

Peer Review

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

Valerio Mazzella¹

1. Integrative Marine Ecology, Stazione Zoologica Anton Dohrn di Napoli, Napoli, Italy

This manuscript entitled "Nitrification in a Seagrass-Sponge Association" by Berlinghof and co-authors describes the association of a sponge and a seagrass in the Mediterranean Sea, analyzing potential nitrification rates, stable isotopes, and prokaryotic communities. While the idea behind the manuscript is solid and scientifically sound, I believe the quality and quantity of the data presented should be improved, particularly regarding the analysis of prokaryotic communities, which I feel is lacking. Additionally, some sections, such as the Introduction and Discussion, should be expanded to include more relevant bibliography.

General Concerns

I could not find the supplementary material.

Data from statistical analyses should be included, or at least the p-values should be mentioned.

Letters indicating statistical significance in Figures 1-2-3 are sometimes missing.

Ensure consistency in citation style throughout the main text.

Avoid starting sentences with numbers or abbreviated species names; check along the manuscript.

Include the authority of the species you are studying in the abstract.

In the introduction, you should better present your model holobionts. You never mention, for instance, that *C.nucula* relies heavily on its associated cyanobacteria for its metabolism. Moreover, there are many sponge species associated with seagrasses (also with other species of seagrasses?) in the Mediterranean Sea, and I missed the specific reason why to choose *C. nucula* (i.e., the scientific question).

Rename Table 1. Environmental Parameters... to Environmental Variables.

Submit the data to a public repository if you have not done so yet.

Major Concerns

I could not find a clear description of the experimental design. How many samples were collected, and under what conditions? Additionally, you sampled at a single site, which has peculiar environmental conditions. Is there a specific reason for this choice? There are also less disturbed meadows in the same area. Why sample there?

In the **Materials and Methods**, you state that Archaea were analyzed separately through sequencing, but I did not see these data included in the main manuscript. These data are interesting and should be presented and discussed.

Microbial Communities

The analysis of prokaryotic communities needs improvement.

I suggest increasing both the quality and quantity of the results in this section. Taxa bar plots alone are not very informative and should be supported by additional analyses (alpha and beta diversity, core microbiome, differential abundance). If you choose to present taxa bar plots, it would be better to use a single taxonomic rank (preferably the highest annotated) and display data sample by sample (i.e., including all replicates) to better visualize intraspecific variability. Additionally, I recommend including a dispersion test. Other plots based on different taxonomic ranks can be placed in the supplementary materials. Results from differential abundance analyses should be included in the main body of the manuscript, as this is a key aspect of the study and warrants in-depth discussion. **DESeq2** typically provides very clear and informative plots that can be used to illustrate differences between experimental conditions, which will also strengthen the discussion.

Figure 5 should be moved to the supplementary materials while keeping the relevant text in the main body, as it is not particularly informative here.

Discussion

Once you expand the results, you should also strengthen the discussion. For instance, you could start the discussion with a better paragraph. For example, the sentence:

"The 16S rRNA gene amplicon sequencing of the sponge-associated microbiome revealed a diverse microbial community, including microorganisms involved in nitrification."

is unnecessary here, as *C. nucula* is already known to be a high microbial abundance sponge. This section should be improved by comparing your results with previously published studies. Currently, the writing lacks a logical flow, and there is a lack of comparison with relevant literature in this field. Overall, the manuscript presents an interesting idea but requires a thorough revision.

Declarations

Potential competing interests: No potential competing interests to declare.