

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.

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Early successional stages of biocrusts are not only important due to the fact of being pioneer colonizers of barren habitats, but also because climatic predictions point to them as possible dominant organisms in areas under strong hydric stress, where later successional stages would not succeed. Up to the authors to use this concept in the introduction in order to remark even more the importance of their study.

We agree with the reviewer that climate change might increase the difficulty of later

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developmental stages of BSC to establish in habitats with less available water and increased temperatures. It might therefore be possible that a climax community dominated by cyanobacteria and green algae will be established. Nonetheless, it still needs to be mentioned, that there are in fact studies of hot and dry habitats, where e.g. lichens or bryophytes are present as the climax community (e.g. Zedda, L., Gröngröft, A., Schultz, M., Petersen, A., Mills, A., & Rambold, G. (2011). Distribution patterns of soil lichens across the principal biomes of southern Africa. *Journal of Arid Environments*, 75(2), 215-220. Or Weber, B., Graf, T., & Bass, M. (2012). Ecophysiological analysis of moss-dominated biological soil crusts and their separate components from the Succulent Karoo, South Africa. *Planta*, 236(1), 129-139.) Nonetheless, we found the comment really helpful and are glad that this point has been brought to our attention. We will include it in our introduction as it strengthens the need for more studies similar to ours. However, it is important to mention that the BSCs investigated in this study are from temperate regions in which climate change will most probably not reduce rainfall as much as that later successional stages of BSC will disappear completely, although a change in community composition is expected. We will include a remark that explains that studies taken in habitats that are more sensitive to changes in rainfall should be investigated. It is discussed now on page 2, lines 19-24.

INTRODUCTION Page 4 lines 4-5: Please clarify this sentence. I think that authors want to say here that depending of the treatment made to the sample (sample with soil, without it, or bare soil) a different response will be found in the gas exchange experiments. But I do not understand the sentence: “We expect that the position and arrangement of the sample inside the measurement system, here a cuvette, will influence the photosynthetic values”. Are the authors analyzing, at some point, how the position of the sample inside the cuvette is influencing gas exchange measurements? I think that the sentence is confusing and is not a good choice to close a, on the other hand, well developed introduction

The referee is correct in pointing out, that we did in fact, not alter the position of the

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sample inside the cuvette. We have removed this sentence from the introduction and discuss the topic in greater detail in the discussion (Page 11, line 15- Page 12, line: 4).

MATERIAL AND METHODS P5, L13: Could authors provide some info about why was this set of temperatures chosen for the experiment?

The chosen temperatures are in accordance with other eco-physiological studies on BSCs (e.g. Weber, B., Graf, T., & Bass, M. (2012). Ecophysiological analysis of moss-dominated biological soil crusts and their separate components from the Succulent Karoo, South Africa. *Planta*, 236(1), 129-139. Or Lange, O. L., Belnap, J., Reichenberger, H., & Meyer, A. (1997). Photosynthesis of green algal soil crust lichens from arid lands in southern Utah, USA: role of water content on light and temperature responses of CO₂ exchange. *Flora*, 192(1), 1-15. Or Lange, O. L. (1980). Moisture content and CO₂ exchange of lichens. *Oecologia*, 45(1), 82-87.) Additionally, they represent the average temperature range of temperate Europe (see e.g. site Homburg in Raggio, J., Green, T. A., Sancho, L. G., Pintado, A., Colesie, C., Weber, B., & Büdel, B. (2017). Metabolic activity duration can be effectively predicted from macroclimatic data for biological soil crust habitats across Europe. *Geoderma*, 306, 10-17.) which the study organisms face most often during a year. We wanted to investigate a broad but realistic range of temperatures to create a very detailed response of the organisms to different climatic conditions. We have added the appropriate information on page 6, lines 3 – 5.

P6, L9: I do not see clear how a one-way ANOVA can be, at the same time, a multifactorial ANOVA. To my understanding, the authors are using a one way ANOVA with type of crust being the factor (meaning that only one factor is being analyzed), and each of the dependent variables analyzed at each moment (NP, DR, WC.being the variable. Is this correct? Probably just a matter of terminology but I see it a bit confusing as written now

We have corrected this sentence. We used a multifactorial ANOVA where we used NP,

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DR, optimum water content range and WCP as the response variables. The explaining variables were temperature and organization form (BSCall, BSCorg and BSCsoil), or an interaction of the two variables. For light compensation and light saturation only a grouped t-test has been performed. This is now clarified on page 6, lines 16 – 19.

P6, L15: A space is needed in “bystatistically”. Besides, which methodology was used to compare these limits?

Space was added. Also, the statistical method was added and described in detail (page 6, line 24-27). Here a multifactorial ANOVA was used, where the explaining variables were organization form and crust dominating species (green algae or cyanobacteria) and the response variable was optimal water content.

RESULTS P7 L23-25. After having a look to Fig. 4 I agree with what is written here, but I think that is falling in contradiction with what is written in the abstract about the issue: “and low or no depression in carbon uptake at water suprasaturation” (abstract L18). I think that the text in the abstract regarding this issue should be changed to fit more accurately what is written in results

The sentence in the abstract was rephrased. Also we rephrased “low” to “minor depression” to emphasize that we mean a depression where the NP is only inhibited slightly. Page 1, line 17-18: “Nevertheless, independent of species composition, both crust types had convergent features like high light acclimatization and minor and very late occurring depression in carbon uptake at water suprasaturation.”

P7 L27-28: I think that what authors want to underline here is that C-BSC and G-BSC water content values are close between them both situations, “all” samples and “dom” samples. But as it is written now it seems that, for example, for C-BSC “all” and “dom” values are similar between them, which does not seem to be correct. Just a small correction would solve the possible confusion. This sentence was rephrased.

P: 8, L. 11-12: “The values for optimum water content between both BSCall are close,

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as well as between both BSCdom which show similar values, independent of species.”

FIGURES Fig. 2. I think that both sub-graphs should be scaled equally at the Y axis in order to compare gas exchange rates between C and G crust types easily The Y axis has been changed according to the suggested amendments.

Fig. 4. Please indicate in the figure legend the amount of light used for the experiment Added the PPFD under which the water curve was measured according to suggestion of both referees.

Fig. 6. This figure is hard to follow for me. I think that the variable “effect size” is a ratio between C and G crust types calculated for “dom” and “all” samples and based on area of each sample and chlorophyll content, but I do not understand why such ratio is called “effect size”. Could authors please provide more explanations about this graph? I do not understand either that bump of the effect size at 25 C for chlorophyll based net photosynthesis. I have read in different parts of the text that authors consider that net photosynthesis has not a statically significant drift with temperature on an area basis, at least for the green algae crust. Does this graph mean that temperature has a significant effect over photosynthesis on a chlorophyll basis but not on area basis? Besides, the figure is supposed to show differences in the effect size for both *N. commune* crusts and *Z. ericetorum*, but I do not see clearly which is which in the graph.

Paragraph in results (P. 8, L 25- P.9 L 8) has been rephrased. We would like to provide some more information here and explain the name “effect size” on the chart. We saw that *Z. ericetorum* crusts always had much lower NP rates than *N. commune* crusts and separated organism, even though we would expect the exact opposite, as the green algal crust had up to 181 times higher chlorophyll rates per area, which would enable the crust to assimilate much more CO₂ than the cyanobacterial crust. We were therefore interested if this higher NP rates of *N. commune* were caused by the eco-physiological features, like a CCM or caused by methodological mistakes. Therefore, we compared the NP rates of both organisms for both reference values, chlorophyll

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and area. If we could detect the same pattern of much higher NP rates in *N. commune* in both reference values, we would prove that eco-physiology was the driving factor. What we did was: We calculated NP for *N. commune* and for *Z. ericetorum* based on area and on chlorophyll each. Then divided the NP/Chlorophyll rates of *N. commune* with the NP/chlorophyll rates of *Z. ericetorum*. Next, we did the same division for the NP rates based on area. If the resulting ratios would have the same size, reference values would have no effect on the higher NP rates, therefore only eco-physiological differences would explain the much higher NP rates in the cyanobacterial crust. Our results showed the opposite: there was an obvious effect on reference values, showing in the always higher bars in chlorophyll based NP rates. We suppose therefore, that chlorophyll calculations as they are used at the moment, are not suitable to calculate NP rates in cyanobacteria, as they exclude phycobilisomes that also are responsible for NP rates. This will result in an overestimation of NP rates in cyanobacteria: In BSC 25 °C we can see that NP based on area supports that *N. commune* does have a seven times higher NP rate than *Z. ericetorum*. But NP based on chlorophyll describes a difference of 42 times higher NP rates for the cyanobacterial crust, resulting in an overestimation of NP rates in cyanobacteria up to six times compared to NP rates based on area. We did not only do this calculation for the separated organism but for the intact BSC systems, too. The much higher value of 42 times higher NP rates if they are based on chlorophyll instead of area is also owed to a temperature dependency of *Z. ericetorum* that we could not detect in *N. commune*, increasing the effect at 25 °C. The graph does not show that there is a temperature dependency visible if the NP values are based on chlorophyll but not on area basis. It only shows that the effect of temperature is stronger on chlorophyll than on area basis, but an effect is visible for both reference values. The shape of the response is only different, because the reference values are of different suitability. The name effect size is originating from the effect that the reference value has on the NP rate, but as this will confuse readers we changed it to “Ratio of NP of C-BSC/ G-BSC”.

DISCUSSION P9 L13-22: Authors discuss in this paragraph about the differences in

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depression of net photosynthesis at high water content between C "dom" crusts and G "dom" crusts, explaining ecologically why makes sense the fact of not finding this depression in Nostoc (C) and finding it in Zygonium (G). After having a look at figure 4, it seems to me that there are more measured points at high water content (over 80% of maximum water content) in Zygonium than in Nostoc (I mean, for CBSC dom it seems that there is a gap between 80% and 100% of water content). Any explanation for this? Could this affect the ecological interpretation of the depression of net photosynthesis at high water contents or authors are using other indicators to analyze this issue?

The displayed data represent normalized water contents, as the absolute water content was different between both crust types and comparison therefore difficult. In the completely oversaturated N. commune crust, the amount of water brought into the measuring system was too much for the system to produce reliable measurement values. This is because of the high cross-sensitivity of the sensor between water and CO₂ molecules. Very high water contents result in unreliable data, as water molecules might mistakenly be detected as CO₂ molecules. Due to the mentioned system limitations it was impossible to measure higher H₂O contents in N. commune. This is clarified on page 8, lines 6 – 8.

P9 L23-27: I have gone to the supplement figure S2 in order to try to follow the detection of the CCM mechanism and its relationship with depression of photosynthesis at high water contents. This is something quite interesting physiologically under my point of view that deserves more research efforts in the literature. I have seen that authors propose (correct me if I am wrong) that the fast changes in differential CO₂ response in the gas analyzer after light changes supports the existence of the CCM in Nostoc, and that this was not found in Zygonium. Do you mean that the response of Zygonium after light changes was different or somehow slower than in Nostoc? Is there any support in the literature for this pattern? (I mean presence or absence of CCMs in cyanobacteria Vs green algae)

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We are glad to provide some in depth explanation on the carbon concentrating mechanism and how it was detected in N. commune but not in Z. ericetorum. 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 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, PPFDs 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. This has been clarified in the caption of Table S2.

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P10 L1-2: I have been following with interest the lack of optimum temperature for net photosynthesis in the green algal crusts because it was something initially unexpected to me. What I see in relation to this in Fig. 3 regarding C and G “dom” subgraphs, is that *Nostoc* follows a pattern of raised net photosynthesis with temperature through all the temperature range and that *Zygogonium* shows a raise up to 17 °C and a decrease at 22 °C (but 22 showed highest photosynthesis compared with 12 °C). I know that authors are supporting their idea of lack of temperature optima in the stats, which I think that is right and interesting, but after looking the graphs it seems to me that it could be perfectly said that *Nostoc dom* has a temperature optima at 25 °C and *Zygogonium* at 20 °C. If *Zygogonium* is less adapted to long activity periods than *Nostoc*, I would expect a concentration of metabolic activity during softer environmental conditions, and this should shift temperature optimals to lower values rather than erase the concept of optimum temperature for net photosynthesis. On the other hand, author's statement of lack of temperature optima in the green algae is supported with the graph 3b for GBSC all, where the link between net photosynthesis and temperature is clearly erratic and defined by a lack of pattern. I just would like to know author's opinion about this, because their approach to T optima concept based in stats is absolutely right to me.

The reviewer has mentioned an interesting point here. It is absolutely possible and likely that *Z. ericetorum* might show an optimum temperature point somewhere between 17 °C and 25 °C, although we can only assume a trend here. As this interval with 8 °C is quite broad it would be very interesting to include this temperature. A lower optimum temperature for *Z. ericetorum* compared to *N. commune* would still be in accordance with our theory, that *N. commune* is wet and active at higher temperatures than the green alga. Additionally, it points towards the algae being able to photosynthesize at high temperatures (for Europe; see climatic data in e.g. site Homburg in Raggio, J., Green, T. A., Sancho, L. G., Pintado, A., Colesie, C., Weber, B., & Büdel, B. (2017). Metabolic activity duration can be effectively predicted from macroclimatic data for biological soil crust habitats across Europe. *Geoderma*, 306, 10-17.). As temperature curves of green algal dominated BSCs are quite rare, this should be done in

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future studies.

And this is a different issue, but it is surprising to me the lack of statistical differences in *Nostoc* between C-BSCall and C-BSCsoil net photosynthesis. It means that the photosynthetic cyanobacteria layer of the soil is not creating any relevant C input compared with bare soil. Interpretations for this behaviour?

The p-value responsible for this similarity is not very far from a statistical difference ($p=0.089400$). We suppose that the high natural variation that has been shown in the high standard deviations of NP is responsible for this similarity. Increasing the sampling size would most likely result in a statistical difference between net photosynthesis input in C-BSCall and C-BSCsoil. As for now, as we cannot detect differences, we have to assume that 1) the low biomass of this very young and not diverse BSC is responsible for this low NP, or 2) that only under optimum conditions a difference can be detected, or 3) that area might not be a suitable reference value to calculate NP rates, although chlorophyll would still not be the better choice here, as it would overestimate NP rates of cyanobacteria and result in a difference that is not real.

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