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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.

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RC 1: Overall, the paper is well-written and the internal logic is consistent. However, I was disappointed that the authors paired state of the art Unisense probes with outdated methods for denitrification and N-fixation measurements, and ignored DNRA altogether. DNRA is an important nitrate loss pathway in seagrasses (Aoki & McGlathery 2017; An and Gardner 2002), but in contrast to denitrification, it returns N to the system as NH4, rather than removing excess N to the gaseous form. Thus it competes with denitrification and potentially exacerbates eutrophication. DNRA could have easily been measured as NH4 from the slurries in this experiment (and could still if there are

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samples in the freezer).

AC 1: We agree with the reviewer that currently there are alternative methods to quantify N fixation and denitrification. We selected the Acetylene Reduction Assay to measure N2 fixation as a cheap and extensively used method, due to the high number of samples resulting from our aim to measure N2 fixation in both vegetated and bare sediments at 4 horizons, along with rates associated to plant tissues at 5 samplings events. Nevertheless, we are aware of the limitations of the Acetylene Reduction Assay and we would include its limitations in the discussion as suggested as well by the other two reviewers. We also agree that it would be very useful to add the DNRA rates in this study to have a more comprehensive picture; however, we regret to inform the reviewer that the DNRA rates cannot be measured at this point. In a newer version of the manuscript, we will include the importance of DNRA in other seagrass systems and discuss the limitations of our conclusions.

RC 2: The helium purging and 15NO3 IPT method is most appropriate for anoxic water columns where nitrification is not expected to play a significant role. Unfortunately, there are severe limitations with using slurries and helium purging when coupled nitrification-denitrification is likely to be important, as it is expected to be near the oxic-anoxic interface or near vegetated roots that actively pump down oxygen. There is a vast literature indicating that coupled nitrification-denitrification is important in coastal sediments (Christensen et al. 1987; Laursen and Seitzinger 2002), including in hypoxic conditions (Gardner and McCarthy 2009). Anoxic slurries destroy natural redox gradients and prevent nitrification (Eyre et al. 2002), which is often the primary NO3- source for denitrification (e.g., Laursen and Seitzinger 2002). The method used here may underestimate actual denitrification rates where there was in situ coupled nitrification and denitrification (van Lujin et al. 1996). Given that the authors did not report ambient NO3, NO2, or NH4 concentrations, it's difficult to know whether direct denitrification played an important role in situ (if there are water samples in the freezer, I would advise running them for nutrient concentrations). In the future, measuring 28N2 fluxes,

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or using a MIMS to measure 28, 29, and 30N2 from intact sediment cores is more likely to account for coupled nitrification-denitrification as well as direct denitrification. By underestimating denitrification, the authors may also have overestimated the importance of anammox. The authors need to acknowledge these shortcomings and try to address them. Although the experimental design has shortcomings, the authors may be able to use the equations in McTigue et al. (2016) to try to correct for their underestimate, although the experimental designs were different.

AC 2: We agree with the reviewer on the limitation of the use of sediment slurries. The use of intact cores would have solved the problem of disturbing the sediment structure and affecting redox gradients, which we will highlight in the discussion section. Following the reviewer's comment, we will state the importance of the coupled nitrification-denitrification in coastal sediments near the oxic-anoxic interface or near vegetated roots which actively supply oxygen, and we will acknowledge the possible underestimation of the denitrification rates reported in our study. We thank the reviewer for pointing out the possibility to improve our work by calculating the coupled nitrification-denitrification using the equations reported in McTigue et al (2016). However, we didn't measure NH4+ and NO3- concentrations in the samples and, unfortunately, we don't have frozen seawater samples to analyze NO3- concentration. Therefore, we cannot calculate denitrification rates in the overlying seawater which are needed to estimate the coupled nitrification-denitrification rates following McTigue et al. (2016).

RC 3: As Reviewer 1 mentioned, there have been many documented issues with N-fixation from ARA, including shifting the microbial community (Fulweiler et al. 2015) and potentially altering rates. The authors should acknowledge these shortcomings.

AC 3: We will include the shortcomings of the ARA following the reviewers' comment.

The new text will read as follow: "Despite the common use of the Acetylene Reduction Assay to measure N2 fixation, it has some methodological limitations that need to be considered. Acetylene is known to induce changes in the microbial community

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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 N2 fixation rates reported here might be under- or over- estimated and need to be carefully interpreted."

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