

Interactive comment on “Revisiting chlorophyll extraction methods in biological soil crusts — methodology for determination of chlorophyll a and chlorophyll a + b as compared to previous methods” by Jennifer Caesar et al.

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1. Some place have awkward English (e.g., “To prove statistical differences between both extraction methods, the Mann-Whitney U-test was performed for moss-dominated biocrusts, as normal distribution of the data was not given.” I believe what is meant is that the distribution was not normal” and “Solely for moss-dominated biocrusts shaking of samples had no effect.”)
2. One cannot say values are higher or lower if they are not statistically different. In

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the Results: “The results reveal that preparatory grinding caused a decrease in Chla+b contents, causing significantly lower values for green algal lichen- and cyanolichen- and a similar tendency in green algae- and moss-dominated biocrusts (Fig. 2). In fact, this preparatory step caused mean Chla+b yields to be ~ 26, 36, 51 and 25 % lower in green algae-, cyanolichen-, green algal lichen- and moss-dominated biocrusts (Supplement Table S4).” If the values for green algae- and moss-dominated biocrusts are not statistically distinct among treatments, one cannot say “. . .this step caused mean Chla+b yields to be ~ 26, 36, 51 and 25 % lower in green algae-, cyanolichen-, green algal lichen- and moss-dominated biocrusts (Supplement Table S4)”. Same for this sentence “In moss-dominated biocrusts shaking had no effect. Chla+b values were ~ 39, 73, and 42 % higher in green algae-, cyanolichen-, and green algal lichen-dominated biocrusts (Supplement Table S5).

3. “Shaking of biocrust samples after each extraction cycle had a mostly positive effect on extraction efficiency, as mean extraction quantities were significantly higher for cyanolichen- and chlorolichen-dominated biocrusts, and showed the same tendency for green algal-dominated biocrusts: As only 2/4 were enhanced, I would say “affected extraction efficiency”, not “mostly positive”.

4. Discussion and Conclusions: Several other labs have also tested different extraction techniques and have reached a different conclusion regarding the best extractant and whether to grind samples. These need to be acknowledged and discussed more thoroughly in both the Discussion and Conclusions, as there is still work to be done to clarify why different results are being obtained. This especially applies to grinding, as I know of at least 4 labs testing grinding versus not, and they all obtained higher chlorophyll extraction with grinding. My lab has tested this multiple times, and even included hand vs mill, cold vs not cold, and still obtained higher values with any kind of grinding (although hand grinding was superior to the mill). The difference may be that we are using a cyanobacterially-dominated biocrust that contains 0-15% green algal and cyanolichens, but I cannot think of why that would affect this question. Regardless,

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there is clearly further work to be done and I would thus not end with “Thus, based on our experiments, we developed a universal DMSO-based chlorophyll extraction method for biocrusts.” Instead, this needs to be state that this issue is still not resolved.

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