

Peer Review

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

Daniel F.R. Cleary¹

1. Universidade de Aveiro, Portugal

I enjoyed reading the study by Berlinghof et al. titled 'Nitrification in a Seagrass-Sponge Association'. It was well-written and addressed an interesting issue. It was an exploratory study, as evidenced by the lack of hypotheses, but still an interesting contribution to the topic of nutrient dynamics in seagrass habitats and the importance of sponges. The results are interesting; for example, PNR is greater in the association (sponge+seagrass) than in the sponge alone or seagrass alone, significantly so in the dark incubation in autumn. I think the authors should discuss this result in more detail in the Discussion section, referring to studies that may have obtained similar results and what the implications are. I also think the authors should place more emphasis on the stable isotope results in the discussion. Provide a short description of what the results entail, for example, with respect to trophic level with the $\delta^{15}\text{N}$ values. For example, the authors note that $\delta^{15}\text{N}$ values for *C. nucula* were lower in autumn than in spring. Describe what the implications of this result are. Also, replace: "Conversely, $\delta^{15}\text{N}$ values *C. nucula* were lower" with "Conversely, $\delta^{15}\text{N}$ values for *C. nucula* were lower". In the sentence below, replace 'but also showing' with: "but also showed". In the 'Organic Nutrient Fluxes' section, starting with "The seagrass was a constant source of daily net DOC flux.....", the authors note that seagrass was a source of carbon in spring and autumn, but observed that DOC fluxes were close to zero in the sponge in spring and autumn, close to zero in the association in spring, but with net uptake in the association in autumn. These are interesting results. How do they fit in with the sponge loop hypothesis mentioned by the authors and the DOM to POM conversion? The microbial analysis was very limited. I didn't see the results of the PERMANOVA analyses nor any ordinations. Maybe they were included as supplementary information. These need to be included in the main text. In the discussion, the authors note the prevalence of *Synechococcus spongiarum*. This is an interesting taxon of which much has been written. It would have been interesting to have sampled *C. nucula* from

other habitats, e.g., deeper water, and to have compared the prokaryotic communities. *Synechococcus spongiarum* may be associated with shallow-water environments, such as seagrass meadows, subjected to high temperatures and irradiance and may provide protection to the sponge from these potential stressors. They also play important roles in primary production. In summary, the results are interesting and provide insight into the sponge-seagrass relationship in seagrass meadows. I would, however, like to see the authors provide some more context for their results in the discussion section.

Other comments:

In the sampling section of the methods, the authors note that they “collected the aboveground part of *P. oceanica* shoots when growing alone, small specimens of *C. nucula* (max. 5 cm Ø) growing alone, and both when growing in association”. How many samples were collected from how many different plants? Please also provide some information on the scale of sampling.

In the analysis section, the authors note that they used PERMANOVA but do not specify the software used or the functions. For example, was Primer+Permanova, adonis, or something else used?

In the abstract, replace PNR was highest in the dark with: ‘PNR were highest in the dark’.

Throughout the manuscript, replace until transported to with: ‘until transport to’ and replace until transferred to with: ‘until transfer to’.

Replace each sample was splitted into with: ‘each sample was split into’.

In the ‘Bioinformatics and data analysis of the sequencing data’, the authors used the Euclidean distance for compositional data analysis as opposed to Bray-Curtis or similar indices that account for zeros. This may be fine, but provide some justification for the choice.

Replace: “sponge was preferential taken” with “sponge was preferentially taken”.

Declarations

Potential competing interests: No potential competing interests to declare.