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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 #1

Received and published: 7 June 2013

This study by Santoro and colleagues examined the dynamics of nitrite in/around the primary nitrite maximum (PNM) in the central California Current through a multidisciplinary approach. With a combination of nutrient profiling and 15-tracer experiment, the authors determined the relative importance of ammonia oxidation, nitrification and assimilatory nitrate reduction to nitrite at the PNM. They measured much higher rates of ammonia oxidation/nitrification, while nitrate reduction rates were low or undetectable. In parallel, with quantitative PCR they found increasing abundance of ammonia-oxidizing organisms towards the PNM and immediately below, as opposed to the shrinking populations of potentially nitrate-reducing primary producers, quantified by flow cytometry. In addition, rate measurements were compared against net pro-

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duction and consumption of nitrite estimated by 1-D modeling. The authors concluded that, contrary to previous conception, assimilatory nitrate reduction was found to be a minor contributor to nitrite dynamics at the PNM, whereas ammonia oxidation is the dominant source of nitrite.

My major concerns for this manuscript lie in the rate determinations. While the described method for rate calculation was the same as the authors used before for nitrification rates (Santoro et al., 2010), it is not clear how the nitrate reduction rates to nitrite were calculated exactly. I presume it was the $^{15}\text{N}/^{14}\text{N}$ content of the nitrite pool being modeled. In that case, the isotopic compositions of nitrite and nitrate would have to be treated separately, but I didn't see these data presented (either as measured or calculated values).

In addition, nitrite assimilation would not be the only output term, but also nitrite oxidation to nitrite. Not only this process ought to be taken into account in the rate calculation, it is also very likely that the ^{15}N -nitrite production was considerably underestimated in the measurements. Given the fairly high nitrification rates measured in/around the PNM, it is possible that a substantial portion of the ^{15}N -nitrite produced from ^{15}N -nitrate reduction has been reoxidized back to nitrate. In theory, the contribution of nitrite oxidation may be corrected by the ammonia oxidation and nitrification rate measurements. However, how, or whether or not, the above-mentioned have been taken into account in the rate calculations, are unclear in the manuscript. Any complications or contribution from nitrite oxidizing organisms would also need to be discussed.

The incubation experiments were conducted over the course 36 hours, but the authors also mentioned that some sampling stations were occupied at night (page 5822 line 17). Have all the incubations experienced the same number of hours in the dark/light? And were the cycles more like 12h light:12h dark:12h light, or 12h dark:12h light:12h dark? Obviously, it is more likely for the photosynthetic organisms to make any noticeable contribution in the former rather than the latter, so the light regime used could potentially introduce biases for/against these photosynthetic organisms.

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The amounts of ^{15}N -amendments resulted in quite a large range of substrate enhancement: 0.5-13 x for ammonium and 0.02-0.23 x for nitrate. Because of the higher degree for ammonium amendment, I wonder if ammonia oxidation were more likely stimulated compared to nitrate reduction, and thus have contributed to the much higher rates measured?

While it is recommendable to employ alternative and independent method to estimate net consumption/production rates of nitrite, due to the high likelihood of horizontal advection in the California Current, I am not convinced that the use of a 1-D reaction-diffusion model was appropriate. Besides, though the authors claimed to have “high”-resolution nitrite profiles, 12 depth spanning 200 m range, meaning every 16+ m on average is not exactly high-resolution in my opinion. There have been quite a few other studies where every 10 m (or down every 1-2 m in the extreme cases) were sampled. More importantly, it is rather apparent from e.g. Fig 5 that the sampling resolution was too coarse for the data to be used to model 1-m resolution (especially panel b which showed only 6 sampling depths over 200 m).

In an attempt to compare between modeled and measured net rates of nitrite, the authors simply added the measured ammonia oxidation and nitrate reduction rates together and then subtracted the nitrification rates as the “measured” net rates. Nevertheless, such mass balance calculation would require gross rates for individual processes, while the ones used are only net rates. Subsequently, the calculated net change became erroneously small, thus giving rise to a misleadingly long residence time. On the other hand, not all nitrite consumption and production processes have been taken into account – e.g. nitrite/nitrate assimilation.

In any case, why were there only three “measured net rates” shown in Fig. 5, when there were four incubation depths shown in Fig 3?

While it was written in text that ammonia oxidation usually exceeded nitrification rates except for one occasion (St. 67.00, 55 m), Fig. 3 showed that rates of the former was

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always greater than nitrification. Please clarify.

With respect to molecular analyses, they seem a little unbalanced with greater effort spent on quantifying ammonia-oxidizing organisms, relative to the assimilatory nitrate-reducing organisms. While, on one hand, I am not certain whether the qPCR and flow cytometry data are truly comparable due to the pros and cons of respective method, there was a lack of data for photosynthetic organisms below the PNM at station 67.155.

Furthermore, as mentioned in the introduction, assimilatory nitrate reduction may also be conducted by heterotrophic bacteria (and likely archaea too). There was, however, no attempt and no discussion to address their potential importance. Perhaps assimilatory nitrate reductase genes, e.g. *nas*, may be used as a biomarker gene to cover more organisms in this functional group?

Overall, I think this study has identified a very interesting topic in marine microbial ecology and biogeochemistry; yet the work did not appear sufficiently well planned and executed. Because of the number of critical issues associated with the rate measurements and calculations, as well as biases in molecular analyses, the conclusion that ammonia oxidation being a more dominant source of nitrite relative to assimilatory nitrate reduction, does not seem to be well supported. More clarification, discussion and probably more data would be necessary to help justify such a claim.

Specific comments:

Page 5808 line 8-12: sampling for nucleic acid extraction vs sampling for DNA extraction – aren't they supposed to be the same? Did you use 2-4L or 1-2L of seawater samples then?

Page 5808 line 17 (also in abstract and throughout the manuscript): please avoid the hype of call it high-resolution, as it is not convincingly so.

Page 5810 line 26: how were the sub-sampling at each time-interval performed? Through rubber septa, or?

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Page 5818 lines 17-19: I'm a little confused with the numbers here. In Fig. 3, the rates for ammonia oxidation were always higher than nitrification rates, but here you mentioned there should be one exception at 55 m. Meanwhile, the number '23 nmol/L/d' became '26' on page 5823 line 8.

Page 5820 line 9-10: if the contribution from nitrate reduction is minimal, at the PNM at station 67.155, for example, the measured ammonia oxidation rates were also very low and you modeled a substantial net production at that depth. How would you explain?

Fig. 3: ammonium profile is missing for station 67.155

Interactive comment on Biogeosciences Discuss., 10, 5803, 2013.

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