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# ***Interactive comment on “Characterization of incubation experiments and development of an enrichment culture capable of ammonium oxidation under iron reducing conditions” by S. Huang and P. R. Jaffé***

**Anonymous Referee #1**

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In this manuscript the authors attempt to link geochemical signals suggestive of anaerobic ammonium oxidation coupled to Fe(III) reduction (feammox) to changes in the microbial community structure across a range of batch and flow-through enrichments. While the results are certainly interesting, the linkages are not completely clear, and certainly leave a number of outstanding questions.

1. When calculating the initial thermodynamics of feammox, the authors describe the use of  $\text{Fe}(\text{OH})_3$  as the structure for ferrihydrite. Why is the formula  $\text{Fe}_2\text{O}_3 \cdot 0.5\text{H}_2\text{O}$  subsequently used in the calculations/discussion?

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2. A range of molecular tools were used in the analysis of enrichments cultures and flow-through reactors. Few details are provided to describe the 454 analyses, or the construction of phylogenetic trees. Please add details on the pipelines used for 16S gene analyses, and the tools used for tree generation.

3. Could the authors please explain further what happened in those batch experiments with ferric citrate, where Fe(III) reduction occurred rapidly but no ammonium oxidation was observed. What was driving Fe(III) reduction in these instances? In the discussion the authors mention that energetics of the reaction are only favorable when Fe(II) is removed from solution via sorption. From this, are we supposed to infer that the lack of Fe(II) sorption is the major reason for absence of Feammox in cultures with soluble Fe(III) sources?

4. An additional chart showing the stoichiometry between Fe(II) production and ammonium consumption across all the 180-day incubation time points would be beneficial. This ratio is discussed for a few select time points (page 12310) currently. This would enable readers to track the linkage between iron and ammonium in these experiments, without having to refer to multiple graphs.

5. There are some discrepancies between the coupling of iron and ammonium in the 180-day main series of incubations. Following the spike of NH<sub>4</sub>Cl on day 125, ammonium is rapidly consumed. Across the time period 120-140 days, only a small increase in Fe(II) is observed. Between days 140-160 ammonium continues to be consumed, and Fe(II) concentrations increase rapidly. It would be helpful for the authors to address these discrepancies in geochemical data, as it detracts for the idea of a 'tight couple' between ammonium oxidation and iron reduction.

6. Trends in the abundance of the Acidimicrobiaceae A6 signal are similarly confusing. Similar ammonium oxidation rates can be identified at a number of points on figure 2b, such as after day 60, and following day 125. Despite similar ammonium oxidation rates, the abundances for Acidimicrobiaceae bacterium A6 are completely different at these

two time points, further clouding the role of this species in catalyzing the feammox process.

The linkages presented here between abundances of certain bacteria, and geochemical trends are too loose, and would be strengthened considerably by a tracking technique (stable isotopes?) to conclusively demonstrate the role of Acidimicrobiaceae A6 in the feammox process.

7. The paper needs editing, either by the authors or a technical editor. There are multiple spelling mistakes throughout the manuscript (e.g. page 12300, lines 5 (through), 14 (from), and 25 (column)).

Minor comments:

Page 12301, line 12: please provide forward and reverse primer sequences, rather than just the target region.

Page 12302, line 4: please change rDNA to rRNA (also page 12305, line 15)

Page 12305, line 18: What do you mean by 'in terms of cell numbers'? I was under the impression that cell counts were not performed, so a different phrase should be used here to describe % of community community.

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Interactive comment on Biogeosciences Discuss., 11, 12295, 2014.

**BGD**

11, C5163–C5165, 2014

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