

Peer Review

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

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Study Summary:

This study investigated the interaction (with potential nitrifier activity?) between the demosponge *Chondrilla nucula* and the seagrass *Posidonia oceanica* in the Mediterranean Sea. The authors claimed that *C. nucula* harbors a diverse microbial community, including potential nitrifiers, which can contribute to the nitrogen demand of the seagrass holobiont. Nitrification rates were highest in the dark, in the case of one event in which *C. nucula* was associated with *P. oceanica*. This association supported up to 8% of the nitrogen demand during seagrass growth and 47% during senescence. The interaction might be mutually beneficial, stabilizing the seagrass ecosystem.

General Comment:

This paper effectively explored the symbiotic relationship between the sponge *C. nucula* and *P. oceanica*, highlighting the role of microbial nitrification in supporting the nitrogen needs of the seagrass. The study combined experimental approaches with molecular analysis (i.e., amplicon analysis—despite it being simple... and SIP) to provide quantitative insights into this ecological interaction. The findings underscored the importance of such associations in maintaining the health and resilience of coastal ecosystems.

Major Concerns:

1. While the study noted higher nitrification rates in autumn, it could dig deeper into the environmental factors (e.g., temperature, light) influencing these seasonal changes.
2. Further analysis should be needed to understand how *P. oceanica* and *C. nucula* compete for nitrogen and how this affects their mutualistic relationship.

3. Although the study identifies key nitrifying bacteria and archaea (they are able to use dark matter), it could explore their functional roles in more detail (e.g., DNA-SIP using ^{15}N or ^{13}C ... or AMO gene). In fact, using 16S rRNA gene amplicon analysis, generally, the abundances of the nitrifying microorganisms have been underestimated due to other microorganisms... (see your Fig. 5)

Minor Concerns:

1. Some graphs could be improved for clarity, such as those showing seasonal variations in nitrification rates.
2. The study claimed human pressure (e.g., tourism) but could expand on how these activities affect the seagrass-sponge association, although we might know that.
3. Including brief definitions for terms like "holobiont" would enhance readability for non-experts.
4. Some references appeared to be duplicated or unclearly linked, requiring reorganization for clarity.

Declarations

Potential competing interests: No potential competing interests to declare.