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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 #1

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General comments

Humbert et al. report emissions and production/consumption processes of N2O in a nitrifying biofilm reactor which simulates a part of a biological waste water treatment system. Although several similar studies have been published, knowledge of key factors that should be controlled to mitigate N2O emission is still insufficient because there are various type of biological waste water treatment and because processes related to N2O depend on many factors.

Major findings of this paper are that N2O is mainly produced by nitrifier-denitrfication in a nitrifying biofilm reactor and that temperature control is more important than oxygen

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concentration or ammonia concentration. They may be worth publishing in Biogeosciences if the authors add implications of their research not only for a specific waste water treatment system but for other systems including natural water or soils.

Although the purpose and conclusion are clearly described, I found several flaws in the manuscript. First, a couple of related studies (Tumendelger et al., 2014; 2016) are not cited and compared with the findings of this study. Second, presentation of results (tables and figures in main text and supplement) is not well organized and is confusing. For example, Table S1 seems to show all the experimental conditions but corresponding results are not shown and figures seem to show only a part of the results. Third, a part of interpretation of isotopic data is not appropriate or based on assumptions that are not clearly explained. Fourth, several sentences are not readable or clear.

In summary, I consider this paper may be acceptable after careful revisions with respect to concerns above and below.

Specific comments

L39 Add Tumendelger et al., 2014 and 2016.

L58–60 I think this statement is vague because equilibrium process is involved in biotic process (e.g., O-exchange between nitrate and water during nitrification and denitrification) and kinetic fractionation also occur in abiotic processes (e.g., diffusion in air or water).

L73 This statement is misleading because many of previous studies cited elsewhere in this manuscript did use combination of isotope data of N2O to analyze production/consumption processes.

L93–94 I think a schematic of the reactor helps readers to understand the experiment and how monitoring of environmental parameters and sample collection were conducted. What is “continuous down-flow counter current mode”?

L102 Here it can be read that the authors made 24 experiments, but in Table S1 total 26 conditions are shown. But in Table S1, the first line in the list of oxygenation tests and the second one for NH4+ concentration tests seem the exactly same condition, and the same for the second of oxygenation tests and the last of NH4 concentration tests. Are these pairs from actually a single experiment? Please explain in footnotes. Also, there is no “n.a.” entry in Table S1 in spite of footnote.

L106 If the numbers in Table S1 are correct, NH4 concentration should be “20.3” –21.1 mg N/L and temperature should be “19.0 to 19.8” C. Please check the data carefully.

L108 How did the authors determine that the optimal oxygen concentration is 21%? Table 1. This table is just showing reduced information presented in Table S1 and is not helpful. I suggest to use Table S1 here.

L131 This sentence seems to explain the calibration of dN and dO for ammonium, nitrite, and nitrate. In the case of N2O, dO cannot be calibrated using nitrate standards because there is a kinetic fractionation during N-O bond rapture in nitrate reduction to N2O. SP is also not determinable using the standards listed here. Please explain more.

L140 Confirm the unit. If concentration is multiplied by flow rate, dimension should be mass per time (e.g., mg N /min).

L147 Consider more appropriate title of the section, for example, “Estimation of the range of nitrogen isotope ratio in N2O produced by each biological process”.

L157–171 It is strange that these sentences describe how to estimate dN values of output NH4+ and NOx-, because in Fig. S2 concentrations and isotope ratios of N-compounds in inflow and outflow are shown as “measured” parameters. If these are really measured, I think it is worth calculating isotope enrichment factors (epsilons) in the studied system and comparing with previous studies.

L171 It seems that produced NOx- (=NOxout – NOxin) is assumed to be derived from reacted ammonium. Then “f” in eq (4) should be “1-f”.

L172–176ãĂĂThese statements are not correct in a strict sense and are misleading. In a closed system, approximation of isotope effect using difference in delta values is valid only when isotope ratio in substrate can be treated as constant as described in Denk et al. (2017). In an open system, it is true that isotope effect can be expressed as the difference in delta values between product and residual substrate that exit from the system (Fry, 2006). But d15Ns in eq (5) can be read as isotope ratio in substrate before reaction (input) and hence is not applicable to open system. Equation (5) can be derived from an equation similar to eq (2) when f=1, but the authors do not state the assumption that f=1 is appropriate in this study. In fact, the value of f decreases as low as 0.2 (Fig. S1).

L182–183 Is “the ratio between ammonium oxidation rate and influent ammonium concentrations” different from “nitrification efficiency” (=oxidation rate/NH4 feeding rate) defined in L140–141? It is odd that ratio of parameters with different dimension is additionally introduced.

L184–186 I cannot understand what this sentence means. Please rephrase.

L191–193 Here, the possible range of dN of outflowing NH4+ and NOx- is shown, but the dN for each timing is plotted as a single value in Fig. S2. How were these individual values calculated with what assumption?

L195–200 In multi-step reaction (in this case, two step reaction of NH3->NH2OH->N2O is considered), overall isotope effect does not necessarily equal to sum of the isotope effect of each step, but depends on substrate availability and ratio of backward to forward flows in the middle step of the reaction (Rees 1973). Please add basis of the authors’s assumption.

L203–204 I believe this is incorrect. The authors did not “observe” the net nitrogen isotope effect for each pathway, but they used literature values.

L224–227 I would like to see the mass balance of N. Judging from Table S2 (which shows results only from NH4+ concentration tests though), increase in NOx- is always lower than decrease in NH4+. Is apparent nitrogen loss explainable by gaseous emission of N2O and NO, or was there significant nitrogen assimilation by the biofilm?

L228–231 I cannot agree that ammonium oxidation rates were “low and stable” for 0–10.5% O2 because two high values were observed at 5% (Fig. 3a). It is not clear whether the authors excluded the two data (because of large error bars?) or not. I understand that these rates are calculated from influent and effluent NH4+ concentrations measured over time as presented in Fig. S2 (again, this figure only shows results from NH4+ concentration tests though), but cannot understand why the error bar (“standard deviation”) for the two data is significantly large. Please explain it as well as detailed procedure for calculating “average and standard deviation” (e.g., how many measured data were used for averaging?).

L234–235 I see 8 data points in Fig. 3d (also 3e and 3f), but Table S1 indicates total 13 data were obtained for oxygenation tests. Does this mean isotopic measurements were not conducted for all samples?

L235–236 I cannot see “similar marked change” in d15N at 16.8% O2 and 21% O2. The two data points for each O2 condition depart each other, and when average is taken, there would be no significant difference.

L238–239 Relatively higher SP value was observed not only at 4.2% O2 but also at 20% O2. But do the authors consider N2O reduction occurs at 4.2% O2 just because SP is larger than the range estimated for N2O produced NO2- reduction? It seems to me that the two high SP data is not significantly different considering the large error bars for 20% O2, and that the upper end of estimated range might be underestimate (see Fig. 6 in Denk et al.).

L244–245 Alternative explanations can be made for the decrease in N2O emission. For example, change in branching ratio between NO2- , N2O, and NO production during

NH2OH oxidation might reduce N2O emission. Are there any other evidences for N2O reduction? I agree that N2O reduction might occur when oxygen concentration is really 0%, but as shown in Fig. S1, measured DO is ca. 1.5 mg/L and this enabled NH4 oxidation. It is unlikely that NH4 oxidation and N2O reduction occur at the same time unless there are specific anoxic sites in the system.

L249 What does “independence of samples” mean?

L249–251 I cannot follow these sentences. Please rephrase and describe why the different trends of d15N and d18O can be explained with reaction rates in more detail.

L259–261 Although NH4+ oxidation rate has linear relation to NH4+ concentration (Fig. 4b), the remaining NH4+ fraction does not (Fig. S1f). It seems to increase nonlinearly. Please discuss why this happened. In addition, I cannot see that remaining fraction of NH4 or NH4+ oxidation rate is “negatively correlated to temperature” in Fig. S1e or Fig. 4a. It seems almost constant irrespective of temperature. Is stated correlation really significant? Please show p values.

L264–267 Although I think there is no significant relationship between NH4+ oxidation rate or remaining fraction and temperature, the authors argue that NH4+ remaining fraction is negatively correlated with temperature whereas NH4+ oxidation rate is positively correlated with temperature. Please explain why this apparently contradict trend was observed.

L267–268 Temperature effect (if any) might be explained with enzymatic activity, but I think NH4+ concentration effect can be explained with kinetics of enzymatic reaction like Michaelis-Menten kinetics.

L274 Based on which parameter do the authors find “stronger” effect of temperature and NH4+ concentration on the N2O emission rate than on NH4+ oxidation”? For example, slopes in Fig. 4b and 4d look similar.

L278–281 Please explain in detail why the authors consider nitrite oxidation (to nitrate)

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is less important than nitrite reduction (to N2O) in this case.

L284–285 In Tumendelger et al. (2014, 2016), larger SP values were reported under aerobic condition.

L285–286 Ambiguous sentence. Do the authors intend to argue that SP value increases with temperature (13.5C<T<19.5C) and that it also increases with NH4+ concentration when T is set around 19C? Please rephrase. I cannot agree with the latter statement because SP value obtained at 42.8 mg N/L is lower than SP at 28.6 (Fig. 5a).

Figure 5 caption. As I pointed out at L228–231 above, it is not clear how the authors made data reduction based on primary data. How “average and standard deviation” were calculated? How did the authors ensure “the steady-state conditions”?

L315–317 Does “stable NO2-” mean that NO2- concentration was constant over time or that it did not depend on NH4+ concentration? If the authors intend to mean the latter, I cannot agree with them because three data points at 20% O2 in Fig. S1g show a large variation.

L323–324 Sect. 3.2 → Sect. 3.3? There, the authors wrote temperature range as 13.5–19.8ïĆřC, and did not describe “exponentially increase”. It seems to me that SP increases with temperature linearly.

Technical corrections

L44 a large “extent”

L67 enriched in “15N at” central position

L81 Add question mark at the end of the sentence.

L103–105 Awkward sentence. Consider other expression than “consisted of”.

L230 and elsewhere. Insert “×” between significant (i.e. 0.35) and exponent (i.e. 10-3)

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Figure 3 legend. Open circle represents NH4 concentration of 25.1, not 25.3 if Table S1 is correct.

L238–239 Rephrase the subject part ("The 15N-SP . . . levels") of this sentence. A higher amount of N2O "was" reduced to N2.

L273 correlation between . . . and the ammonium oxidation rates (delete "to")

L333 a larger "extent"

References

Rees, C. E. (1973), A steady-state model for sulphur isotope fractionation in bacterial reduction processes, Geochim. Cosmochim. Acta, 37, 1141–1162.

Tumendelger, A., S. Toyoda, and N. Yoshida (2014), Isotopic analysis of N2O produced in a conventional wastewater treatment system operated under different aeration conditions, Rapid Communications in Mass Spectrometry, 28(17), 1883-1892, doi:10.1002/rcm.6973.

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