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Reply on RC2

Capucine Baubin et al.

Author comment on "The function and composition of active bacterial communities diverge during the hydration and desiccation of desert biocrust - a field study" by Capucine Baubin et al., SOIL Discuss., https://doi.org/10.5194/soil-2021-88-AC2, 2022

Comment on soil-2021-88
Anonymous Referee #2

Referee comment on "The response of desert biocrust bacterial communities to hydration-desiccation cycles" by Capucine Baubin et al., SOIL Discuss., https://doi.org/10.5194/soil-2021-88-RC2, 2021

Reviewer comments to Baubin et al. “The response of desert biocrust bacterial communities to hydration-desiccation cycles”

General comments: In their manuscript, Baubin and coauthors investigated the presence of bacteria and the chorophyll concentrations as related to hydration-desiccation cycles in biological soil crusts. This is a very interesting and timely topic, as water is considered as one of the dominating factors influencing the microbial composition in biocrusts, and the scientific community just starts to understand the functional roles of different bacteria. However, the manuscript suffers from some major flaws and the results are only superficially analyzed, as described below. Thus, major reviewing is needed before the study can be published in SOIL.

Specific comments:

• Sampling methodology: As the first sample was taken in June and the next ones were taken in January of the following year, it would be important to know the climatic conditions before the first sampling and also between the first and the follow-up samplings, as this information is necessary to thoroughly interpret the results. Information on the habitat, where these samples were taken, is missing. What was the surrounding vegetation, how was the geomorphology, what about fog and dew at the site? This information is necessary to the reader to interpret the results.
Following the reviewer’s comment we have added the requested information to the materials and methods section (ln 119-126):“The study was conducted in the long-term ecological research station in the Negev Desert Highlands (Avdat, 30°86'N, 34°46'E, Israel; Figure 1). In this site, the annual rainfall ranges from 20 to 180 mm (average of 90 mm) that extends from October to April (www.data.lter-europe.net). The soil in Avdat is mostly loess sediments in leveled Byzantian agricultural terraces cleared of rocks

(Bruins, 2012). Vegetation cover is about 25% dominated by dwarf shrubs, mostly Haloxylon scoparium (Shelef and Groner, 2011). Dew and fog in the area were estimated at 0.1 - 0.2 mm per day (Kidron, 1999; Hill et al., 2020) and occur year round on approximately 200-250 days (Zangvil, 1996)."

• I see a major general problem in the presentation and discussion of the results. In this study, the RNA and thus the active organisms were investigated. Thus, the results do not give information on the community composition, but on the composition of active bacteria in biocrusts at different time points before, during and after a rain event. The argumentation thus has to be adopted throughout the manuscript.
We have replaced "community" with "active bacterial community" throughout the manuscript.

• Relative abundance at order level: In figure 3, it is hard to tell the colors apart from each other (e.g. Chitinophagales vs. Cytophagales). At the latter also a "p" is missing. It also seems that the sorting of the orders varies from one time point to the next. As an example, in T[R] it seems that "unclassified" is next to the Actinobacterial orders, whereas in T[1] to T[3] it is all the way at the bottom. In the text, only the changes from T[0] to T[1] are described, but the changes from T[R] to T[1] are largely ignored. This is unfortunate, as there are major changes that need to be interpreted (and look quite interesting)!
Figure 3 was fixed ("p" added to Cytophagales and Chitinophagales, Unclassified at the bottom of each bar, and a better colours distinctions for some orders, Proteobacteria in Figure 3 were grouped, coloured in shades of brown and put under the Actinobacteria group, the Chloroplast order was removed as it represented plants and the values in Table S and p-values were changed accordingly). In addition, the. In the manuscript, the changes explained are between T[0] (before rain) and T[R] (during rain) as they are the most pronounced changes. The differences between T[R] and T[1] are not significant, and therefore we did not focus on them afterwards. However, following the reviewer's comment we added the following (ln 287-303): "Figure 3 shows the active bacterial community composition at the order level for each sampling point. The community is mostly composed of the phyla Cyanobacteria, Actinobacteria, and Proteobacteria in addition to six other phyla (Figure 3; Table S6). During the dry season (T[0]), biocrust community composition differed significantly from the community depicted during the rain event (T[R]) (Figure S3 and S4, Table S7). The differences were shown mostly in orders belonging to the Actinobacteria and Cyanobacteria phyla (Figure 3; $p < 0.05$, $\chi2 = 36.7$ and $\chi2 = 49.8$, respectively Table S7). The relative abundance of Cyanobacteria, dominated by the Cyanobacteriales, increased during the rain event(T[R]) (from 21% to 41%, Table S6; $p < 0.05$, $\chi2 = 49.8$, Table S7). While the relative abundance of the Actinobacteria, dominated by Micrococcales, decreased during the rain event (T[R]) (from 52% to 20%, Table S6; $p < 0.05$, $\chi2 = 36.7$, Table S7). While the biocrust water content decrease after the rain, In the following days , no major changes were detected in the biocrust community (Figure 3; Figure S3 and S4, Table S6 and S7). These patterns were supported by the alpha and beta diversity analyses (Figure S3 and S4). The community diversity significantly differed before the rain (T[0]) and during (T[R]) and after the rain (T[1-3]) (Figure S3, $p < -0.05$, F-value = 10.96, Table S10 and S11). The diversity in the later timepoints (T[R, 1-3]) did not differ ($p>0.04$, Table S11). Similarly, the RDA showed that T[0] was separately clustered from the other time points (Figure S4, F-value : 5.75, Table S12 and S13).". We have also added "T[0]" and "T[R]" whenever "before rain" and "during rain" were written.

• Temporal changes in microbial function: the results shown in figure 4 are hardly discussed at all. This is hard to understand, as they look quite interesting as well. It also would be good to include the statistical results in figure 4, as this will facilitate an interpretation of the results without the necessity to search in the supplement.
Following the reviewers comment we have added letters to Figure 4 to denoting the

statistical results. We also added text to the results section (ln 418-424): "Figure 4 shows the predicted function based on the taxonomic composition using Piphillin displayed in copy number (CN). The values were significantly lower ($p < 0.03$; $\chi 2$ and p-values in Table A9) in the dry season (T[0]) compared to the hydrated soil (T[R]-[3]), except for light and energy sensing pathways (Figure 4; Table S8). The results suggest that the rain event enhanced activity of the biocrust communities including C and N fixation (phototrophy and organotrophy), as well as preparing for the eminent desiccation by activating stress response pathways (DNA repair and ROS-damage prevention) and persistent mechanisms (sporulation and DNA conservation) (Figure 4; Table S3)."
In addition, we added the following text to the discussion section (ln 449-460): "The increase of water content leads to a "metabolic window" (Leung et al., 2020) for quick energy reservation following the "pulse-reserve" paradigm proposed for plant adaptation to desert ecosystems (Noy-Meir, 1973). In this framework hydration of the biocrust community increased the potential activity of gene groups linked to assimilation of carbon and nitrogen (Figure 4; Table S9). We also detected an increase in the potential motility of the community upon hydration (Figure 4) that could facilitate cells interactions in the soil aqueous phase (Dechesne et al., 2010). Yet, while the community is exploiting of brief water abundance, it also prepares to the unavoidable desiccation and associated stresses, by increasing potential stress mitigation mechanisms like ROS damage prevention and DNA repair (Figure 4; Table S9). In addition, the community prepares to persist during the long draught through potential activation of sporulation and DNA conservation mechanisms (Figure 4; Table S9). These strategies correspond to reports of sizeable fractions of spore forming bacteria in desert biocrusts (Meier et al., 2021; Nunes da Rocha et al., 2015)."

• In the discussion (line 269 ff.) the authors wonder why the response to desiccation is slower than the response to hydration, but the results show that desiccation also happens much slower and thus it seems reasonable that the organisms stay active over longer time- spans. This then, indeed, could also be caused partly by EPS, but the argumentation has to be adopted accordingly.
The representation of hydration and desiccation in Figure 2A might be misleading as the X axis is not linear. Soil water content decreased from 16% to 3% within three days of the rain event. Most of the desiccation occurred during the first day (decline of 16% to 6% in the soil water content). Likewise, Kidron and Tal 2012 reported that the biocrust rapidly desiccates because the soil surface, is in direct contact with the atmosphere and thus dries rapidly, immediately after a rain event in the desert (Kidron and Tal, 2012).

• Looking at the statistics of the water contents (Table A4, A5) it seems highly questionable that the water content of T[0] versus T[R] and of T[R] and T[1] should not be significantly different. Thus, the statistics need to be thoroughly checked again!
We have inverted the statistics of the water content and chlorophyll and have corrected the error. For better clarity, we have also added letters on Figure 2 to denote the significance the statistics in the figure.

• There are multiple language problems throughout the text, which need to be fixed by a native speaker or professional language editing.
The revised manuscript was proofread by a native speaker.

Technical corrections:

• Line 35: The term biome does not completely fit for "arid environments". Better write "drylands"
Modified

• Line 42: Biocrusts are not only fixed by EPS, but also the organisms themselves, as e.g. fungal hyphae, entangle soil particles and thus stabilize the soil matrix.

The sentence was modified (ln 47-50): "Biocrusts are soil surface matrices of phototrophic and heterotrophic microorganisms that bind soil particles, using extracellular polymeric substances in cyanobacterial biocrusts (Kidron et al., 2020; Belnap et al., 2016), or fungal hyphae in lichen and moss biocrusts (Pointing and Belnap, 2012)."

• Line 43: I don't think that "desolate" is always the correct term for biocrust habitats. They could also stabilize the soil in regions where e.g. succession starts again.
The word "desolate" was removed

• Line 47: Biocrusts could be a main source of C and N and a strong contributor to soil respiration, but I would not say that this always is the case!
The sentence was modified as followed:" Desert biocrusts are the main source of carbon and nitrogen (Agarwal et al., 2014), and strong contributors to soil respiration (Castillo-Monroy et al., 2011)."

• Line 52: It is critical to speak of cryptogams as a "seed bank". They do not produce seeds and they mainly outlast as desiccated plants and not as seed-like structures or spores.
The sentence was modified as such: "To that end, photosynthetic members of the biocrust community either form a seed bank of species that can spring to life when soil water content increases (Murik et al., 2017; Lennon and Jones, 2011; Kedem et al., 2020) or cryptogams that desiccate and remain dormant until the next rain event (Péli et al., 2011)."

• Line 121: The section is named "Chlorophyll concentration and water content", but the calculation of the water content has been described before.
"water content" was removed from the title

• Line 178: please write the version, company and location of the company in brackets; the description, what R is, is not needed.
The description was removed and the following was added :"(version 4.0.0, The R Core)"

• Line 187-189: It is written that at T1 the sampling site was greener than at any other sampling time, but in the referred figure, only pictures of T0 and T1 are shown. It would be very interesting to also see images of the other time points (T2, 3, 4)
Pictures of the soil during the other time points were added to Figure 1

• Line 196: The term "However" does not make sense here, as also the other parameters, that were described before, behaved in the same manner.
Removed

• Figure 2: It would be very helpful to include the statistical results in the figure (letters a, b, c…). The number of replicates should be added in the legend.
Letters were added to Figure 2, 4, S1, and S2

• Line 263: Delete comma at end of line
Removed