

# ***Interactive comment on “High denitrification and anaerobic ammonium oxidation contributes to net nitrogen loss in a seagrass ecosystem in the central Red Sea” by Neus Garcias-Bonet et al.***

**Neus Garcias-Bonet et al.**

neus.garciasbonet@kaust.edu.sa

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General comments:

RC 1: This paper contributes significantly to the understanding of nitrogen cycling in seagrass meadows. There are few studies quantifying annamox, denitrification, and n-fixation in seagrass meadows. The authors did a great job quantifying annamox and denitrification rates in this system and presented most of their data in a clear and concise manner.

AC1: We thank the reviewer for this comment.

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Specific comments:

RC 2: The authors did not address the known issues with using the Acetylene Reduction Assay technique to measure nitrogen fixation (Fulweiler et al., 2015; Mohr et al., 2010). I would like to see these issues addressed in the paper.

AC 2: We thank the reviewer for this comment and we will include the methodological limitations of ARA in the discussion section, specifically addressing the known effect of acetylene on the microbial community composition reported by Fulweiler et al 2015. Regarding the technical issues reported by Mohr et al. 2010, we took into consideration the potential underestimation of N<sub>2</sub> fixation rates due to the delay in substrate equilibration when it is added as gas. Thus, to avoid the issues raised by Mohr et al, we performed our ARA incubations by adding acetylene-saturated seawater to our incubations, following Wilson et al. (2012). We will specifically mention this in the materials and methods section, following the reviewer comment.

In a newer version of the manuscript, we will include the following text in the discussion reading the ARA limitation: "Despite the common use of the Acetylene Reduction Assay to measure N<sub>2</sub> fixation, it has some methodological limitations that need to be considered. Acetylene is known to induce changes in the microbial community composition in marine sediments, especially in sulfur- and sulfate-reducing bacterial groups (Fulweiler et al 2015). The effect of acetylene is species specific, and, therefore, the N<sub>2</sub> fixation rates reported here might be under- or over- estimated and need to be carefully interpreted."

Similarly, we will include the following text in the methods section to specify why we added acetylene as acetylene-saturated seawater. The new text will read as (line 210): "The acetylene was added in the form of acetylene-saturated seawater to reduce the acetylene equilibration time and therefore avoid potential underestimation of ethylene production rates".

RC 3: It was also very difficult to tell the difference between the brown (sulfide) and

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dark red (oxygen) in figure 1.

AC 3: We thank the reviewer for pointing this out and we will change the color code accordingly to avoid confusion.

Technical corrections:

RC 4 1. Line 50 "estimated in" should be "estimated at" 2. Line 51 same as above

AC 4: We thank the reviewer for pointing this out and we will amend the text accordingly.

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