

Peer Review

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

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Comments to Berlinghof et al.

The manuscript entitled “Nitrification in a seagrass-sponge association” by Berlinghof et al. used a variety of approaches to investigate nitrification (potential nitrification rates and inorganic N fluxes) and the underlying nitrifying microbes in the sponge *Chondrilla nucula* associated with seagrass. The biogeochemistry part of this study is solid and informative. However, I believe there is some room for the authors to improve the microbiology part (i.e., the nitrifying communities) to help us gain more insights into nitrifiers in the not-well-studied sponge *Chondrilla nucula*. See

Major comments

Fig. 5. Is it possible to include AOA in the plot? That would represent a more complete picture of nitrifying communities in the habitats. Also, it would be helpful to indicate in the figure legend the processes mediated by the taxonomy groups, e.g., Nitrosococcaceae involved in ammonia oxidation and Nitrospiraceae in nitrite oxidation.

It is also interesting to see that the relative abundances of putative NOB (Nitrospiraceae) are higher than those of ammonia oxidizers (Nitrosococcaceae). If this is still true when AOA is accounted for as ammonia oxidizers, it may indicate that the metabolism of NOB is not tightly coupled with ammonia oxidizers. This could be something interesting to explore to gain a deeper understanding of nitrifiers in the investigated habitats.

For the section “Microbial community structure,” can any more information be given about the archaeal community? Could similar results be presented in Fig. 4? I noticed that archaeal 16S rRNA gene amplicon sequencing was performed and AOA were detected in the samples. More information regarding the prevalence of AOA and probably other archaea could be presented.

It is very interesting to see that only Gammaproteobacterial AOB were presented in Fig. 5. Were any Betaproteobacterial AOB (e.g., in the family of Nitromonadaceae) detected in the samples? Betaproteobacterial AOB have been the major AOB detected in many other sponges. More comparison of AOB community with other sponges is also helpful for us to appreciate the uniqueness of this sponge microbiome.

Minor comments

Is the nitrogen demand of the seagrass holobiont mainly nitrate? More background information in this regard (in the Introduction section) could help more readers to deeply understand the importance of nitrification/nitrifiers in the seagrass ecosystem.

In the Abstract, “PNR was exclusively measured when the sponge was present (alone ...)”. The word “measured” should be changed to “detected”.

Declarations

Potential competing interests: No potential competing interests to declare.