

Interactive comment on “Eco-physiological characterization of early successional biological soil crusts in heavily human impacted areas – Implications for conservation and succession” by Michelle Szyja et al.

Michelle Szyja et al.

michelle.szyja@web.de

Received and published: 12 December 2017

Comments to the Authors The paper of Szyja et al. aims to characterize ecophysio- logically early successional biological soil crusts in heavily human impacted areas. For achieving this they choose two locations with a different type of BSC: one dominated by a cyanobacteria and the other by a green alga. Overall, I found the paper represent- ing an interesting contribution to scientific knowledge of BSC ecophysiology because: 1- there are at present not many data available about ecophysiology performance of these type of BSCs and 2- The comparison of the response between bare soil, intact

C1

BSC and isolated compo- nent is novel and very interesting. Nevertheless, I found some important problems as how the work is presented. The main problems are in the methodology where the experimental design (mainly number of replicates in each experiment) is not clear and in the results, where some of the figures are quite confus- ing. The question about whether the NP rates should be expressed on a chlorophyll or surface basis is not relevant here and, obviously, will differ if comparisons are made between cyanobacteria and green algae. In my opinion the number of references (85) exceeds the needs of the paper. Beside some minor/typographic errors (i.e. check subscript in CO₂ throughout the text), in general, the paper is well structured, the dis- cussion is good and conclusions clear but it needs to show results in a way that they appear more conclusive. In conclusion, I find the paper interesting and scientifically sound but taking into account the amount of data and how they are presented I don't think it reach the standards of BG. I have some comments and suggestions that I think will improve the paper.

Major and minor comments

TITLE I suggest removing the second part of the title (implications for conservation and succession) as it does not reflect the content of the paper. Second part of the title has been removed. We agree with the referee that it has no connection to the contents of the paper.

ABSTRACT There is no reference in the abstract to one of the main points in the work that is the differences found between response of intact BSCs and of it isolated dominant components. We agree with the referee that this topic was not getting enough attention in the abstract. We have included information regarding this topic in the actual version and also moved this section to end of the abstract to underline its significance for the interpretation of the data (lines: 21-24, page 1).

Page 1. Line 20. I suggest to remove the sentence beginning “Nevertheless, a major. . .” See comment above. This is a response to the general remark throughout the

C2

referee's comment about removing the part of the study where we investigated differences in NP rates if the used reference value is either the chlorophyll content or the sample surface area.

We consider this partial aspect of the study worth mentioning, even though these coherences are well known for eco-physiology experts. Nevertheless, by being a potential contribution to the special issue on biocrusts in Biogeosciences, a major benefit of this manuscript is to reach a broad biocrust readership and also non-physiological experts. We see this as an opportunity to introduce and explain this topic to a new audience, especially because the choice of reference value is variable, depending on the investigated organisms and research question. Basing NP rates on chlorophyll content will result in an overestimation of NP rates of cyanobacteria dominated BSC compared to other crust organisms or biocrust types. We agree with the reviewer, that a comparison of gas exchange rates between different publications was, of course, not the main goal of this study. Nonetheless, we want to provide a suggestion on how to avoid discrepancies in interpretations of gas exchange data. In the actual version of the manuscript we have taken great care to clarify this point and give explanations as to how our findings may influence study design and data evaluations of similar studies in the future (Page 12, Lines: 5-20).

INTRODUCTION

Page 1. Line 29. Please rewrite the sentence "Investigations. . ." As it is now is contradictory. Are there abundant or few investigations in cyanobacteria?

Sentence has been rephrased so that it is easier to understand now (P 2-3, L 34 - 4 and P 2, L. 1-3).

Page 2. Lines 5 to 20. In my opinion the concept of arrested succession should be introduced at the beginning so it is clearer for the reader.

We have restructured and reorganized the paragraph. The concept of arrested suc-

C3

cession is now presented in a clearer way (P 2. L 9-18:)

Page 3. Line 9. Reference Reisser et al. 2007 is not in the list.

The reference Reisser et al. 2007 has been corrected to Reisser, 2007.

Line 23. I suggest to change "or" for "and a"

Redrafted.

Lines 25-26. Were these "in situ" measurements carried out? I think it would be better say "would allow"

Was corrected.

Line 32. Colesie et al. 2014b not in the list. "Higher" than what?

Colesie et al. 2014b was corrected to Colesie et al. 2014 (without b).

Sentence has been rephrased to provide a comparison: P. 3 L. 32-33: "A higher physiological flexibility is predicted for cyanobacteria and green algae compared to bryophytes and lichens which would enable both organism groups to cope with a wider range of abiotic stresses. "

Page 4. Line 4. The sentence is confusing and I think is not relevant here. I assume that when authors refers to system they refer to BSC and not to the measurement systems. The treatment or position in the cuvette is another question. Of course there will be variability between samples, but here the comparison is between isolated individuals (green algae or cyanobacteria), soil biocrust and soil. I suggest removing this sentence.

As suggested by referee #2 and #3 this sentence has been removed and the topic is now discussed in the discussion section (P. 11 -12; L 15 – 4).

MATERIAL AND METHODS

Page 5. Line 2. Check reference Honegger 2008. Is 2003 and also it refers to green

C4

algae photobiont but not to cyanobacteria.

Reference Honegger has been removed. Reference for *N. commune* (Tamaru et al., 2005) and green algae (Seckbach, 2007) have been included (P. 5, L. 3).

Line 11. n=6. It is not clear to me how the sampling or subsampling was made. From each 6 of C-BSC and 4 of G-BSC you take 3 subsamples?

We agree with the reviewer that this section in the methods was not written clearly. We have rearranged the whole section and tried to clarify terminology as well as the description of the different measurement series and units (P.5, L 32- P.6 L 3).

Line 12. First, you need to indicate how the saturation light was determined.

We rearranged the methods part and put the determination of saturation light before the determination of water dependent photosynthetic response (P.5, L. 17-31).

Line 16. Delete "from the"

Deleted.

Line 19. Should not be a new paragraph.

Deleted.

Line 21. I understand that the weighing was during the dehydration cycle to have the full response, but not between them. Please explain this.

Because of the sample being located in a closed, gastight cuvette "during" the CO₂ exchange measurement, it can only be weighted once this reading is taken and the cuvette is open. For detailed description please see: Photosynthesis and Water Relations of Lichen Soil Crusts: Field Measurements in the Coastal Fog Zone of the Namib Desert; O. L. Lange, A. Meyer, H. Zellner and U. Heber; Functional Ecology; Vol. 8, No. 2 (Apr., 1994), pp. 253-264. We have tried to clarify this in the completely rewritten methods section (P 5 – 6; L. 30-5).

C5

Line 23. I suggest new paragraph. "To obtain the net response to light. . ." n=3. Are the samples BSC or species individuals? It is not clear from the text and in Fig. 2 they appear as individual species measurements. In agreement with the previous comments we have rearranged this whole section for more clarity and transparency in the used methods.

Figure description was also changed. N=3 represents BSC samples, not species individuals.

Line 25. How the optimal temperature was obtain? Are there any regressions done for this? Data is not show. Please explain.

Prior to the light dependent gas exchange experiments the operation temperature was checked by testing if the light saturation point was independent of temperature, by testing if a difference was visible between 17 °C or 25 °C, with n=3 replicates each. As no difference for the light saturation point could be detected (grouped t-test; p-value for C-BSCall: 0,095; p-value for G-BSCall: 0,597), the operation light for the water dependent gas exchange experiments was therefore set to 985 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for C-BSC and 1260 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for G-BSC, which represent the results from the 25 °C measurement. This has been clarified (P. 5, L. 24-28; P 6, L1-2).

Line 29. Should not be a new paragraph.

Deleted.

Page 6. Line 10. Include "of the two types of BSC" after "levels". The analysis as it is explained is confusing as there were different number of samples and subsamples for the different experiments. For the drying curves there were 6 C-BSC and 4 G-BSC and from each of these all, dom and soil. But in the light curves there are only 3 CBSC and 3 G-BSC without distinction of components. So, I understand that BSCall, BSCdom and BSCsoil cannot always be the explanatory variables.

The reviewer is absolutely correct in pointing out that the statistical data analysis is

C6

written in a confusing way. We acknowledge showing us where we have described it poorly. We included the suggestion of the reviewer and rewrote the paragraph about the light curves, where we only did a grouped t-test, as correctly pointed out, we could not have organization level as a dependent variable (P. 6, L. 16).

RESULTS

The adjustments of the curves in Fig. 2 doesn't look very good, especially that of *Z. ericetorum*, showing an increase in the response and no saturation following the points and not the line. Please check this. Also, how were the light parameters (compensation and saturation) calculated, from individual adjusted curves or from one curve? It should be explained in material and methods. There are no supplementary tables or graphs showing values of these parameters.

According to suggestion of the referee we have included the necessary information in the material and methods section (P. 5, L. 27-29) but would like to give further information for the reviewer to follow our argumentation: The mathematical formula used to fit the curve is the so-called Smith function. It is the standard curve to fit light curves of BSC organisms as it: "makes it possible to calculate apparent maximum quantum yield of CO₂ fixation (Φ , initial linear slope of light response curve), NP_{max} (the theoretical maximal rate of NP at saturating PPFD), PPFD_{sat} (the light intensity allowing 90% of NP_{max} which represents a realistic estimate for light saturation [...]), and PPFD_{comp} (the light compensation point of CO₂ exchange)" (from Lange, O. L., Belnap, J., & Reichenberger, H. (1998). Photosynthesis of the cyanobacterial soil crust lichen *Collema tenax* from arid lands in southern Utah, USA: Role of water content on light and temperature responses of CO₂ exchange. *Functional Ecology*, 12(2), 195-202.) The calculation for the compensation and saturation point were as follows: The original data from the GFS 3000 work sheet were put into the graphics program Sigma Plot. These data only had the following light intensities with corresponding NP rates: 0, 25, 50, 100, 300, 500, 1000, 1500, 1750, 2000 photons m⁻² s⁻¹. Calculating light saturation and compensation points from this data set is not very accurate, therefore a curve

C7

fitting is being done with the Smith function. The Smith function provides 259 points for our curves interpolated between the actual measured points. Light compensation is calculated by creating a linear regression line from the last negative to the first positive point, that is created by the Smith function. With the obtained formula for the slope the intersection with the x-axis can be calculated, which represents the light compensation point, where respiration equals assimilation. Light saturation is done by calculating 90% of NP and looking in the fitted curve for the NP value closest to the NP_{90%}. The corresponding, calculated light value is then considered the light saturation. Each light curve measurement produces one light saturation and one light compensation point. The given values (P. 7 L. 4-11) were means of three measurements each. To simply say that the Smith function is the standard tool to use for BSC light curves is not sufficient, so we would like to provide the R² values of the single curves, to prove that the formula is fitting for the data: C-BSC: 0.98; 0.98; 0.98; G-BSC: 0.95; 0.97; 0.97.

Page 6. Line 4. From Figs 4a and b they don't contribute to NP response.

That is correct and exactly what we wanted to prove. We wanted to remove the soil crust organisms and only measure the organisms and soil separate. With not having many photosynthetic active cells in the soil we also did not expect a contribution to NP response.

Line 22. Here it is said G-BSCall and C-BSCall but not in Fig. 2. Please clarify. I suggest changing "almost twice as much" for "higher"

Rephrased.

See comment above, description of light curve was clarified, description of Fig. 2 has been clarified also.

Line 24. Why organisms? Is it not BSC? It is not reasonable that the difference in compensation point was twice as much but then there were no significant. As comment above please explain how this analysis was done.

C8

Corrected to BSC. Analysis was explained more in detail in material and methods as suggested by the reviewer. We want to emphasize that one of the main conclusions of the study is that these BSCs (and mainly the separated dominant organisms) show a very broad range of responses to different environmental parameters, also to light. In this case it means that the standard deviations are so big that even though the values for G-BSC are higher than for C-BSC, there is no statistical difference between the means.

Line 26. The same discussion will apply for the saturation points. From Fig. 2 we can understand that there is no saturation at 2000 μmol (just a few lines before it is said that maximum NP rates were reached at 2000 μmol).

Sentence was rephrased, and the following information added: The GFS3000 cannot increase the PAR above 2000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. (P. 7, L. 5). Therefore, we need to conclude that the highest NP rates we can measure are at 2000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Additionally, the saturation point is calculated as 90% of NP (see P. 5 L. 27-28) and is not reflected by the slope of the curve.

Page 7. Line 2. Include "dominated BSC" after "commune". Refer here to Supplementary tables. Line 6. Include "dominated BSC" after "ericetorum". Refer here to Supplementary tables.

Included both. We refrained from putting the sentence in P. 7 L. 22 at the end of each paragraph and left it at the end of the segment.

Line 8. Delete "an" Water dependent photosynthetic response. In my opinion better than exemplifying graphs, average data of all replicates should be represented. Differences between just two samples are not relevant. Also, curves shown in Figures 4 are very difficult to understand as it is not normal the fluctuation around 80% water content. It must be an artifact that could be masked using averages. Also the water depression is not clear.

C9

Using exemplifying graphs for presenting water dependent photosynthesis data is a common and well-accepted style in BSC, lichen and bryophyte physiology literature. Many studies on BSC, as well as lichens make use of this, because it allows a better understanding of the processes and clearer graphical design. One complication that comes with mean values plotted in these graphs, is that they vary not only along the y- but also along the x-axis, which would require a demonstration of standard deviations in two directions, which is clearly complicating the graph and the message that should be transported with it. Rather than "masking" effects, as suggested by the reviewer, by using mean values this is an option to precisely describe and demonstrate processes that are otherwise easily overseen. Plotting the response curves against a standardized water content scale (%) allows comparisons between samples with highly divergent water contents and is a tool used in review articles and book chapters. As an example to show that even overlaying single curves will not produce a clear picture, we plotted 5 NP curves of C-BSC (all, dom and soil; 25°C) on normalized water content (Fig.1). In this graph we used normalized NP rates instead of total NP rates, as we have strong variations between samples. It is obvious that all the curves for C-BSCdom are very similar, but the ones in C-BSCall vary so much that a clear pattern cannot be shown with this kind of graph. This is because of the strong variations in the soil (shown in C-BSCsoil).

In order to clarify which water contents were optimal (90% NPmax) we have included highlights in the graph and provided a table with the mean water contents after which NP is slightly inhibited (below 75%). Selected literature regarding this topic and showing the same type of graphs: - Lange, O. L., Belnap, J., & Reichenberger, H. (1998). Photosynthesis of the cyanobacterial soil-crust lichen *Collema tenax* from arid lands in southern Utah, USA: Role of water content on light and temperature responses of CO₂ exchange. *Functional Ecology*, 12(2), 195-202. - Lange, O. L., Büdel, B., Heber, U., Meyer, A., Zellner, H., & Green, T. G. A. (1993). Temperate rainforest lichens in New Zealand: high thallus water content can severely limit photosynthetic CO₂ exchange. *Oecologia*, 95(3), 303-313. - Lange, O. L., Green, T. A., & Heber, U. (2001). Hydra-

C10

tion—dependent photosynthetic production of lichens: what do laboratory studies tell us about field performance?. *Journal of Experimental Botany*, 52(363), 2033-2042. - Green, T. G. A., Nash III, T. H., & Lange, O. L. (2008). Physiological ecology of carbon dioxide exchange. *Lichen biology*. Cambridge University Press, Cambridge, 152-181. - Lange, O. L. (2001). Photosynthesis of soil-crust biota as dependent on environmental factors. *Biological soil crusts: structure, function, and management*, 217-240. - Green, T. A., & Proctor, M. C. (2016). Physiology of photosynthetic organisms within biological soil crusts: their adaptation, flexibility, and plasticity. In *Biological Soil Crusts: An Organizing Principle in Drylands* (pp. 347-381). Springer International Publishing.

Line 16. Change Table for Tables.

Changed.

Lines 26-28. Data shown in the text of ranges of optimal WC seem different from the ones in Fig. 5 (i.e. upper limit never coincident). Please check.

The referee is absolutely correct. We accidentally used the wrong values in the text, although discussing the correct ones and also doing the statistical analysis with the correct values. We corrected the text passage (P. 8, L. 10-15).

Page 8. Line 5. I would rather delete this subsection as discussed above.

We would like to include this topic, as explained in the comment above.

Line 20. Table S6

Changed.

DISCUSSION

Line 25. BSCs photosynthetic organisms

Changed.

Page 9. Line 5. Delete “none” and better G-BSCall and C-BSCall. What does it means

C11

physiological flexibility to water gain?

We have clarified the interpretation of this result and explain now that: “both organism groups take the same functional role in the BSC consortium and can operate at near optimal conditions over a variety of different water contents, as it would also be expected for highly stress tolerant crust pioneer species” (P. 9, L. 26-30).

Line 18. I suggest delete sentence beginning “A depression. . .” as it has already said before.

Deleted.

Line 23. I don't see the detection of a CCM from Fig.S2.

Referee #2 also asked about an explanation as to how we were able to detect that *N. commune* had a CCM and *Z. ericetorum* did not show one. We will copy part of the answer here, as it also describes how a CCM can be seen in gas exchange data: In general, it is known from literature that most green algae as well as all cyanobacteria do possess an inorganic CCM (Raven, J.A., Cockell, C.S., De La Rocha, C. I. The evolution of inorganic carbon concentration mechanisms in photosynthesis. In: *Phil. Trans. Soc. B.* (2008)). Although the mechanisms have multiple evolutionary origins, the function is the same: CCMs accumulate CO₂ around rubisco. While the mechanisms behind the accumulation might be different, the photosynthetic response is the same, which can be seen in supplement figure S2 (a): There is a strong peak in carbon uptake as soon as the light is turned on, which flattens itself after a few minutes into a straight line. Usually the uptake of CO₂ during photosynthesis looks like a sudden drop of the CO₂ concentration in the measurement system gas. Afterwards the assimilation curve stays on the same level. This can be seen in S2 (b), in the downward curve just before the black arrow marks the peak in the upwards curve. If a CCM is present, this pattern is changed. As soon as the light is turned on more CO₂ is accumulated than would normally be the case under continuous conditions of water content, light and temperature. This is because the reservoir around rubisco is filled up, which can

C12

be seen as a sudden peak in the picture S2 (a; marked by a black arrow). As soon as the light is turned off again, CO₂ that has not been used during photosynthesis is released again, which is shown with a sudden increase of CO₂ in the measurement system gas. Here the same applies: More gas is released than normally would. After a few minutes this peak drops again, under light and in dark conditions and a continuous respiration or assimilation can be detected. We were unable to detect the same pattern in the green algae BSC, even under heavy manipulation of the measurement conditions, which included different temperatures, water contents, PPFs and time intervals of measurement. Therefore, we conclude that no CCM can be detected in *Z. ericetorum*. As this was the first study to test this for this species, we provide a first insight in how this green alga photosynthesizes.

Additionally, we want to provide the publication where the method of detection was used for the first time: Badger, M. R., Pfanz, H., Büdel, B., Heber, U., & Lange, O. L. (1993). Evidence for the functioning of photosynthetic CO₂-concentrating mechanisms in lichens containing green algal and cyanobacterial photobionts. *Planta*, 191(1), 57-70 We have clarified this topic in the text of the supplement material in order to make the phenomenon understandable for a broad readership (Figure description of S2).

Page 11. Lines 2 and 7. Species name in italics.

Changed.

Lines 16-26. In my opinion this question is not relevant as it is obvious.

We would like to refer to our comment at the beginning of this letter, that we consider discussing the differences between these two options is an important information and of interest for a broad BSC readership.

CONCLUSIONS

Page 12. Line 3. The authors conclude that there is a relative temperature independence of NP but the results show significant differences in the response of NP to

C13

temperature.

Here we disagree with the referee, according to the statistical tests, there is no significant difference in the response of NP to temperature, except for the one mentioned by us (25°C for C-BSC_{dom} C-BSC_{all} and C-BSC_{soil}).

Line 5. In general, the question about physiological plasticity should be avoided because there are no experiments proving this.

We agree with the reviewer that plasticity was not measured in the presented study and rephrased the sentence for clarity and a more precise ecological interpretation (P. 12, L. 24-26).

Line 6. To incorporate the results into global scale carbon cycle models, the work should better provide numerical data sets (i.e. tables).

Part of the publication process for BGS is to upload the original data to an online database, so that it can be accessed easily, therefore the numerical data of this publication will hopefully be made available.

REFERENCES There are too many for the paper. As mentioned above some cited literature in the text is not in the list. Please check references through the list.

Checked and corrected the literature to fit the text.

Some literature has been removed, as single cited publications behind some statements should be enough. Still, the number of publications did not decline a lot, also because we needed to include some sources because of added comments from the other referees.

Table 1. Following my suggestion about Chlorophyll question then this should not be included.

We would like to include this part of the publication, but we put it in the supplemental material as Table S10. See comment at the beginning.

C14

Figure 2. Legend. The second sentence is not necessary, just n=3.

Removed.

Figure 3. Legend. What do you mean by . . . of one of the group only? Please indicate what vertical bars represent.

“Of one group only” has been corrected to “organization levels”, to make it clear that one BSCall, BSCsoil or BSCdom was being compared. Also vertical bars were described as being standard deviations.

Figure 4. See comment above. Indicate PAR

This has been changed.

Figure 5. This graph is very difficult to understand. See comments above. What does the letters mean? Why n=24 here?

The letters on the graph represent statistical differences between each lower limits (letters a-c) and between all upper limits (d-g). As described in p. 6 L. 24 -27: the optimum water content was compared by statistically testing if the upper and lower limits between the both BSCs and their components differed. This means we compared if the lower limits differed from one another, then we compared if the upper limits differed. We have now included a clear definition of optimum water content (water content at which 90% of NP is reached to water content at which it decreases below 90% again). The optimum water content is calculated independent of temperature, as it is not influenced by temperature. Therefore, we have pooled all readings (6 samples times 4 temperatures) for C-BSC. The sampling size for G-BSC was missing, which is 16 (4 samples times 4 temperatures). We clarified this in the text (P. 6, L. 6):

Figure 6. As suggested above I would not include this graph.

We would like to include this part of the publication. See comment at the beginning.

C15

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-369>, 2017.

C16

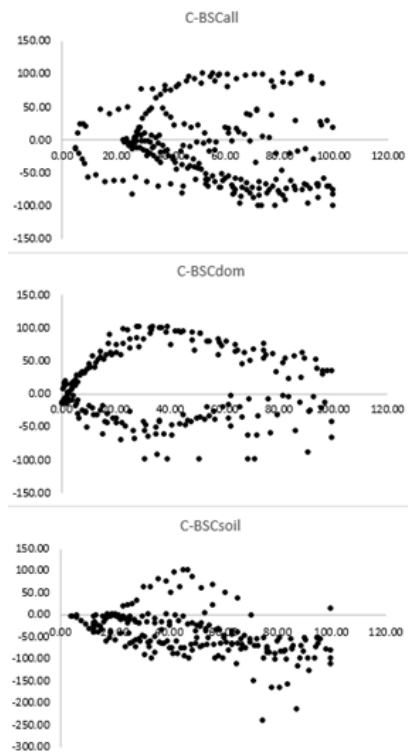


Fig. 1. Fig. 1: Normalized NP rates plotted against normalized water content for 5 samples of C-BSC at 25 °C.