

Interactive comment on “Isotopic evidence for alteration of nitrous oxide emissions and producing pathways contribution under nitrifying conditions” by Guillaume Humbert et al.

Anonymous Referee #2

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The authors report the use of stable isotopes of N₂O (bulk and site specific $\delta^{15}\text{N}$), complementary to N₂O and dissolved inorganic nitrogen concentrations to identify the key processes producing N₂O in a biofilm reactor used in a local wastewater treatment facility. They showed that nitrite reduction was the primary N₂O producing pathway in the reactor irrespective of the experimental conditions (i.e. different percentage of O₂, temperature and initial NH₄⁺ concentrations). Temperature, however imposed the greatest effect on N₂O emissions compared to the other factors by simultaneously promoting hydroxylamine oxidation pathway.

This study contains interesting dataset particularly on the factors controlling N₂O emis-

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sions; which could have broader implications to other systems. As such, I think this study has the potential to be an interesting and helpful addition to the literature but to make it so will require a concerted effort. This is because the manuscript is not very well-written. Most part of the manuscript is confusing with either no or invalid justifications were provided for the assumptions made. For example, (1) experimental conditions presented in the tables are different from the ones presented in the graphs but no explanation was provided as to why some of the data points were ignored; (2) some of the interpretations on the trends are misleading and were not supported with statistical analysis; (3) rates of processes were not well-defined and some of the terms were randomly introduced in the discussion without prior definition of the terms; (4) there was no clear distinction on which part of the results were depicted from the literature and which part was obtained from the study; (5) in the method the authors mentioned that they analysed the d15N of nitrate, nitrite and ammonium, they then indicated in the later section that they hypothesized/estimated the values from the proposed equations.

Specific comments:

Line 16: The authors argued in the text that nitrifier-denitrification was the main N2O producing pathway, remove heterotrophic denitrification if this is true

Line 17: Method/procedure to estimate nitrite oxidation rate was not discussed/mentioned. Not clear what you mean here. Consider revising the sentence.

Line 18: State the sub-optimal condition.

Line 19: You mentioned that heterotrophic denitrification could be present, if so, how do you know the N2O was produced from NH4+ not from other substrates given that the inflow also comprised of NO3-?

Line 28: Is there a more recent estimate for N2O emission? WMO?

Line 80: Biofilm reactor is only introduced here and no other explanation on its importance. Perhaps a sentence or two should be included to emphasize on the importance

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of these reactors (e.g. are these reactors commonly used in waste water treatment plant and how the efficiency of the reactors affect N₂O emissions, why only nitrifying reactor is considered).

Line 94: What do you mean by down-flow counter-current mode? More explanation is required especially for non-expert readers.

Line 98: Is the feeding solution described here the same as your inflow solution? If yes, why the inflow solution comprised of other DIN species not only NH₄⁺ as described. As written, the biofilm is only fed with NH₄⁺ not NO₃⁻ and NO₂⁻ so where did these species originate from?

Line 102: 24 or 26? There was a total of 26 experimental conditions listed in Table S1

Line 104: I suggest the authors consider removing the first two conditions for the O₂ test because the different NH₄⁺ concentrations could be compromising the effect of dissolved O₂ on the N₂O production. Remove from graphs as well if these data points were included in the graphs.

Line 108: What is the optimal DO level and how was this determined?

Line 110: Check the numbers and cross check with Table S1. Some of the values are different

Line 129: What were the protocols and standards for determination of d15N, d18O and d15N-SP of gaseous N₂O? At present, this part of the method is missing.

Line 140: check the units between AOR and N₂O-ER. The reported units are not consistent

Line 140: the authors did not seem to discuss on nitrification efficiency throughout the manuscript, please remove this from the method if this is not needed to avoid confusion. Instead consider including the calculation for nitrite oxidation rates as this was briefly mentioned in the abstract and at some stage of the manuscript.

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Line 181: Why was the text being placed in supplementary section? These sentences should be moved to the main section.

Line 183: What is the suboptimal condition? Please define and explain how this condition was obtained and on what basis this condition was considered suboptimal.

Line 183 – 186: Not quite sure what you meant here. Please rephrase.

Line 191: The $\delta^{15}\text{N}$ values of the outflowing NH_4^+ and NO_x were estimated from equation? Were they not measured using the same method as the $\delta^{15}\text{N}$ of the inflowing NH_4^+ and NO_x ? If you did measure the outflowing $\delta^{15}\text{N}$ of NH_4^+ and NO_x how did that compare to the ones estimated using the equations? Where did you get the ε value from and why only 0 and 1 were used for f , given f should represent the fraction of NH_4^+ or NO_x remained in the reactor.

Line 195: I do not see the importance of discussing the net isotope effect of the overall ammonium oxidation here given the main focus of the discussion point here is the importance of hydroxylamine oxidation versus nitrite reduction. Furthermore, I doubt the validity of the assumption made by the authors in estimating the overall net isotope effect of ammonium oxidation to nitrous oxide. The net isotope effect relies heavily on the initial $\delta^{15}\text{N}$ and the availability of the substrate and do not necessarily associate with the total ε from different part of the processes. Even if the $\delta^{15}\text{N}$ of the substrate is the same, different bacteria culture or organisms tend to generate different fractionation effects. Furthermore, the values cited by the authors especially for the net isotope effects related to hydroxylamine oxidation to N_2O were not found in the cited references! Please recheck. It is perhaps more interesting to look at the separate effect of the two processes (i.e. ammonium oxidation to nitrite and hydroxylamine oxidation to N_2O) on the overall processing of NH_4^+ in the reactor. You should have enough data to estimate the net isotope effect of ammonium oxidation to nitrite and discuss how that compares to the literature value?

Line 201: Which method are you referring to? And on what basis that the authors think

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that d15N-N2O values here refer specifically to hydroxylamine oxidation? Were these d15-N2O values obtained from all the experiments? Or from a specific experiment (ammonium concentration or temperature or DO)?

Line 203: Did the authors observe the net isotope effects or the values were depicted from previous studies? Be more specific. If they did observe the net isotope effect in this study then why not just use these values in the rest of the discussion?

Line 206: And again, where did you get these values from? Are the values in brackets represent the averages of the d15N of respective analytes? Please specify

Line 207-209: I don't think I quite get what you mean here. Explanation is needed on how ammonium oxidation influences the denitrifiers and why is that relevant to the d15N-N2O derived from nitrite being similar for both bacteria? What if NO₃ not NO₂ was used as a substrate for denitrifier?

Line 229: Not entirely true because some of the rates were high.

Line 230: To me, there was no increase in N₂O emission for the same NH₄ concentration for different DO condition. I think the authors should carefully consider the trend by comparing the data points for the same NH₄ concentration. Can you please include the slope values of the trend line so that it is easier to compare if there was an increase in the tested parameters.

Line 234: Why only 8 SP values are presented? But you have 13 data points for N₂O concentration. Justification is required.

Line 237: Describe the processes rather than just the bacteria – if you mean nitrifier-denitrification and heterotrophic denitrification, mention this at the start

Line 239: But there was high SP value at O₂ higher than 16.8%. If what you said were correct that nitrous oxide reduction was driving the high SP value at low O₂ (4%) then what is driving the high SP at 21% O₂?

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Line 249: What were the independence samples? How were they defined/sampled? How do you know that the N2O at different O2 condition were not from the same origin? Can't they be a mixture of N2O from different processes? And not quite sure what you meant by '...then partially reduced'.

Line 261: Concentration of NOx increased for which experiment (temperature or NH4 concentration)?

Line 261: Why are these values different from the ones presented in Fig. 4c and 4d? Were you referring to the same thing?

Line 275: I agree that there was a stronger effect of temperature on N2O emission compared to NH4+ oxidation rate but for the effect of NH4 concentration, this could only be controlled by the very high NH4+ concentration – indicating a possible effect of NH4+ concentration on these points.

Line 276: Don't think the authors can draw conclusion on the N2O processes based solely on the NH4+ oxidation and N2O emission rates. Suggest discussing these processes after the discussion on the d15N values.

Line 280: You should also calculate nitrite oxidation rate the same way you did for ammonium to support the contention you made here.

Line 294: Optimal here means? Why was 21% O2 considered optimal? Justifications/explanations are also required as to why 23.8 mg/L was chosen for the temperature experiment?

Line 329: I assume you mean heterotrophic denitrification here? Why? You have just discussed that denitrifiers were sensitive to O2 and can be excluded as an important process contributing N2O at the high O2 conditions.

Figure 1: Very nice figure but references used for the ranges of the d15N d18O and SP should be included in the caption of the figure

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Figure 4: Include slope, p and r² values for each of the plot. Not clear on how the errors on the NH₄⁺ concentration plot were derived. And why only for NH₄⁺ conc, did you see any variations for the temperature experiment as well? You have not mentioned elsewhere that replicates samples were collected. If this was how the error bars were derived state that in the method section.

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