique allows a discrimination only between green algae and cyanobacteria, because chlorophyll a in green algae is the same as in bryophytes. For this reason, bryophytes are included in the total organic carbon content.

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