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Interactive comment on "Nitrification and its oxygen consumption along the turbid Changjiang River plume" *by* S. S.-Y. Hsiao et al.

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In general, there are aspects of this study that are highly interesting and can contribute meaningfully to nitrification research in the form of nitrification rates, the co-correlation of relevant environmental parameters, and the relative distribution and potential activity of AOAs and β-AOBs, particularly in estuarine environments. However, the authors neglect most of the interesting discussions pertaining to this set of results as well as previous relevant studies, in pursuit of a set of alternative discussion arcs that are, in my opinion, tenuously supported by the data. Although the idea of reactive Fe or Mn participating as alternative electron acceptors for ammonia oxidation is highly intriguing, certainly plausible in this sort of environment given the right conditions, and deserving of further research, by ignoring more logical conclusions, some inherent biases in their

methods (which the whole field suffers from), and previous high profile studies, the authors veer off the main crux of their research topic. However, by re-focusing on the meat of their biological data and 'fleshing' out these details, the authors can hopefully regain the narrative and provide us with more logical and meaningful conclusions.

Specifically, the focus of this study should be concentrated on the results pertaining to the relative distribution and activity of AOAs and β-AOBs throughout this estuarine system, and how certain prominent estuarine features, such as salinity gradients and estuarine turbidity maxima (ETMs), affect these components. Certainly, by demonstrating a clear difference between particulate and planktonic nitrification rates and the quantity of ammonia-oxidizing microorganisms (AOMs) between these fractions in an estuarine setting, the authors have neatly confirmed and re-affirmed that ETMs, and the particles associated with them, can be hot-beds of nitrification activity. The method of partitioning between the particulate and planktonic fractions in both of these analyses is also not commonly done and a strong point of the data set. And yet, the authors cite only two studies from freshwater river systems in their discussion of the correlations between TSM and nitrification, when a rich amount of research on nitrification in estuaries is much more relevant. For example, high nitrification activity is commonly associated with intermediate salinities (Berounsky and Nixon, 1993) and ETMs (Owens, 1986), although there are also cases where increasing salinities decrease nitrification rates (Rysgaard et al., 1999; Brion et al., 2000, Cébron et al., 2003). Phytoplankton have also been hypothesized to lyse upon contact with saline water and release POC (Lara-Lara et al. 1990). In the case of a quantitative AOM physical affiliation with particles, again, this study demonstrates what has heretofore only been sparsely observed in analogous systems (Wuchter et al., 2006; Woebken et al., 2007 (in an OMZ so it serves as a contrast); Galand et al., 2008).

Salinity, ostensibly also seems to have an effect on the relative distribution and abundances of AOAs and β-AOBs, and so it would be nice to see what the correlations here are in comparison to previous reports (Mosier and Francis, 2008; Bernhard et al.,

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2010). In particular, it would be nice to see some discussions regarding these results as they pertain to potential differences between sediment and water column parameters with a potential focus on the varying substrate affinities of AOAs and β-AOBs (Martens-Habbena, 2009). Since it is not a very large dataset (although still passable) it may help to add some more sites for some more qPCR analyses of AOMs, provided the samples for DNA isolation were taken for more than the sites listed. By the looks of it, more NRb measurements were made than qPCR analyses. In a similar vein, if light transmissivity was measured by the CTD in this study, the authors could further explore the relationship they postulated to exist between light and nitrification, in particular by looking at the specific nitrification rate (the daily rate of nitrification divided by the corresponding ammonium concentration). Of course, all of these correlations between environmental parameters and the biological data (qPCR copy numbers and nitrification rates) should be first analyzed using multivariate statistical tests, such as canonical correspondence analysis or correlation matrices, such that co-correlating complications can be avoided and dominant factors can be teased out for further discussion and analysis. This was a major pitfall the authors fell into and it led to the postulation of weakly supported conclusions.

In their postulation of reactive Fe and Mn as potential and likely roles as alternative electron acceptors for ammonia oxidation or nitrification (a distinction which needed to be elaborated) in the seasonal periods of hypoxia/anoxia in this system, the authors ignored some fundamentally known pieces of information. Firstly, in the absence of oxygen or in anoxic micro-niches on particles, NO3- and NO2- are much more energetically favorable electron acceptors than Fe or Mn, and are commonly used by heterotrophic denitrifiers in breaking down organics. In addition, the process of anaerobic ammonium oxidation has been known for quite some time now and has been found in many diverse habitats, even associated with particles. This process is carried out by bacterial members within the family Planctomycetaceae and involves the oxidation of NH4+ by NO2- to produce N2 gas. By only measuring AOMs and hypothesizing that reactive Fe and Mn are involved as alternative electron acceptors, it can

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be easy for readers to mistakenly conflate that the authors are proposing a process involving AOMs, that if it were to occur, most likely would involve completely different and uncharacterized organisms and mechanisms. Second, at no point in any of the incubations would the O2 concentrations have been depleted by the measured CR rates, since the maximum CR rates (10.79 μM d-1) were about 5 times lower than the lowest oxygen concentrations (58 μM). Although the authors do point out that the O2 consumption from nitrification would commonly exceed the amount of O2 needed by other heterotrophs for the breakdown of organics, this assumption can be violated by a few factors: 1) as mentioned by Referee 1, the CR rate may be underestimated; 2) relatedly, since the nitrification rates were conducted in the dark, AOMs are relieved from light inhibition thereby potentially inflating in situ rates; 3) AOAs are known for their extremely high substrate affinity for NH4+ and O2, that they may even be able to outcompete heterotrophs for both substrates thereby forcing the heterotrophs to use alternative electron acceptors such as NO3- and NO2- ; 4) this scenario could then fit with anammox bacteria operating in anoxic micro-niches on particles; 5) if a significant proportion of ammonium does not come from immediate or adjacent organics; 6) since the nitrification rates combine both the production of 15NO2- and 15NO3- and ammonia oxidation only uses 1.5 moles of O2 per mole of ammonia oxidized, by not separating the rates into two processes, one can slightly overestimate the O2 consumption from nitrification (although to be fair, ammonia-oxidation is the rate-limiting step and most nitrite produced should be eventually oxidized). Finally, a more interesting analysis would be to calculate the cell-specific ammonia-oxidation rates for the AOAs and β-AOBs, respectively, from the derived qPCR numbers and nitrification rates, by assuming the typical amount of amoA copy numbers per cell for each type of organism (Norton, et al. 2002), and comparing them to the cell-specific rates found in cultured representatives (e.g. Könneke et al. 2005; Prosser, 1989). One can then theoretically determine how much either the assayed AOAs or β-AOBs are capable of contributing to the measured nitrification rates. Depending on how close these numbers are to each other, a discussion could then focus on the inherent biases in the DNA

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extractions, primer coverage, and the nitrification rates (e.g. $^{15}NH_4^+$ tracer rates vs. $^{15}NO_3^-$ isotope dilution rates vs. rates derived from bulk changes in NO_2^-/NO_3^-). Only at this point would it be more appropriate to invoke other potential actors – that is if the rates and AOMs do not significantly match up, even by accounting for biases – such as anammox, heterotrophic nitrifiers, and even Fe/Mn mediated processes. Again, one could at least go back to the existing DNA extracts and assay them for the anammox marker gene nirS, although these numbers should not be correlated to the nitrification rates, since anammox bacteria would have converted labeled $^{15}NH_4^+$ or $^{15}NO_2^-$ into N_2 gas.

Minor comments and technical notes:

It may be more helpful to express the amoA copy number concentrations in ml^{-1} notation and using orders of 10 (e.g. $7.6 \pm 0.5 \times 10^4$ copies ml^{-1}) so that comparisons with previous studies are easier.

Ammonia is oxidized by AOMs, not ammonium.

Would it be possible to quickly re-calculate the nitrification rates based on the equation found in Ward and O'Mullan (2005) and also found in the supplemental methods of Beman et al. (2011)? This is just to ensure your rates agree between different calculation methods and should not be too complicated, unless you are missing crucial pieces of information.

In lines 9-11, paragraph 1 of page 8695, is it possible to clarify what is meant by 'unraveled factors'.

Finally, the difference in slopes in Figure 4b (13 vs. 0.33) is a difference of around 40-fold, instead of 5-fold, right?

Interactive comment on Biogeosciences Discuss., 10, 8685, 2013.