

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

In the revised version of their manuscript entitled “**Clumpy coexistence in phytoplankton: the role of functional similarity in community assembly**”, the authors have addressed most of my comments and made substantial changes in order to clarify the paper. I would like to thank them for this new version. The ‘Results’ section is now better documented and the analysis have been detailed in order to help the reader to better understand the study. Although I trust that this paper has the potential to be of interest for the community, the manuscript is still perfectible in both its content and style.

**[1] About the form:** When I have to review a paper, the main points to evaluate are both the interest for the scientific community and the scientific accuracy and rigor. From my point of view, it is not crucial and fair to evaluate a paper on the writing, especially as I am not an English native speaker. I would suggest that the authors get editing help to improve and clarify their manuscript, however. The text is not fluent, with shortcomings; frequent typos, missing words, ~sentence fragments lead to an annoying manuscript: e.g. lines 86, 92, 96, 99, 106 (x2), 115, 132, 168, 184, 196, 256, 263, 376, 387, 432.

It would be useful to ensure homogeneity in the text. For example, the words volume/biovolume is used alternatively in the document; I think the use of “*biovolume*” when reference is done to the volume of species, would be more correct.

**[2] About the methodology and the results:** The use of ‘functional traits’ (see line 187) is not correct. By definition – in a numerical way, at least - a trait is called functional if a relation with the environment is found. This is the interest of the RLQ method: to study the relationships between species traits, species abundances and environmental conditions (Dolédec et al. 1996). The RLQ method is therefore applied to first identify the traits responding to the environment, and consequently being functional, assessing the significance of the R and Q relationship by a randomization test. And then, only the functional traits must be used to calculate functional community metrics. A minor comment: why did the authors apply different numbers of permutations for their tests: 999; 49999; 99999?

I wonder why the authors applied first a RLG and the fourth-corner method, and then the approach that combines both analysis; why not applying directly the second method? A sketch diagram describing the different steps of the methodology used for analysis would certainly help the reader to better understand (see, for example, Breton et al. 2017, in MEPS; 10.3354/meps11974).

When looking at the results from the RLQ analysis, I have major concerns about the low correlation values found when environmental values have been correlated to species traits, the highest correlation being  $r=-0.10$  (p-value, for each correlation, are missing); such low values cannot be used to assess a patent relation between traits and environmental parameters (see, for example, Breton et al. 2017 for comparison). When looking at the literature, many authors performed data transformation on the environmental values before performing the RLQ analysis (e.g. log-transformation, root-transformation), especially because environmental parameters show different units and/or range of variation; applying a preliminary transformation on the environmental dataset could help to clarify the results.

“[...] *we first calculated the functional redundancy (FRed)*”: here, only functional traits have been used for calculation, but please, clarify which/or the number of traits. If all traits have been considered (cf. the ‘Results’ section), the low correlations, values considered to assess a trait as functional must be clearly discussed. This possible limitation needs also be discussed when functional distinctiveness has been calculated (Lines 240-259).

“*We did not weight the clump-centroid by species abundance and 254 calculated FDis using all species pertaining to the significant clumps*”. Could the authors explain why they have proceeded in that way?

“*We thus tested  $H_2$  by conducting Mantel tests with 1000 randomizations, whenever possible [...]*”. Could they authors better explain why “whenever possible” as the possible restriction is not clear”? As mentioned above for Pearson correlations, Mantel correlation values are quite low. This must also be interpreted and discussed in the manuscript.

**Figure 3:** it would be helpful, for the readers, to explain “loess curves” in the ‘Methods’ section as no information has been given in the current version of the manuscript.

“[...] by applying a Principal Coordinates Analysis (PCoA) in the species-by-traits data table”. how many axes were retained from the PCoA?

**[3] About the Discussion:** Because this study is rich in analyses, results and concepts, it would greatly help if the authors could put into context their results; for example, by adding the numerical values or by referring the reader to the corresponding figures that explain how they conclude on their preliminary hypotheses. In some parts of the Discussion, vague sentence could be redrafted to improve that sections. For example: **Line 390.** “[...] comprised species from the same MBFG found in previous studies from different systems”. Add more information about this statement: which species? Which ecosystems? Could this information be more relevant by drawing possible hypotheses that explain why such a similarity has been observed?

#### **Other comments:**

**Lines 100-102.** “Despite the importance of body size, the use of a single trait as a proxy for the niche may not evidence species differences generated by hidden niches and impair the understanding of clumpy patterns”. My apologies, but this sentence is still not clear (from my viewpoint) because of a short-cut. Feel free to correct me if I am wrong, but my interpretation of the concept is that body size can be used as a proxy for niche difference, not a proxy for the niche (see Wilson 1975, The American Naturalist and references therein); When the authors state that “generated by hidden niches”, I wonder if it isn't rather: “generated by hidden/unknown niche axes (i.e. ecological dimensions of the niche), i.e. considering body size only is not sufficient.

**Lines 172-185. Phytoplankton samples.** First, please avoid the use of biovolume and volume when they both refer to biovolume. Could the authors clarify how they have calculated the significance of their counting as I do not understand the meaning of the p-value and which test has been performed to calculate the significance. When using ‘density’, please add the unit (i.e. cells L<sup>-1</sup>). “using the appropriate geometrical forms”: please clarify how you have inferred the appropriate form to each species. Finally, more information about the use of the “Morphological Based Functional Groups” would be helpful (in the Appendixes?)

#### **Minor comments:**

**Lines 54-56.** “[...] at small spatial scales, [...], as the environmental heterogeneity is expected to be small”: I am not convinced that environmental heterogeneity is small at small spatial scales, especially when considering the cardinal influence of micro-habitats, physical turbulence processes, advective forces, vertical stratification, etc... Physical factors often vary substantially at small spatial scales: e.g. Bell et al. 1993 (and references therein).

**Lines 76-77.** “However, within a clump, trait differences may be important to species performance in the niche space”: please, clarify this sentence.

**Lines 84-86.** “However, although two functionally redundant species most likely show high similarity in trait combination, functional distinctiveness is not directly linked to redundancy, and such metrics are often”. Please, finish that sentence.

**Line 99.** “[...] and other eco-evolutionary processes”. Please, clarify this statement.

**Line 116.** “[...] water flow that affects the morphology, sedimentation patterns”: what is morphology in this context?

**Line 132.** “[...] scales positively [...]”]: “are positively related to [...]” or “increase with functional redundancy”?

**Lines 139-141.** “Therefore, species with the most distinct trait combinations with respect to their clump peers are less likely to share same ecological requirements, and by consequence, attain higher abundance.” Does this statement is in line with the Tilman' concept (1982)?

**Figure 1.** Please explain “Posse” and “Bingen” (the weather stations?)

**Line 165.** “[...] *water samples were taken and kept frozen (one or 2 weeks) [...]*” Does this difference in the conservation time could have impacted nutrient concentrations?

**Lines 170-171.** “[...] *can be found elsewhere (Graco-Roza et al. 2020)*”. “can be found in Graco-Roza et al. 2020”

**Lines 183-185.** “*Species were then classified into Morphological Based Functional Groups (MBFG) according to (Kruk et al. 2010)*” More information about the MBFG (i.e. the main criteria added in Table 1) would help to better understand how the authors have classified the species.

**Lines 195-196.** “*The p-values were corrected with 49999 permutations of the sites and the species. I do not understand this sentence and permutations “of the sites and the species”.* Please clarify or revise this sentence.

**Line 263.** “*RLQ analysis; Figure 3;  $P < 0.05$* ”. The authors must refer to Figure 2, not Fig. 3.

**Figure 2.** Please, clarify the meaning of the asterisks on panel C

**Figure 3.** Using cumulative curves or cumulative histograms would help the reading.

**Line 312.** “[...] *Pseudanabaena sp. 4*”. Please, clarify the meaning of ‘4’ for that species.

**Figure 4.** The legend of this figure is – from my viewpoint – too detailed when comparing with the description of the Results in the current version of the manuscript.