Qeios

Peer Review

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

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The manuscript titled “Nitrification in a seagrass-sponge association” by Berlinghof et al. explores nitrification processes, including potential nitrification rates and inorganic nitrogen fluxes, as well as the associated nitrifying microbial communities within the sponge *Chondrilla nucula* found in seagrass habitats. The study integrates both biogeochemical and microbiological evidence to support its conclusions. However, I believe there are areas where the manuscript could be further refined and improved.

**Introduction:**

“The sponge can be found growing in very close association with the seagrass, attached to the lower part of the leaves.”

Are there any references provided? Additionally, when the authors mention that sponges attach to the lower part of seagrass leaves, it may cause confusion since different seagrass species have varying leaf structures. It would be better to specify the seagrass species here for clarity.

I recommend that you clearly outline the specific research questions and hypotheses at

the end of the introduction, as they are currently not well defined in the manuscript.

The authors mention that they conducted experiments under both light and dark conditions; however, they did not provide justification in the introduction section for why both conditions were included.

**Methods:**

“DNA from sponge and seawater samples was extracted using the Qiagen DNeasy Powersoil Kit (Qiagen).” Here, kindly indicate the country of origin alongside the name of the kit. Additionally, please specify the number of samples (n) for each type used in the microbial analysis.



doi.org/10.32388/HZ8XoH

Please use the primer references for bacteria and archaea.

It is unclear whether the samples were rarefied or not. Please provide clarification on these aspects.

**Results:**

Figure 4 is difficult to interpret in its current form. To enhance clarity, please improve the visualization by increasing the font size. In the figure legend, bacterial phyla are mentioned, but what about archaea?

Although the authors mentioned in the Methods section that they analyzed both bacteria and archaea, they did not provide details on the specific methods used for analyzing archaea. Additionally, the results of archaea analysis are not presented in the Results section. Therefore, I request the authors to clarify the methodology used for archaea analysis and to include a detailed presentation of the archaea results in the manuscript.

“We found a significantly higher relative abundance of nitrifying families in the sponge communities compared to the water column (Table S15).” Could you confirm if any statistical tests have been conducted? If so, please include the P-values here. Without this information, it may not be appropriate to state "significantly higher."

Figure 5 could be presented using a different type of visualization, such as a box plot, for clearer representation.

**Discussion:**

“This study of potential nitrification rates (PNR) and inorganic nutrient fluxes is the first to show that DIN provided by nitrification in the sponge Chondrilla nucula can be taken up by the seagrass Posidonia oceanica.”

The authors have already provided the full scientific name in detail, so it is not necessary to repeat it here. Please use the abbreviation instead and ensure consistency throughout the manuscript.

“Potential nitrification rates tend to be higher during the warmer seasons in salt marshes and estuary sediments.” Needs a citation.

“We found the ammonia-oxidizing archaea (AOA) Candidatus Nitrosopumilus, and studies showed that they are stable associates of many sponge species.” Which data?

## Declarations

**Potential competing interests:** No potential competing interests to declare.