Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2017-482-RC1, 2017

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Microbial Biobanking Cyanobacteria-rich topsoil facilitates mine rehabilitation" *by* Wendy Williams et al.

Anonymous Referee #1

Received and published: 18 December 2017

GENERAL COMMENTS The article title Microbial biobanking cyanobacteria-rich topsoil facilitates mine rehabilitation, by Wendy Williams and collaborators, explores a very important topic in ecological restoration: the rehabilitation of degraded soils provoked by mining activities. And it is relevant because the work was done in a dryland, and drylands are highly impacted by human activities, which are responsible for the continuous loose of ecosystem goods and services provided by healthy environments. The experiment (or the group of experiments) looks like to be well-designed and the methods, poorly explained, are, in general, correctly applied. Actually, the aspect the most I like of the manuscript is the use of both classic and novel methodologies to explore, describe and study the community of microorganisms, mainly cyanobacteria. It is not common at all to find works that use both approaches at the same time. But the prob-

Printer-friendly version

Discussion paper

lem is that the methods are not well described, so it is really difficult to understand at first sight what authors have exactly done. And because of that, the results are not clear, and everything in this section is a mess, which it is a pity because results are really relevant for the field of degraded soils in arid-lands, and their restoration. Finally, the discussion section could have a great potential and impact in the current literature on the topic, but it seems to me that the way in which authors have organized this section deeply penalizes the whole work. In its current condition, I do not recommend this manuscript for publication. Moreover, I recommend the authors to read the manuscript with attention because they will find some parts difficult to understand: sometimes it is necessary to include punctuation, and some other times they wrote the manuscript fast with little attention to the meaning of the sentence within the paragraph. But, if authors make a great effort and change everything I do not like in the manuscript, I will take a look again with a lot of pleasure.

SPECIFIC COMMENTS (by section) ABSTRACT Although well written in general, I miss more specificity in the section. For example, any general result of the study is provided, so it is difficult for any potential and/or interested reader to decide how to do with the manuscript (whether to continue reading it or not). So, please, provide important results within the section (the community structure that you found in your study, the level of chlorophyll a, etc.). Regarding the first sentence of this section, I think that mining rehabilitation does not require any key solutions, but the degradation provoked by mining activities, so please rewrite this sentence. In lines 21-22, you say that "...a range of attributes that contribute to their resilience and survival in arid environment.": well, this is true, but not only for the organisms living here but for any of them living anywhere, so please remove this idea, which is not relevant for your study, and not only here but in some other parts of the manuscript too.

INTRODUCTION In my opinion, this is the best section of the article. It is well written, it is easy to follow and understand, and both the general problem in rehabilitation programs in arid lands and the specific goals of the current work are provided (although

the specific goals are difficult to understand), all along with good but old references. In this sense, please, provide some other recent references (in this section especially in line 10, page 2). And provide some extra references all along the manuscript too. For example, you have not included any reference of recent works focused on restoration of degraded soil in arid lands with cyanobacteria-based methodologies. You provide some references of rehabilitation works, but most of them related with works that include mosses only. I want to highlight several problems that I see in this section. Please, do not mention biofilms too much, this term is more restricted to aquatic environments, and you work with soils (line 24, page 2). In line 27, page 2, you say chlorophyll, but what type of chlorophyll you measured? I suppose is a, but you don't say anything about it, even in the M&M section, so we need to suppose that you measured chl a. Please, bear in mind that this pigment is the only photosynthetic pigment of cyanobacteria, and this is why you measured it. And you do not say why you measured this pigment. Again, it is necessary to suppose that you measured it as a surrogate for biomass, which is correct, but this is not the only way to estimate biomass in an indirect way. So, as I will say later, be much more specific, you have to provide clear information and data. In line 32, page 2, please replace "was" by "were". In line 9, page 3, you say that "Research into. . .is rare". Compared to? Please, be more specific here too. In line 22, page 3, replace "in a plant-available" by "in a biological-available". Not only plants will benefit with the presence of N2 fixers in soils of arid lands. What do you mean with the idea of the sentence in lines 24-25, page 3? I really think that this work has only focused on the scale of community of microorganisms, right? But because the methodology is obscure, I am not sure about it. And perhaps I am not able to understand this sentence because you are not clear enough when you write it. In any case, try to read the section again and look for sentences to be improved, there are some of them too long to understand.

METHODS This section is, in general, a mess. And it is a pity because the work that you have done has a lot of potential, as I said before. The section needs to be rewritten again, starting from the beginning. For example, you say that you were working in 10

sites, but in Figure 1 we have 11. It is really difficult now to know how many points, sites, samples, etc. you were working with. Please, imagine that you are a reader instead of an author, and rewrite the section according to it. You have to write in an easy way, in order for any potential reader to understand what you want to say. If I understood the manuscript, you were working with natural crust samples, and also with stockpile samples. But this is not said at the very beginning of the section, and you should write it. Even in the previous section you have to mention that you worked with both kind of samples. In line 26, page 4, what you mean with crust types? It is essential that you define the crust types here, because you use them all along the manuscript. In line 2, page 5, it is not enough to cite this paper. You have to provide clear, concise specifications about the properties of your different biocrust types. In line 11, page 5, what kind of tool you used to excise biocrust and take samples? Again, please, be more specific. In line 3, page 6, again, what kind of chlorophyll you were working with? I suppose that it is a, but you do not say anything about it. Even if you provide a reference that you followed to estimate chl a concentrations, please briefly explain what you did, what kind of devices, if any, you used, etc. Be more specific again. In lines 10-11, page 6, you say that "The measurement was taken at the point when the crust was broken. . .". This does not have any sense to me. You have to measure your variable with the penetrometer in intact soil cores, otherwise your results are not meaningful or representative. When explaining the measurements of photosynthetic activities, please, do not give technical explanations, but provide information on what you did. Now, it is difficult to assess what you have done. I know that new molecular techniques change constantly and you need to be an expert to provide up to date specifications on what you do. But in your case, you have not said enough to understand what you did with your samples to identify microorganisms in your samples. The only point of all the process that you followed which is clear to me is the first one: the extraction method of the DNA of the soil. But after that you have not provided any information. For example, how you built the libraries for amplicon analyses? What kind of steps you followed in the PCR cycles? What kind of technique you used for sequencing the samples? Please, be more

Printer-friendly version

Discussion paper

specific here, because this part is, apparently, central for the rest of the manuscript. Because a good point of the manuscript, as I have said before, is the use of classic and novel methodologies to identify cyanobacteria, I would merge both subsections in the M&M section saying that the community structure of cyanobacteria was analyzed by using both approaches and... In any case, it is not clear now if you used both approaches for both type of samples (biocrusts and stockpiles). In my opinion no, but because everything is a mess in this section, any future reader will have the same problems that I have now to understand the methods. Finally, please, specify what kind of statistical analysis was used for each specific question of the work. Because the questions of the work are not provided in a clear way, to specify statistical analyses is challenging in the current form of the manuscript. Please, also specify the significance level you chose, this is really important. Go step by step, now this subsection looks like a salad of statistical tools to address unclear questions.

RESULTS This section has a great potential, but I think that there is lot of room for improvement in the interpretation of the results. Perhaps because the methodology is not appropriately explained, this section is hard to follow, and patterns in results difficult to discern. Please, present your results step by step, use your experiments (or what you want) to do it. Otherwise, it is really difficult to extract the important information that you saw after your excellent experimental design. In line 24, page 8, do not start the section saying "In Table 1 we present...". This is not elegant. In line 25, page 8, what you mean with "...ecologically significant differences..."? Differences can be (or cannot be) significant, and after this you provide an ecological/biological explanation. But not the other way around. In line, page 9, again, specify the type of chlorophyll that you measured in your samples. In the same line, you forgot to put a number of chl concentration, and this means that you did not pay enough attention when you wrote the manuscript, and you did not spend enough time for editing it. This is very serious. Now, I have to say that it is much better to use surface units instead of weight units when pigment concentration data are reported, because it is much easier to compare different places by surface than by soil weight. And this is because the surface is

always the same but the weight of a soil is highly variable depending on its texture. So, please, if possible, express all pigment data by surface. In line 6, page 9, you introduce the site T2, but I think that I haven't seen it before in the manuscript. Please, double-check just in case. And be sure that you describe in the previous section of the sites, places, treatments, etc. in an easy way to follow. In line 7, page 9, do you know what the Levene′s test is used for? I do not think so, but it is normally used to test the homogeneity of variances among groups, not for testing the potential differences in the mean of the dependent variable. Please, remove it from here and explain in the previous section what exactly you did to analyze your data. In line 8, page 9, you introduce a result of a Student-t test, but you did not say anything about it in the previous section. So, please, state in the data analyses section what you did, and be precise because this is really important. I am not sure, because it is not really well explained, if you estimated the community structure of cyanobacteria using classic and novel (molecular) techniques in both type of samples (the three stages and the stockpiles). Please, state in the previous section, in a clear way, what type of technique you used for each kind of sample. In line 19, page 9, you say "... and the unicellular genera ...", but what genus or genera. Again, you haven′t paid any attention to edit the manuscript, and it looks like that you want to publish it not matter at all. In line 20, page 9, differences in richness, evenness or diversity, according to what? Explain in the previous section what you did, otherwise it is impossible to follow the results here. In lines 24-25, page 9, the sentence is OK, but it does not belong to your manuscript, so please focus on what you have done, nothing else. In lines 26-30, page 9, you just describe what we know about biocrusts. This is not a result at all, so please remove it. And be careful when you say subsurface cyanobacteria, explain that they can survive under these conditions although they normally need light to be alive. In lines 7-8, page 10, you suddenly say that the soil texture can influence the community structure, which is totally true, but the problem is that you do not say anything about it before, so I have to suppose that you measure soil textures but I can′t see any result in the manuscript. In lines 8-9, page 10, the ideas are opposite. Which idea is the

Printer-friendly version

Discussion paper

correct one? In line 16, page 10, you introduce an idea about the soil textures, please, do it before. In this subsection (3.5) there are too many numbers when you present your data on community structure. Please, sum up this information and only show the real important numbers, otherwise nobody will understand anything. In line 1, page 11, you say "...2YO topsoil...", and although I think that I am able to understand what you mean, it is difficult, and any reader wants to think, just to read and understand your work. In line 13, page 11, you did not sample any Stigonema, you observed it under the microscope. In line 16, page 11, and in other parts of the manuscript, when you show results of data analyses, please indicate what kind of analyses you used, because now it looks like a salad of numbers. And, please, again, in the data analyses subsection of the previous section, state what you did, why you did, etc. In line 1, page 12, area? Why area? You have not measured any variable by area, or you have? Please, be concise and explain what you did. In lines 19-20, page 12, please remove the whole sentence, this information is not really relevant for your work. In lines 21-22, you suddenly start talking about growth rates. So I can suppose that you measured them. But you did not say anything before!!! Please, rewrite the whole M&M section and, after it, rewrite this section again.

DISCUSSION The most important section of any paper is poorly organized, hard to follow, and there is a number of comments where I believe the results are being over-interpreted. So, I recommend rewriting the whole section again. In lines 4-5, page 13, you state that "...species having a range of attributes that contributes to their...". This is OK, it is logical and can be applied to any community in the world. We know it. But you have not measured any of these attributes, and this is not the most important contribution of your work. Actually, this is not a contribution at all. So, please, start this section describing what you have discovered or seen after your excellent field and lab work. In lines 8-9, page 13, again, do not use "biofilm". And instead of "microbe" use "microorganism" all along the manuscript. Do not include any summary of results at the very beginning of this section. Leave it for the next section, the conclusion section, if you want to include this kind of summary. In general, I have the impression that you are

not discussing your discoveries in this section. You are just stating ideas that are well-known for people working with biocrusts. And it is a pity, I have to say again, because you did a hard work and you worked with degraded soils after mining activities in drylands, which is really interesting. So, please, make an effort and rewrite this section, showing first your main discoveries and second using what we know to understand what you saw. In lines 15-16, page 14, you start talking about salinity gradients, but you have not said anything before in the M&M section. And this is a recurrent problem of the manuscript. Please, correct it. In lines 12-13, page 15, what kind of chlorophyll are you talking about? And the presence of chlorophyll is indicative of the presence of an organic layer, but not of an organic layer of EPS. Do you really know what EPS means? Please, make an effort and read more literature on the topic. It is well-known that Microcoleus is the pioneer bacterium in the establishment of biocrusts in North America and Asia. Because this result partially contrasts with what you have seen, it would be great if you include a paragraph discussing why you have seen this difference. You can then talk about biogeography, dispersion, etc., and the paper will have a very interesting ecological perspective. Lines 13-16, page 16, are difficult to understand. In lines 24-25, page 16, you are not discussing your own results anywhere, you just say what we know, but this is not the goal of a discussion section. Again, in the subsection 4.5, I do not see any of your results discussed along with what we already know. You just state a lot of ideas, all of them correct. But this manuscript is not a review manuscript, but an original paper I suppose, so, please, include your main discoveries and, simultaneously, discuss them with what we know, but highlighting what you think is really novel after your excellent work.

CONCLUSION In general, and not only in this section, please include references of cyanobacteria-dominated biocrusts. During the last 10 years, a lot of work has been done on this topic, not only in pristine places but also in degraded soils. In any case, you need to rewrite this section, in its current form is difficult to distinguish between your novel discoveries and the well-known patterns and properties of biocrusts.

TABLES Remove Table 2 and make a plot (barplot) with these data. In Table 4, I do not know which one is the dependent variable of the analysis. So, it is impossible to interpret the table and its results. And this again reflects a big problem of the manuscript: you did not spend enough time double-checking all the info provided and, as a consequence, the manuscript is not consistent and has a lot of mistakes. And this is serious if you pretend to publish it. In Table 5, how did you measure the diversity? Or numbers here are richness data? Please, specify, I do not understand.

FIGURES In Figure 1, how many sites did you survey? 10 or 11? Please, be homogenous all along the manuscript. This is important. In Figure 4, I miss the y-axis name. This is a typical error when you are student, not your case. Same in Figure 5, be serious please... Same in Figure 6... In Figure 7, transform the plot and make a relative abundance one (like the Figure 6). With the new plot, the box called "unclassified/other" will have less weight. In any case, I think that you haven't done too much effort in identifying all these "other" OTUs, but... In Figure 11, use the info that you provide in the caption to discuss the results in the manuscript, this is not the place to do it. In Figure 13, I think that diversity is really richness, right? Please, double-check it. Same in Figure 14. Do you really know the difference between richness and diversity? I now have serious doubts.

FINAL COMMENTS In this manuscript, the introduction is well written, but a bit repetitive sometimes. The rationale for the study of degraded soils after mining activities, and its rehabilitation, is clear. And the combination of classic and modern approaches to study microbial communities of biocrusts is interesting. But the manuscript needs a profound change, starting with the methods and finishing with the conclusion, and also including the figures and the tables. Authors need to improve the quality of the manuscript because they did a great job in a current field of work. It would be a pity if they do not do it because we will not have access to important ideas and discoveries on restoration processes mediated by biocrusts in degraded soils of drylands after mining activities.

Printer-friendly version

Discussion paper

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-482, 2017.

Printer-friendly version

Discussion paper