

Peer Review

Review of: "Nitrification in a Seagrass-Sponge Association"

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The manuscript from [Berlinghof](#) et al. entitled "Nitrification in a Seagrass-Sponge Association" focuses on the interaction between the Mediterranean sponge *Chondrilla nucula* and the phanerogam *Posidonia oceanica*, in terms of nitrification processes and nutrient exchange, and takes into consideration the holobiont concept for both organismal systems. The authors apply incubation experiments with isotopically labeled nutrients (ammonium) and perform nutrient and stable isotope analyses, together with microbial community characterization only for the sponges and the seawater, to address this ecological interaction. From their findings, they conclude that there is a beneficial effect in this sponge-plant interaction, reflected in increased nitrification rates, likely driven by sponge-associated bacterial and archaeal nitrifying taxa, when both holobionts are in association. They further suggest that the dissolved organic carbon released by *P. oceanica* could be used by the sponge, while the inorganic N released by *C. nucula* may sustain the seagrass. Overall, the study sounds very interesting, and the experimental approach seems solid and comprehensive in the approaches chosen. However, in its current status, the work seems incomplete and analysed and discussed in a very superficial manner. In particular, the microbiome data seems to be added as decoration. I think the authors can grasp many more interesting outcomes and integrate all their datasets much better to produce a better product. In particular, the microbial community analysis should be taken with a bit more love, and the discussion with more hunger.

More specific observations:

Abstract: Add the authorities of the species used. Introduce better all the analyses performed to address your question (e.g., nutrients and stable isotopes).

Introduction: You talk a lot about the holobiont system of *Posidonia*, but then you do not analyse the microbiome associated with the plant. Why? You either should justify why not including a plant microbial

community characterization in the study, or you should give less weight to the plant microbiome. Instead, for the sponge, you do the opposite; you do seem to be interested in the microbiome, since you do analyse it in *Chondrilla*, but then you do not introduce some essential microbial functions, like the photosynthetic reliance/capability of this sponge due to cyanobacterial symbionts. These aspects are key to include, as they are the most known microbial taxa in this sponge holobiont.

Methods: In general, the Methods are very well explained in some points, but very lacking in others. The replication pattern in the experimental design is not clear: how many individuals of what were examined for such and such measurement, and also, if they were the same specimens or different. Again, the microbial community analysis is missing information. Details are required for the sequencing laboratory (where did you sequence?), the conditions of the sequencing, and also the bioinformatics analysis; more detail there, how did you remove chimeras, with which program or plugin did you annotate taxonomy, etc.? Also, how did you treat the bacterial and the archaeal sets? Did you analyse them separately, did you merge them somehow? I think the microbiome can be analysed in more depth, adding other analytic measurements, maybe using compositional data analysis, PCA, or other ordination for beta diversity, and maybe correlations. The barplots with relative abundance alone look a bit poor; you can squeeze more results from there, surely. Justify why the plant microbiome was not analysed, and also why you only analysed the sponge microbiomes in spring and not also in autumn.

Results: Where is the Supplementary data? You should explain, or cite how you determined that such and such taxa has nitrification activity. For Fig. 5, for the Average relative abundances of nitrifying families, did you run a statistical test to support the plot?

Discussion: The Discussion needs work. As written, there are many parts just exposed as results, but not discussed. You need to confront and discuss your data with other available research on the topic in the literature and position yourself according to your findings. What is Table 2 doing here? Remove it and send it to Results. Also, remove all citations for figures in Discussion, and unless strictly essential, specific numbers referring to measurements are to be mentioned in the Results, not here. Here you need to discuss. The microbial data needs to be better integrated and correlated with the rest. Explain how you determined the nitrification capabilities of the microbial taxa selected as nitrifying microbes. Finally, in rewriting the Discussion, include a strong concluding paragraph that is sound and gives relevance to your work, while proposing upgraded approaches for the future.

*All this said, I give a low vote because it is not a finished, refined manuscript; it needs a bit of work and meditation. But potentially, it can be great!

Good luck in improving it.

Declarations

Potential competing interests: No potential competing interests to declare.