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Interactive comment on "Annual net primary productivity of a cyanobacteria dominated biological soil crust in the Gulf savanna, Queensland, Australia" *by* Burkhard Büdel et al.

Prof. Dr. Büdel

buedel@rhrk.uni-kl.de

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Anonymous referee 3 GENERAL COMMENTS This work deals with the metabolic activity and gas exchange of a cyanobacterial biocrust in the Boodjamulla National Park of Australia. The authors have identified the main species in the biocrust, have carried out lab measurements of net photosynthesis (NP) and dark respiration (DR) as related with variable conditions of light, temperature and water content (WC), and have carried out one-year monitoring of NP and DR under field conditions, recording also a series of micro-climatic data. The work is very interesting, in the cutting edge of knowledge, fully matches with the scope of the BG special issue, and is well made and well writ-

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ten with almost the only exception of some aspects in the methods section, which has consequences in part of the results. However, I think all my comments can be resolved with a few changes. The results includes the time duration of metabolic activity of the biocrust, the time proportion of NP vs DR, and an explanation of the timing of these processes. It should be noted that the authors provide with annual net CO2 flow data. They also provide evidence of strong seasonality of these biocrust-atmosphere gas exchange processes, and of the positive annual balance of NP despite the relatively short time during which NP is achieved, showing that the net incoming CO2 flux produced by NP is larger than the flux due to DR, since the duration of NP seems shorter. The positive annual balance of CO2 is important because it is not obvious, having into account the low frequency of the conditions in which positive NP can be observed in situ in biocrusts in most of the sites. Discussion and Conclusion are based in the results, include substantial contributions to knowledge and, along with the Introduction, show the experience and up-to-date knowledge of the authors.

Thank you for the positive valuation.

SPECIFIC COMMENTS Methods The sampling is a bit confusing. How many samples/replicates were used for the experiment of CO2 exchange under controlled conditions in lab? It seems that three replicates were used but the sentence in lines 12 and 13 of page 4 introduces some doubts. It is unclear what '9 different samples' are. If I have understood well the main text, I suggest rewriting that sentence, for example: "For every independent variable (light, temperature and water content), a different set of three samples/replicates was used". About the samples used for the one-year monitoring, taking some replicates in each measurement time would have been better. I think that it would been possible with only one cuvette (measurements in a series of replicates can be done in a short enough period to avoid that daily gas exchange variation had a significant effect in part of the replicates with regard to the others).

Answer: you are right, that was confusing and we rephrased the whole part as follows: "2.3 Environmental manipulations For the analysis of the effect of the different

environmental factors (light-, temperature- and water content; termed environmental manipulations throughout the text) on net photosynthesis, samples were air dried in a 10 cm Petri-dish, sealed and transported to the laboratory, where they were stored frozen (-20°C) until used for the measurements. This treatment had been tested in our laboratory many times with lichens of many different geographical origins, including the tropics and resulted in high survival rates (roughly 95%) compared to dry storing in herbarium cabinets or boxes in the laboratory. Earlier gas exchange measurements on biocrusts, cyanobacteria, byrophytes and lichens before freezing and after thawing and re-moistening resulted in identical rates (unpublished laboratory tests). Prior to the measurements, samples were allowed to defrost at 23°C for 12 h in an air tight box at low light intensities (« 50 μmol photons m-2 s-1) in order to avoid decondensation. Subsequently samples passively dehydrated and were kept at 23 °C and natural day-night cycles ($\sim$150 μmol photons m-2 s-1) for 2 days. Light-, temperature- and water content related NP-measurements were performed using three independent replicates each. CO2 gas exchange measurements were conducted under controlled laboratory conditions using minicuvette systems (CMS 400 and GFS 3000, Walz Company, Effeltrich, Germany). The response of net photosynthesis (NP) and dark respiration (DR) was determined independently for light, temperature and water content (WC). Samples were weighed between measurements and WC was calculated later on as mm precipitation equivalent after final determination of the samples dry weight (exposed 5 days in a desiccator over silica gel at the end of the measurement). To obtain the NP response to light, fully hydrated samples (n=3) were exposed to stepwise increasing photosynthetic active radiation (PAR) from 0 to 2500 μmol photons m-2 s-1, near optimal temperature (32°C) and ambient CO2 concentration. The light cycle (about 30 min duration) was repeated until the samples were completely dry (after 3–4 h). Light saturation was defined as the PAR at 90% of maximum NP. The temperature related NP and DR were determined at increasing temperature steps, 22, 27, 32, 37, 42, and 47°C, while light was constantly at 1500 μmol photons m-2 s-1 and WC was constantly at optimum (n=3). The influence of WC on NP and DR was determined at constant,

nearly saturating light (1500 μmol photons m-2 s-1) and six different temperatures (22, 27, 32, 37, 42 and 47°C) using three replicates. Samples were completely soaked with water and exposed in the cuvette. Then, NP and DR were measured in short time intervals (roughly 10 minutes) until the samples were almost dry and did not show any NP nor DR reactions. After each time interval, the fresh weight of the sample was determined using a balance and the corresponding WC to each data point calculated using the dry weight of the sample (see above). In all experimental manipulations, the CO2 exchange rates of the sample were related to chlorophyll a content. For chlorophyll determination, the samples were ground to small pieces and then extracted two times with di-methyl-sulfoxide (DMSO) at 60 °C for 90 minutes. The chlorophyll a + b content was determined and calculated according to (Ronen and Galun, 1984). 2.4 Field monitoring of CO2 gas exchange As there was only one semi-automatic cuvette system available, we could not replicate the measurements. To partly overcome this problem, we used several samples over the year. Samples were placed in a basket of thermoplastic resin with drainholes in the bottom to avoid standing water during rain events. The basket had a fixed size and all samples had exactly the same exposed surface of 16.5 cm^2 (Fig. 1 d). All samples used were tested for a comparative large NP and DR rate under the given environmental conditions for two measurements (1 hour) in the cuvette system and only those were used that had more or less identical NP and DR rates. Fourteen samples were used during field monitoring (Table 1) and exposed in a random mode. The “random” mode was determined by the ability of access (climatic conditions, days off) by one of us to the investigation site during the whole measuring period. Water content of samples of the experimental manipulations and those of the field monitoring is always expressed as millimeter water column. As it was impossible to remove the sample after each measurement from the monitoring cuvette system to determine the fresh weight corresponding with the measured value by weighing it with a balance (measurements every half an hour, nobody of us could stand at the site for the whole period), the only method getting matchable values between field monitoring and controlled experiments is to express it like rainfall in millimeters water column. Field

monitoring of the biocrusts CO2 gas exchange was recorded using a semi- automatic cuvette system (ACS) as described in detail by Lange (2002). Full technical details of the ACS (Walz Company, Effeltrich, Germany) are given in Lange et al. (1997). We therefore focus on some major topics of the procedure here. The whole device is composed of two major parts, first the cuvette system itself that is exposed in the natural environment of the biocrust (Fig. 1c) and secondly the controlling and data acquisition unit together with two infrared gas analysers (IRGA) for CO2 ambient and CO2 samples (Binos, Rosemount, Hanau, Germany) and a pumping unit regulated by mass flow controllers (Fig. 1e). For safety reasons a data printer and a graphics plotter were added as well. The soil crust samples were exposed on the lower part of the cuvette (Fig. 1d, arrow). When the upper lid was open (H in Fig. 1d), the sample was fully exposed to the natural environment. Measurements were taken every 30 minutes during which the cuvette was closed for 3 min. We recorded the CO2 exchange of the sample and absolute ambient CO2 partial pressure as well as mass flow, air temperature, the sample surface temperature, air humidity, and ambient photosynthetic radiation at the samples level. Net photosynthesis and DR were related to the area covered by the biocrust".

However, Fig 3 suggests (showing the time overlaps among bars of different colours) that sometimes through the year only one sample was measured, whereas in others, two, three, four or even five samples were measured. Were there any replicates at certain times of the year? If this is so, I think that this diversity in number of replicates along the year requires some comment. If this is not the case, the Fig 3 should be corrected or explained. Independently from the number of replicates during the monitoring period, the use of several different samples throughout the year would have been probably necessary, because the cyanobacterial biocrust samples have a limited resistance to handling and, after a series of measurements, they should be replaced. (Due to this fact, this is not properly a case of repeated measures over time). But again Fig 3 shows how the duration of the different samples is very different; in some cases the same sample appears to have been used repeatedly during even five or six

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weeks, but in others, only once. Is this related with the difficult to understand the last sentence of section 2.2 (page 4, lines 20 - 21)? Please, explain better how, “for the one-year monitoring”, you used 21 samples (page 4, line 16) and, though from those 21 used samples, only 11 were selected “for the long-term monitoring” (page 4, lines 20-21). Do you mean that those selected 11 samples were used repeatedly over time while the other 10 were used only once? Why?. By the way, the caption of the Fig 3 should be completed; a caption must be self-explanatory. I think we can assume that replacing the samples along the year for the monitoring is acceptable, apart from possibly necessary, since the monitoring was made only on carefully selected samples of the brown cyanobacterial community dominated by Symplocastrum purpurascens, and each sample includes probably billions of cyanobacterial individuals, being a good representation of the whole community. Besides, the microbiota at a certain sampling point could change enough along the year, which decreases the importance of always sampling at the same point. On the other hand, it would be advisable to state explicitly the number of times in which measurements were taken during the monitoring (and when), avoiding the reader having to speculate or discover this from the figures. For example, writing, “twenty-five measurements were taken between November and April, once per week, on the dates shown in the Fig 3”.

Answer: We omitted figure 3 as it was confusing. We replaced it with table 1 that says what sample has been used for what time period. We took great care that all samples hat 1) the same surface area and more or less the same surface community of cyanobacteria (excluding lichens and bryophytes) and all showed a comparable range (plus/minus 5%) of NP and DR rates. Of course, we could not guarantee that the microbiota were comparable, but at least they respired at more or less the same range.

The sentence (page 4, lines 24-25) “The response of NP and DR to WC was determined for light, temperature and WC” could be better written, to avoid the expression ‘the response to WC was determined for WC’. I am not sure I have understood this paragraph, particularly after seeing Fig 4. According lines 30-31 of page 4, the

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temperature-related NP and DR were determined by varying temperatures while keeping constant both light and WC; whereas the WC-related NP and DR were determined (lines 1-2 of page 5) at constant light and different temperatures (in addition to different WC, it is supposed).

Answer: We rephrased the sentence as it was indeed confusing (see content of metarial and methos above)

However, according the Fig 4, it seems that temperatures and WC were not crossed. On the other hand, the first step of the procedure was determining the effect on NP and DR of light in every level of WC, for constant optimal temperature. I wonder whether the determination of the effect on NP and DR of temperature by itself (for constant optimum conditions of light and of WC) was the procedure for establish that optimal temperature. If so, then this should be explicit and constitute the firs step. If not, why is studying the effect of temperature by itself important since the effect on NP and DR of WC was determined for every temperature (keeping constant light)? I do not think these experiments were badly done, only that this paragraph is difficult to understand and raises doubts. Since to test the effect of each of these independent variables, at least one of them remained constant, the design is not fully factorial. Probably the triple interaction is significant and, in such a case it would be interesting to understand the biocrust functioning under natural conditions, to study the NP and DR response to that triple interaction, rather than the responses to every independent variables more or less separately. Nevertheless, meanwhile, this work provides very valuable information.

Answer: we indeed measured the WC response curve at 22, 27, 32, 37, 42, and 47 °C. But show only the WC curve at 47°C. For all curves shown in figure 4, always to variables were kept constant. For the WC we kept light and temperature constant, for the light curve temperature was kept constant while WC was kept in a narrow range (e.g. 0.77 to 1.10 mm for the optimal NP, and for the Temperature curve we kept water content in a narrow range and light constant. In figure 8 (new figure 7 in the revised manuscript version, see attachment), we plotted all NP-measuring points of biocrust

from one year against light and air humidity as an integrating variable of temperature and precipitation and got a nice pattern where one can see how many wet up and dry down cycles the biocrust underwent during the year.

In page 4, line 31, is 1500 mol photons m2 s-1 a saturating light? And, how is “optimal WC” defined? A (very short) definition appears only much after, in the line 15 of page 6. On the other hand, is the whole procedure described in the last sentence of this paragraph (lines 2-4 of page 5) repeated for every temperature? This is almost obvious but I think that to say it explicitly would be better.

Answer: optimal WC is that water content of the biocrust or of an organism where it reaches 90% of its maximal CO2 uptake rate. 1500 μmol photons m-2 s-1 is not fully saturating but we choose this value in order not to photoinhibit the cyanobacteria as the experiment lastet for several hour and under this circumstances the chance of photoinhibiotn is rather large. We rephrased that part of the methods for better understanding.

Results The lines in Fig 4 b are not attributed to any WC levels. A series of lines similar to those of graph from Fig 4a are expected here, if I understood adequately the methods. Caption of Fig 4 does not help to understand this; in the part referred to graph 4b, any reference to the WC levels is missing. If the graph from Fig 4b refers to the effect on NP and DR of the temperature while keeping constant both light and WC, a value of (optimal) WC is lacking in the graph. What are the lines of Fig 4c, has each sample one line of NP and one line of DR? How? Where are the six different temperatures, since in the graph 4b temperatures and WC are not crossed?. Why the graph 4c shows 47 C as constant? experimental temperature whereas, according the main text (page 5, lines 1-2), six temperature were crossed with different WC levels?.

Answer: The graph 4b indeed shows the reaction of NP and DR to an increased temperature while light and WC were kept constant (WC = 0.77-1.10 mm). This is now explained in the text and the figure legend.

Why was the graphed experiment made at 47 C instead of at the optimal temperature (32C)?. Perhaps the authors plotted a graph for every temperature and only show the last one; but, in such a case, what are the lines of Fig 4c?.

Answer: We measured the reaction of NP and DR to increasing WC at 22, 27, 32, 37, 42, and 47 °C but do show only the curve measured at 47 °C as the other curve show only have different NP ranges but expose a similar pattern, meaning that they always remain in a certain range of WC for optimal NP.

The wording of Methods and/or Results should be a bit improved.

Answer: we agree and tried to improve wording considerably

Conclusion: In page 10, line 29, the sentence “three months having a negative balance probably due to regrowth of the biocrust” is hard to understand since, by default, ‘re-growth’ implies growth, and growth requires net CO2 assimilation. Besides, I think that this sentence about the regrowth requires an explanation, defining what exactly means ‘regrowth’ in this case, since this is closely related with the hypothesis presented at the end of the introduction (page 3, lines 12-14). Perhaps the authors used here the word ‘regrowth’ to refer to the recovery of metabolic activity after the latent-life span of the dry season.

Answer: You are right, this is misleading. We used the words reestablishment (of the biocrust) and resurrection (of NP) insetad.

It would be also advisable a better definition of that hypothesis in the Introduction

Answer: yes, we did that in the Introduction too and avoided the word “regrowth” Technical corrections Page 4, line 2; the expression ‘(factorial design)’ would be better than ‘(factorial analysis)’. Indeed, the experiment was factorial (although not fully factorial); but, no statistical analysis is explicit in the Method section.

Answer: we omitted this term and rephrased with “For the analysis of the effect of the different environmental factors (light-, temperature- and water content; termed environ-

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mental manipulations throughout the text) on net photosynthesis".

Page 4, line 19: 'NP'and 'DR' appear in that line, whereas they are defined after, in lines 24 and 25 of that page 4

Corrected

Page 5, line 27: "2" is lacking after 'Fig' Page 6, line 15: A dot or a semicolon seems advisable just before the last word of that line.

Corrected

Thank you very much for your effort and helping us to improve the manuscript

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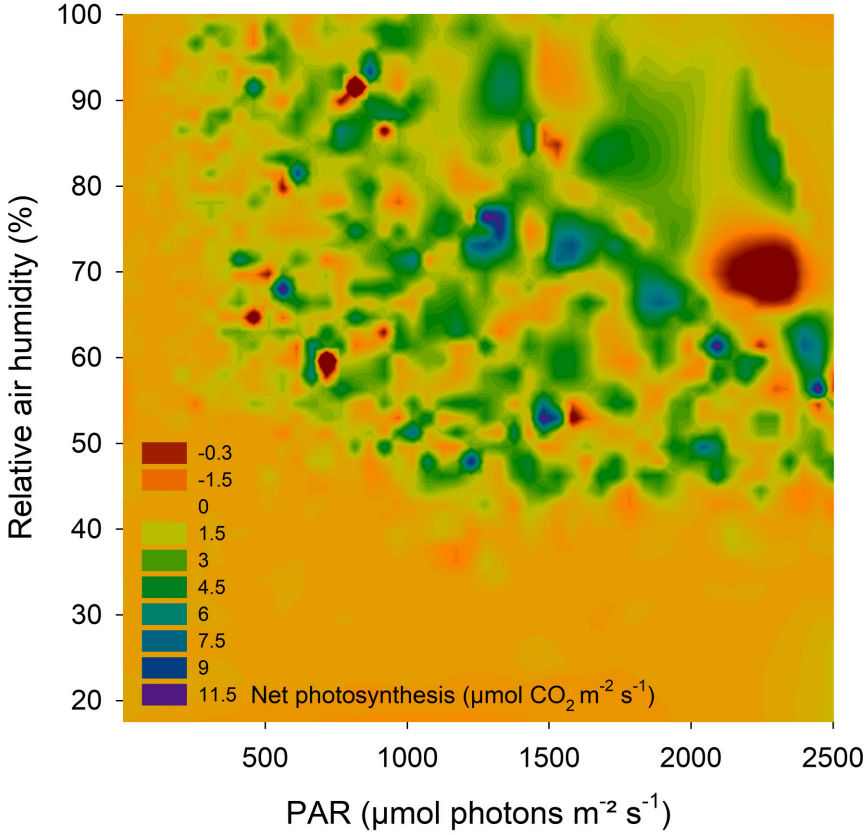

Fig. 1. New figure 7

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