### Review of Graco-Roza et al. - Comments to the Authors

I would like to thank the authors for the revised version of their manuscript entitled "Clumpy coexistence in phytoplankton: the role of functional similarity in community assembly". In its current form, I found this paper interesting and the authors have made an important work in order to clarify their study, especially the 'Methods' and 'Results' sections. They have addressed all my previous comments and I think that the paper will be a valid contribution to the literature on this topic, providing quantitative information about the mechanisms that promote species coexistence and explain community structure. There are a few (minor) points that I would like the authors to address and/or discuss in the final version of the manuscript.

# **Abstract**

- [1] The authors could clarify the meaning of "clumps" in a clearer way, *i.e.*, "a group of coexistent species in a community" to help non-specialist readers to better understand.
- [2] line 40: "In sum" might be removed from the last sentence.

### Introduction

- [3] line 51: "exogenous spatio-temporal mechanisms".
- [4] lines 82-88. I found this paragraph very interesting and relevant. The authors might clarify that the challenge that they highlight here if I am correct is mainly related to a statistical/numerical viewpoint.

#### Methods

- [5] line 161: "based on the location of steep slopes the river elevation profile". Please, correct this sentence.
- [6] lines 161-162: "located close to the sampling sites" might be replaced by "located in the upper and lower courses of the river".
- [7] line 190: "100 individuals of the dominant species". Please clarify how which basis were determined the dominant species.
- [8] line 200: "was measured, and the presence of aerotopes, mucilage, flagella, and siliceous exoskeletal structures". A part of the sentence is missing.
- [9] lines 186-208. The authors might refer to Table S1 in this section. Please, correct "Mophology based functional group" in Table S1 and use the same abbreviates than in the main text (e.g., "vol" vs "V") and the same units.
- [10] lines 186-208. Please clarify in this paragraph if the classification into MBFG you performed here to group species was based on a numerical approach or only on threshold values to assess species in each group.
- [11] I really appreciate the sketch diagram that links statistical analyses and the three hypotheses. I would suggest to add this figure directly in the main paper as it will greatly help the reader to follow the flowchart of the analyses and the link with the results. The first steps of the work, e.g., calculation of species biovolume, data transformation, etc... might be added to the figure to be as exhaustive as possible.
- [12] lines 210-214. This paragraph might be replaced at the end of the 'Methods' section.
- [13] lines 219-221. Please, specify a), b) and c) for each matrix/data frame.
- [14] line 238 vs line 264. Could the authors clarify why two different log-transformation approaches were applied and why a log10+x1 transformation was not performed for the individual volume of species?
- [15] line 314: "and one by diving the actual  $F_{Dist}$ " might be replaced by "and one by <u>dividing</u> the actual  $F_{Dist}$ ", if I am correct.

# **Results**

- [16] I suggest the authors (and this applies to the other sections) to replace "temporal" by "seasonal" (e.g., lines 358, 381) throughout the paper, as the study was performed on two seasons in a single year. For Figures 3 (line 401) and 4 (line 425), it might be helpful to specify: "**Figure 3.** Seasonal distribution of phytoplankton…" and "**Figure 4.** Spatial distribution of phytoplankton…"
- [17] For Figures 3 and 4. I suggest the authors to sort the species code from the highest to the lowest biovolume, if possible; e.g., Figure 3. A3: 028 -> 012 -> 026 -> 140.
- [18] lines 446-448. For consistency, R<sup>2</sup> values might be mentioned following the same format than the one used in Tables 3 and 4.
- [19] Table 4: "Dependent variable: log<sub>10</sub> Biovolume".

#### **Discussion**

- [20] The 'Discussion" section is very interesting and well-written. I found that the link between the results, previous studies and theory is clearly highlighted by the authors. If of interest I suggest the authors to consider the paper "Size differences predict niche and relative fitness differences between phytoplankton species but not their coexistence" by Gallego et al. (2019; 10.1038/s41396-018-0330-7) that is quite in line with the conclusions made in this study: "size is more than a key trait controlling physiological and population-level aspects of phytoplankton, it is also relevant for community-level phenomena such as niche and fitness differences which influence coexistence and biodiversity".
- [21] I suggest the authors to add a few lines (or a short paragraph) about potential limitations/assumptions related to their dataset, especially the length of the time-series (could the conclusions be related only to the sampling frame or could the conclusions be generalised?) and the consideration of the two contrasted seasons (wet *vs* dry). It has been shown that annual phytoplankton succession is strongly influenced by the interaction between the niche of species and environmental conditions, at the community level "Annual phytoplankton succession results from niche-environment interaction" by Caracciolo et al. (2021; 10.1093/plankt/fbaa060). Did the strong and erratic seasonality of phytoplankton species, the phenological signature of each species, influence the analysis and ensuing conclusions?