

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 #2

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The current manuscript addresses one of the remaining mysteries of the nitrogen cycle: ferric iron dependent ammonium oxidation. Unfortunately the current manuscript only presents circumstantial evidence, which is not convincing.

Introduction: Line 9: what do the authors mean by “conventional removal of nitrogen”?

Line 11: What are these saturated with?

Lines 11-14: This sentence contradicts the preceding sentence. How can nitrification and denitrification occur if there is no O₂ and/or oxidized nitrogen species? Further-

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more, it is incorrect. The presence of compounds does not mean much, what is important is fluxes. Nitrite hardly occurs in high amounts in oxygen minimum zones and in wastewater, but microorganisms that convert nitrite are very important in nature and form the basis of wastewater treatment.

Results and Discussion:

I do not agree with most of the discussion.

1) The observed ammonium oxidation activity can also be explained by oxygen leakage to the used system. The ammonium oxidation rates are so low that a small amount of O₂ leakage would be enough to establish a small AOA or AOB community that would be able to convert the same amount of ammonium. The experiments are conducted in anoxic bottles; however, the authors cannot exclude O₂ leakage because the incubations were not conducted in an anaerobic chamber. It is conceivable that every time the authors sampled their batch incubations, they introduced O₂ to the bottles.

2) The employed methods and the presented data set do not allow the identification of any microorganism that performs this reaction. Based on phylogenetic inferences and intensities of DGGE bands, one cannot establish or exclude the involvement of any of the detected microorganisms in the observed reaction. There is no evidence linking the activity to the presence of the detected microorganisms. There is no reason to believe that the increase in the population of Acidobacteria, Actinobacteria and betaproteobacteria is not merely coincidental. These microorganisms are found in all natural ecosystems and there are many members of these groups that can perform a multitude of different reactions.

3) Even if the authors presented conclusive evidence, which they do not, for the iron-dependent ammonium oxidation activity, this would still not mean that these microorganisms are growing on this reaction. It could well be a side reaction of any microorganism.

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- 4) I do not see the point of DGGE. It is a very crude method with so many drawbacks that there is no place to list here. Decreases and increases in DGGE bands and their intensities do not mean anything. Furthermore, rRNA or mRNA amount does not mean that organisms with more RNA are more active. There is no direct correlation between RNA, levels of protein expression and activity. I would remove the whole DGGE section.
- 5) The authors use acetylene to inhibit ammonium oxidation. Acetylene inhibits both anaerobic and aerobic ammonium oxidizing microorganisms, methane oxidizers and denitrifiers. The effect of acetylene that the authors describe could well be due to the inhibition of the denitrifying community. The authors state that acetylene did not affect Fe(II) production, which strongly suggests that this activity is uncoupled from ammonium oxidation. If the organisms in the incubation were converting Fe(III) coupled to organic acid oxidation, indeed they would not be inhibited by acetylene.
- 6) The authors suggest that the nirS is increasing due to nitrite produced through ammonium oxidation coupled to iron reduction. Of course, nitrite could have been produced via nitrate reduction or normal aerobic ammonium oxidation. The authors should also measure nirK abundance.
- 7) The decrease in the amoA gene does not mean that AOA or AOB are not responsible for ammonium oxidation activity. As I stated before, only a small amount of AOA or AOB would be enough for this activity. Further, I wonder if the authors considered checking their samples for acidophilic ammonium oxidizers. Surely, in their samples there is no free ammonia, but only ammonium (due to low pH).
- 8) The bicarbonate-amended samples have marginally higher rates. Furthermore, the authors cannot exclude the fact that there are still slowly released organic compounds in their samples.
- 9) Please remove all the speculation based on acetylene experiments. Acetylene is a very crude inhibitor and inhibits many, many reactions. Furthermore, without any genomic or biochemical data one cannot speculate on the pathway of any reaction.

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Moreover, the authors use the word “pathway” wrongly throughout the manuscript.

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