Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2017-413-AC1, 2017

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.

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Received and published: 15 December 2017

Title: You can trim the title simply into: Carbon content and structure of intact Arctic, Antarctic

We would be pleased if we could keep the original title because it makes a point out of the novel and unique visualization technique. The first part of the title also helps a broader audience to conceive the intention of the study.

Introduction: Page 1 Lines 25-26. The sentence is not well written. Please modify. Page 1 Lines 29. Place a sentence connector. For example: Thus, plant cover is

sparse as well as decomposition rates and biodiversity are generally low. Plant cover can be also moss and lichens which are sometimes very abundant. Here, you probably want to say vascular plant cover. Page 1 Lines 29 - Page 2 Line 1. This sentence is too complex. It is better to divide it into two.

The section has been redrafted, see page 1 lines 25 to 29.

Page 2 Line 1. I don't think it is correct to write that instead of the vascular plants BSCs occur in polar and alpine regions. You can see vascular plants there and vice versa you can see BSCs in other types of environment. Page 2 Line 2. The word "algae" is too complex because this term includes macro-, microalgae and cyanobacteria. Write something like eukaryotic microalgae.

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 3. You wrote that "cyanobacteria especially are important players within these intimate associations." However, the explanation why are they so important to compare to other organisms is very poor. For example, if you write about EPS, you can add that production of EPS promotes the stabilization of the soil surface, moisture retention, and protection against erosion.

Additional explanations with references were added such as soil stabilization by EPS (Belnap J (2003) Biological soil crusts and wind erosion. In: Belnap J, Lange OL (eds) Biological soil crusts: structure, function, and management. Springer, Berlin Heidelberg New York, pp 339–347), and nitrogen fixation by heterocytes (Fay, P., Stewart, W. D. P., Walsby, A. E., & Fogg, G. E. (1968). Is the heterocyst the site of nitrogen fixation in blue-green algae?. Nature, 220(5169), 810-812.), please see page 2, line 3 to 5.

Page 2 Line 13. Cryoturbation is a process, but not an environment. You can write:

e.g., cryoturbated soils.

Changed to cryoturbated soils (page 2 line 13).

Page 2 Lines 8-9. It cannot be similar for green algae, because they have different functions to compare with cyanobacteria. Either write what is similar exactly or delete it.

Removed

Page 2 Lines 15-17. What's about other cyanobacteria contributing to C- and N- cycling? Nostoc, for example. Response to reviewer: Chroococcidiopsis and Scytonema are named as well-known examples. For sure there are more taxa that contribute to C- and N- cycles. Scytonema was replaced by Nostoc, to use a more common example on page 2 line 16.

Page 2 Lines 15-18. Provide references for 2d and 3d. Response to referee: Reference for the whole paragraph is given in line 9 (Weber et al., 2016). The following reference was added: Potts, Malcolm. "Desiccation tolerance: a simple process?." Trends in microbiology 9.11 (2001): 553-559.

Page 2 Lines 20-21. Modify the sentence into: Therefore, a large proportion of important ecosystem services, such as erodibility (Belnap and Gillette, 1998; Bowker et al., 2008), soil formation (Rillig and Mummey 2006), soil moisture (Belnap, 2006) and C and N- cycling (Shively et al., 2001; Tiedje 1988; Kowalchuk and Stephen 2001), are influenced by cyanobacterial communities.

The section has been redrafted as suggested at page 2 lines 20 to 21.

Page 2 Line 24. Whose biomass? Response to reviewer: Cyanobacteria provide an initial structural integrity and accumulate biomass by growth.

Page 3 Line 1. I don't understand this sentence. Please clarify which carbon you mean here.

Sentences were redrafted at page 3 line 1: Soil is the largest pool for carbon in general. Three times the amount of carbon that is accumulated in above ground biomass, as well as double the amount of carbon fluctuating in the atmosphere as CO2 is stored in soil (Schlesinger and Andrews 2000).

Methods: Page 3 Line 27. and from 2 to 4 âŮęC in July. Can you clarify in brackets what are frost days and what are ice days?

Explanations are added in brackets, page 3 line 27: Frost day: Minimum day temperature below 0°C. Ice day: Maximum temperature below 0°C.

Page 3 Line 33. with a polar tundra climate in both?

Response and to reviewer: Yes. Geopol and Ny-Alesund are only 8 km apart from each other. The term tundra usually refers to the continuation of permafrost.

Page 4 Line 2. "of" instead of "off" Page 4 Lines 7-9. Either divide the sentence into several sentences or place the sentence connector.

All corrections inserted

Page 4 Line 19. Indicate the type of samples you collected: soil, soil crust or vegetated soil. How many samples did you collect. Page 4 Lines 19-26. How did you choose the samples? Randomly? Page 4 Line 27. It is not clear which part of BSC you chose. Surface or part below surface?

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? Added at page 5 line 2: chlorophyll-a

Page 5 Line 8. You should explain what is 530/30? Response to referee: This is a

common expression that is used to explain the width of the bandpass filter. This filter is set to 530 nm with a width of 30 nm, meaning that it ranges from 515 nm to 545 nm.

Page 5 Lines 10-11. How many centimetres? Response and to reviewer: The sample collection part (page 4, line 22) was redrafted, all samples were taken with a Petri-dish with a thickness of 1 cm. All samples had the equal thickness of 1 cm but with varying relations of BSC to soil.

Page 5 Lines 13-15. Either divide the sentence or place the sentence connector. Sentence was divided

Page 5 Line 18. How the solution was prepared? 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 22-23. Modify the sentence, it is hard to understand in the current state. Page 5 Line 26. although they are BSC organisms. Page 6 Line 1. and available online. Page 6 Lines 1-3. Use the past tense as you started to use in Method section. Page 6 Lines 6-7. Correct the sentence. It is not well written. Page 6 Line 6. You have already used the word voxel in previous sentences Page 6, Line 2). You should put explanation of abbreviation at first time you use this word. Page 6 Line 7. Take care of the tenses you use throughout the text. If you use past then use it everywhere in the methods.

Tenses have been changed, sentences redrafted and all corrections were inserted. Please see page 6 lines 1 to 7. 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. How do you visually estimate it? Sometimes you cannot see small microalgal or cyanobacterial cells by eyes. Page 6 Lines 11-13. Again, the sentence is

not well written. Modify it.

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 or data with normal distribution. Results

Check the tenses you use here. It should be either past or present tense. Page 6 Lines 28-29. Check punctuation throughout the manuscript including these two lines.

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

Page 6 Line 30. Since you write about EPS here, maybe it would be useful to indicate it (for example with arrow) in the Figure 1.

Arrow was added to indicate EPS and the reflectance as suggested.

Page 7 Lines 1-2. Divide the sentence into two. The section has been redrafted

Page 7 Line 3. You write that Nostoc is found on top. I see on the figure 3 that white triangles are in the upper layer but not on the top. Also from the figure it is very hard to understand where exactly the surface of BSC is. Maybe you can show that. Also on the figure 3 you indicate Nostoc. However I don't understand the way you decided that it is these cyanobacteria. Especially Fig 3b.

Response and to reviewer: Figure 3 shows cross sections of the BSC. The beginning of the fluorescence signals indicates the BSC surface. The dark black background above this is the agarose matrix. Soil surfaces are rough and therefore the surface is not a smooth line. If you zoom into the images you can see morphological features such as rounded heterocytes within the characteristic arrangements of Nostoc-like trichomes (especially in Fig. 3a). Due to further light microscopy based investigations

(paper in preparation) we know that also the structure indicated in figure 3b is Nostoc gelatinosus.

The following sentence was added to the figure caption of figure 3: The beginning of the fluorescence signals indicates the BSC surface. The dark black background above this is the agarose matrix.

The following sentence was changed in section 3.1, line 2: Macroscopic thallus forming Nostoc species were found on top and within the BSC and their identity was checked by light microscopy.

A scale was added to figure 3 to clarify where the surface is as well as bars that indicate PIL and PAL proportions for each panel. Additionally, a zoom was added to figure 3 as figure 3e where the Nostoc thalli, bryophytes, filamentous cyanobacteria and green algae are highlighted.

Page 7 Lines 7-8. Delete this sentence. You have already mentioned it. Removed

Page 7 Lines 11-12. Correct the sentence. For example: The highest values range between 25 and 40 mg C cm-3 in all soil crust samples. Page 7 Lines 18-19. Divide the sentence into two sentences. Page 7 Line 20. The sentence is too complex and it is hard to read.

The section has been redrafted, please see page 7 line 11 to 20.

Page 7 Line 22. Here you talk about bryophytes but I don't see bryophytes in the Fig. 6 where you show who is responsible for total organic C.

Response to referee: Total organic carbon was obtained by loss on ignition. During that process BSC samples are burned at 500 °C. All biomass gets lost without discrimination between heterotrophic bacteria, plant litter, lichens, cyanobacteria, bryophytes etc. Therefore bryophytes are included in total organic carbon. The CLSM technique allows only a discrimination between cyanobacteria and green algae based on phycobillins. Chlorophyll a is shared by green algae, cyanobacteria and bryophytes.

Page 7 Lines 23-24. I don't understand the sentence. Cyanobacteria occupy 7-23% of BSC in general? Or BSC organisms?

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).

Page 7 Line 25. accounted instead of differ Replaced

Discussion: The discussion can be trimmed. Some information is not so important for this manuscript. For example, the paragraph is too long and could be trimmed. I also feel that discussion is mostly focused on the results obtained from Hochtor. Maybe you should also discuss a bit more arctic and Antarctic BSCs.

Response to reviewer: The discussion section is shortened. This paper will serve as an introduction to future work in these ecosystems and therefore the discussion includes ideas for further investigation where Hochtor plays a major role.

Page 7 Line 27. You applied CLSM, to measure what?! Add in this sentence. Page 7 Line 30. Which is instead of this is. Page 8 Line 1. You have already mentioned in the beginning of the discussion that you used CLSM for the first time. No need to repeat it again. Page 8 Lines 9-13. This sentence is too long. It is hard to read.

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 Lines 19-20. Was it detected somewhere else or only in Hochtor? This sentence is confusing. Was it found in your study? Or it was found only in 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. Write something like this: 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.

Correction was included as suggested at page 8 line 22-24.

Page 8 Line 24. Can you write a sentence with a small conclusion coming out of this finding.

Sentence was added at page 8 line 24: This shows that the role of cyanobacteria within microbiome studies that are based on DNA proportions might be underestimated.

Page 8 Lines 26-27. Which literature exactly? Put references. References added at page 8 line 26 to 27: Belnap, J., Lange, O. L.: Structure and functioning of biological soil crusts: a synthesis. In: Biological soil crusts: structure, function, and management. Springer Berlin Heidelberg, Germany, 471-479, 2001

Page 8 Lines 27-28. You write that light regime could be a responsible factor for the differences in the crusts thickness. However, in these sentences you point that light conditions are similar for all studied crusts.

Response to referee: Light regimes are comparable across the four sites but at Hochtor situations are fluctuating strongly. Light regime parameters were added to the description of the sampling sites and highlighted again at page 8 line 28: Hochtor: 600-1500 PAR (μmol 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 (μmol 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 (μmol 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 29-30. Reference? The following sentence was added at page 8 line 30: This is a suggestion which ongoing investigations will attempt to clarify.

Page 8 Line 34. Therefore Page 9 Line 13. cyanolichen, chlorolichen and bryophyte crusts Page 9 Line 16. Better not to use a colon. Rather start a new sentence.

All corrections were included

Page 9 Lines 21-24. Reference? 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).

Figure 2. I don't see the point to show Fig 2a and 2c. It is clear from the text that the green algae don't have phycobillins.

We agree with this suggestion and combined figure 1a-d with figure 2b and 2d. The new figure 1 contains now figure 1a-d (Nostoc) with arrows that indicate the EPS, 1e showing the red panel of the green algae and 1f showing the light microscopy image of the green algae. Wavelengths of the applied lasers for each panel have been added to the caption of figure 1 and appropriate paragraphs in the text have been fitted.

Figure 5. Can you present Depth scale in mm. It would be easier for the reader. The legend of the figure is too long. I see this text already in the results. Figure 6. The

same here. The legend is too long and should be trimmed. Inorganic carbon instead of anorganic. Corrections were included and the scale was changed from μm to mm in figure 5.

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