Qeios

Peer Review

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

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I read with great interest the manuscript titled "Nitrification in a Seagrass-Sponge Association." The authors investigate how nitrification processes function within the association between the sponge *Chondrilla nucula* and the seagrass *Posidonia oceanica* in the Mediterranean Sea. The authors used biogeochemical approaches to measure nitrification rates, organic and inorganic fluxes, and also performed sponge microbial community analysis. The findings emphasize the importance of this association in stabilizing nutrient dynamics in seagrass ecosystems. The study is interesting and valuable to a wide audience. However, I suggest some modifications to improve the clarity and discussion of the presented findings.

Abstract

Please add details about the organic fluxes (i.e., dissolved organic carbon measurements) when you talk about the analyses performed, rather than mentioning them only at the end as a result.

Introduction

The topic has been contextualized well. However, I believe some changes could be made to help the reader better appreciate the current state of knowledge on the subject and more easily follow the study's context.

I suggest a better reorganization of the first paragraphs to help readers follow the flow of the text. Consider introducing why you discuss marine sponges right after starting with seagrasses. You could establish this connection by including literature on the percentage cover of sponges (e.g., [XX]%) found in seagrass meadows. This would also help justify why *C. nucula* was used in the study.

I suggest moving the sentences on the importance of the prokaryotic community in sponges and DIN fluxes to the section where you talk about the sponge microbial community.

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I suggest starting the paragraph with "*In the Mediterranean Sea, a similar association can be found between the demosponge Chondrilla nucula and the endemic seagrass Posidionia oceanica*."

Please consider adding more recent publications on nitrate and nitrite release in sponges to provide a better state-of-the-art overview of the topic (e.g., Maggioni et al. 2023, *Marine Pollution Bulletin*, Lopez-Acosta et al., 2019 *PlosOne*).

Methods

The methods section is generally well-structured; however, additional details are needed to improve the reader's understanding.

Study site and sampling

Specify how many samples were collected for *P. oceanica*, *C. nucula*, and their association. Additionally, clarify the distance between the collected sponge specimens, as this could impact microbial communities or genetic diversity. Was there replication for colonies, or are the samples assumed to be genetically distinct?

Incubation experiment with stable isotopes

Please avoid starting the sentence with acronyms (e.g., PNR).

The description of the incubation setup is a bit unclear. Please provide more specifics about the number of chambers per condition (light, dark, enriched, non-enriched, spring, and autumn) and how many samples were in each chamber. A schematic or photo of the setup would help clarify this.

If I understand correctly, the authors performed light and dark incubations. Why, then, are the results for nutrients and organic matter not presented for both conditions (Fig. 2, Fig. 3)?

Did you remove the epibionts from the sponges before incubation?

Potential nitrification rates

Please define the abbreviations (SG, SP) when they are first introduced.

Stable isotope analysis

Please clarify this point. Are these samples the same as those used in the incubation chambers? How many samples were there?

Results

I did not see the supplementary data.

I recommend including information on the environmental parameters that differed significantly between spring and autumn, as this could aid in the interpretation and discussion of the results.

Organic and inorganic Nutrient Fluxes

These are interesting results. When the authors say "*close to zero*", consider performing a simple test to determine whether your fluxes are actually statistically different from zero (e.g., using a one-sample t-test to test your rates against a population mean of zero).

The light/dark treatment results are missing for organic and inorganic fluxes (Fig. 2, Fig. 3). These results could provide valuable insights into sponge holobiont metabolism and should be included if sampling was performed (please clarify this in the Materials and Methods section).

Microbial community structure Please include more details on the statistical tests used to support these results.

Discussion

I think some parts of the discussion could be further developed by making more comparisons with existing research to hypothesize or draw conclusions.

Seasonal differences in PNR and nutrient fluxes

Please add a reference here: "*Potential nitrification rates tend to be higher during the warmer seasons in salt marshes and estuary sediments*"

The authors mention that changes in environmental parameters could affect P. oceanica metabolism. What about changes in *C. nucula* nutrient recycling under different conditions? (See below.)

Potential effects at the holobiont and ecosystem level

Move Table 2 to the results section.

The nutrients and DOC flux results are interesting; I believe more attention could be given to their discussion. Could you elaborate further on the hypotheses regarding the impact of different environmental conditions (e.g., changes in fluxes between spring and autumn) on the sponge loop, sponge nutrient cycles, and the sponge-seagrass association? Environmental changes and nutrient availability could influence sponge metabolism and their capacity to recycle nutrients. Recent studies have shown that environmental changes and increasing temperatures may be key drivers of changes in sponge organic and inorganic fluxes, with consequences for nitrogen and carbon balance in ecosystems (e.g., Maggioni et al., 2024 *Limnology & Oceanography*; De Goeij et al., 2017 *book:Climate Change, Ocean Acidification, and Sponges: Impacts Across Multiple Levels of Organization*). I also suggest expanding the

discussion on the stable isotope results by comparing them with similar studies and/or other marine organisms. Additionally, consider discussing the differences in the dark and light results.

Microbial community

Do you have any analysis of the soil microbiome near the sponges? Additionally, do you have any hypotheses regarding the origin of the *Nitrospiraceae* found in the sponges but not in the water column?

Please justify why the plant microbiome was not analyzed. Additionally, it would be useful to explain the absence of sponge microbial analysis in autumn, as this additional analysis could help clarify some of the variations observed between the two seasons.

Minor comments

1. Figure 2 is missing statistical letters on panels a, b, and f.
2. Please add in the figure legend which statistical tests correspond to the letters.
3. Figure 4 is unclear. For example, increase the font size of the bacterial phyla names.
4. Please standardize the reference style in the text.
5. Fiore et al., 2010 and Rix et al., 2015 are cited in the main text but are missing from the references list.

Declarations

Potential competing interests: No potential competing interests to declare.