

## ***Interactive comment on “Measurements of nitrite production and nitrite-producing organisms in and around the primary nitrite maximum in the central California Current” by A. E. Santoro et al.***

**Anonymous Referee #2**

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The study by Santoro et al. presents a broad dataset on the development and existence of the primary nitrite maximum (PNM) in the California Current. Nitrite is definitely a central switch with regard to the nitrogen cycle and thus this study identified a not necessarily novel but nevertheless hot topic in the area of the marine nitrogen cycle. To approach the dynamics of the PNM, Santoro et al. performed nutrient measurements, molecular detection and <sup>15</sup>N incubation experiments. The dataset they present compares nitrate reduction and ammonia oxidation as key producing processes. Santoro et al. identified the oxidation of ammonia to nitrite as the responsible process for nitrite formation, here, based on rate measurements and the detection of a high number of ammonia oxidizers. This challenges the classical view that nitrite is mainly formed by

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assimilatory nitrate reduction and adds to the growing knowledge of the importance of ammonia oxidation- particularly by archaea- in various marine systems.

Overall, this study is well presented and the multidisciplinary approach is a good way to investigate a complex topic like this one. The topic is appropriate for a publication in Biogeosciences.

However, I have several general comments and questions:

Rate determinations: p. 5810: Although the authors argue that added nutrient concentrations were more or less around the ambient concentrations, an addition of nutrient one order of magnitude higher than present before always arises the problem that you measure potential rates instead of real ones. Moreover, the treatments for nitrate and ammonia supplementations are not really comparable, and it might happen, that ammonia oxidation is triggered stronger compared to nitrate reduction. How would you argue, here? Can you really exclude that you trigger the one or the other process with this? Could you also add some statement on how the nitrate reduction rates are calculated, this is a bit unclear.

The incubations were performed over 36h. Is there any bottle effect to expect, here? Did you follow up the oxygen consumption in your incubations, which would be highly interesting anyway, and if so, how does oxygen behave compared to ammonia oxidation? Is there any influence of daylight on the rates (which would be expected)?

What about further nitrite oxidation to nitrate? Of course, there is always the problem, that you can't screen for this process using molecular tools, however, is this nitrite-loss term neglectable?

Fig.5: Why do you only present three out of four incubation experiments?

Molecular methods: p. 5813 ff.: My major concern on this study is, that you might be missing one or more groups of important organisms, as you screened only for selected ones. How do you know about the diversity of the ammonia oxidizers and

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the nitrate reducers.? Did you perform any molecular screening based on sequences, e.g. a clone library? If so, it would strengthen your study enormously to present that data, as it is completely unclear, whether you identified all important organisms or not. Particularly with regard to the ammonia oxidizers, you only quantified the beta proteobacteria and the archaea. Are there no gamma proteobacteria around, that might oxidize ammonia? The way you describe your screening for ammonia oxidizers gives me sort of a feeling that you want the AOA to be the important group, here. It would be good for the argumentation of your study to present this a bit more balanced. p.5817: The other thing, I have concerns, is on the comparability of flow cytometry to qPCR, how can you assure that both methods are quantitatively comparable?

Specific comments:

p. 5808: The paragraph on DNA filtration and extraction is a bit confusing, please clarify, if there is any difference concerning nucleic acid filtration and DNA sampling, which filters, filtration times and volumes were used.

Same page: Which volumes did you sample for flow cytometry?

p. 5816: The first datapoint you present is Chlorophyll, but no method is provided for this, please add one sentence.

Figures: fig. 3: Rates in the text and in the figure do not compare at 55m fig.5: What happened to the other incubation experiment?

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