

## Comment on bg-2022-75

Anonymous Referee #2

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Referee comment on "Diversity and assembly processes of microbial eukaryotic communities in Fildes Peninsula Lakes (West Antarctica)" by Chunmei Zhang et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-75-RC2>, 2022

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The article "Diversity and assembly processes of microeukaryotic community in Fildes Peninsula Lakes (West Antarctica)" by authors Zhang et al. is an impressive effort to characterize and interpret protist communities in hard to reach and understudied ecosystems. The authors analyze protist communities from the same lakes every austral summer for three years. This provides a unique opportunity to understand how stable these communities are over time. The authors discuss the dominant taxa—Cryophyta, Cryptophyta, and Chlorophyta—and how their abundance relates to environmental factors and is influenced by biotic interactions, and whether the community assembly processes are mainly deterministic or stochastic. Overall, the authors conclude that environmental factors contribute little to community composition, interactions are mainly positive between taxa, and stochastic factors primarily shape community assembly. This is a unique study, due to its temporal component, and it documents important aspects of rapidly changing ecosystems in Antarctica. Below are comments that I believe will help improve the manuscript.

General comments:

My main concern is the pre-filtering step in the methods and how that might influence the subsequent results and interpretation. The methods state that the water was pre -filtered through 20 micron mesh-size to remove "mesoplankton and large particles" and then biomass was collected onto a 0.2 micron pore-size filter. This step actually removes all of the microplankton and leaves behind the nano and pico plankton. This has obvious

implications for the title and the language throughout the manuscript, but also has more important implications for the interpretation of the results. The authors note that there is less diversity in these samples than in similar studies, which I suspect could be due to more aggressive filtering? The authors also note in the introduction that diatoms have been studied in Antarctic lakes previously but they do not report finding significant proportions of diatoms in their samples, which could also be an artifact of the size fractionation in this study. Finally, the authors report mainly positive relationships in their co-occurrence network analysis. Again, I think this may be due to the size selection, as microeukaryotes are more likely to graze nano and pico eukaryotes. However, the observation that there seems to be more niche-overlap than competition between nano and pico eukaryotes remains very interesting. Lastly, I feel that it is incorrect to refer to the positive interactions as symbiotic without further evidence documenting symbiotic relationships between the node OTUs being discussed.

Technical comments:

In the methods and throughout, I suggest choosing one spelling for each lake and sticking with it for consistency.

Line 123- I suggest abbreviating temperature just as Temp, similar to using Sal for salinity. WT adds an unnecessary additional acronym. And please complete the statement YSI Model 30 ... what type of instrument, a CTD?

Line 143- "PCR products were pooled and purified using the DNA gel extraction kit." I think this statement is a mistake as pooling should not occur at this step ...

Line 148- please provide more information regarding the sequencing. The first line of the

section states the instrument model, but which version chemistry was used? How many base pairs were sequenced (300?)? And paired or single end?

Line 149- the bioinformatics methods are a bit dated. For instance, why did you use qiime instead of qiime 2? Why OTUs instead of ASVs? Likewise, the SILVA database used is not the most recent and you might also consider using the PR2 database, which is curated specifically for protists. To be clear, I do not necessarily recommend redoing the analysis with more up to date methodology, but I do recommend justifying your decisions with an explanatory sentence.

Line 161- here and elsewhere the OTUs index is referred to and I am not sure what this means. Perhaps you are referring to richness?

167- define MNTD at first use

179- Bray-Curtis distance or dissimilarity, Not similarity

181- I think you may want to scale these variables, especially for variance partitioning (z-scores)

219- a range of 0.9 to 7.14°C does not feel similar

222- molarity is mols per liter, so the units "uM L-1" is incorrect. Only uM should be reported.

226- the *a* of chlorophyll *a* should be italicized

227- salinity needs units (PSU?)

223- the Good's coverage is calculated based on singletons, so please clarify that it was calculated before quality filtering. Also, providing rarefaction curves in the supplemental material will increase confidence in adequate sequencing depth and coverage.

246- SAR should be defined at its first appearance, I'm assuming stramenopiles-rhizaria-alveolates supergroup?

251- 70.09% Arthropoda is .... a lot. Potentially fecal material since the samples were filtered through such fine mesh? I would consider excluding this sample unless the remaining sequencing reads still reach OTU saturation after the Arthropoda reads are removed. In general, you might consider removing metazoan reads early in the analysis.

267- still unclear what the OTU index is

285- define UPGMA at first use

287- rather than saying "clustered into one clade," I think it is more correct to simply say "clustered together"

345- while it is true that the taxa found in the samples are small cells and their small size makes them better adapted to low nutrient conditions, I think that it is hard to say whether they were more or less abundant than larger cells since all the larger cells were removed by pre-filtering with 20  $\mu$ m mesh. As such, this section probably should not take up so much space or prominence in the discussion.

363- I am not sure what is meant by "forming temporary groups"— maybe change the word choice?

378- please clarify whether the other studies you are referring to used similar size fractionation

426- "indicating that species coexistence was achieved mainly by symbiotic relationships between species" — I think this is an overstatement and not supported by the data.

465- unclear which "channel" is being referred to, more context is needed

478- what is "ecological scheduling"?

484- the statement regarding extreme conditions exerting less selection pressure seems incorrect?

511- again please be careful about assuming that positive co-occurrence patterns equate to symbioses, it seems niche-overlapping is more likely

520- please provide the PRJ number to make it easier to access all the sequences.

Figure 1- Please also include a large map that places the region in regional context

(probably include Antarctic peninsula and tip of South America)

Figure 3-The significance indications of letters are not defined in the figure caption. What do "a" , "b", and "ab" mean?

Other comments:

English editing is needed throughout the manuscript. Below are a few edits that stood out to me.

125- nutrient to nutrients

189- opening sentence needs to be rewritten

190- "OTUs represented Occurred" ??

217- Result to Results

310- "still keeps a high proportion" needs to be reworded

353x reference mistake "F R Pick"- remove 1st initials

406- "the nonconsecutive of environmental factors among different expedition seasons was deficient in our study" as is, I cannot make out the meaning of this sentence.