

We would like to thank the referee for the effort and time she/he put in to review our manuscript. We are grateful for her/his valuable comments and will make every attempt to carefully address these comments that will improve the quality of the revised manuscript. Hereafter, the points raised by the referee are written in black, whereas our responses are shown in blue. Citation of our corrections that will take place in the revised version of the manuscript are highlighted in grey.

The authors report the use of stable isotopes of N₂O (bulk and site specific d¹⁵N), 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 emissions; 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;

The isotopic measurements were not conducted for all samples. However, the concentration data were all presented to capture the whole dynamic constrained by environmental conditions tested. We added sentences in the method section 2.2 and in the caption of Fig. 6 for improved clarity.

(2) some of the interpretations on the trends are misleading and were not supported with statistical analysis;

Please see our specific answers below. When lacking, we added statistics to support our statement in the results section.

(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;

We carefully defined the processes rates in the Methods section in consistence with those used further in the manuscript.

(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;

We carefully checked our manuscript and used appropriate terms throughout the manuscript to better specify where data came from.

(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.

The isotope ratios of N compounds in inflow and outflow were measured only for the three experiments shown in Fig.S2. Although these experiments allow calculating isotope enrichment factors (epsilons), these epsilon values unlikely describe the whole range of operating conditions tested in our study. Therefore, we think that using isotope ratio estimates from the literature is more appropriate

than values based on a limited number of experiments. We added sentences throughout the manuscript (in 2.5 and 3.1 sections) to better explain this and hence to improve its clarity.

The isotope effect of reaction step can be determined from the isotope composition of substrates or products. Although being performed on a few tests here, the obtained value can only be applied to a limited number of environmental conditions. The use of estimates from the literature seems therefore suitable.

The $\delta^{15}\text{N}$ of inflow ammonium, nitrite and nitrate were $-3 \pm 0.1 \text{ ‰}$ ($n = 3$), $-15 \pm 0.1 \text{ ‰}$ ($n = 2$), $6.9 \pm 0.3 \text{ ‰}$ ($n = 3$) respectively during ammonium concentration experiments (Fig. S2). The $\delta^{15}\text{N}$ of outflowing NH_4^+ and NO_x^- were estimated from Eqs. (1-4) with $f = 0$ or 1 , $\epsilon_{\text{ao}} = -30 \text{ ‰}$, the highest $[\text{NH}_4^+]_{\text{in}}$ (62.1 mg N L^{-1}) and the lowest $[\text{NO}_x^-]_{\text{in}}$ (1.4 mg N L^{-1}). They ranged from -3 to 27 ‰ and from -32 to 7 ‰ , respectively, which encompasses a few isotope compositions measured in the outflow during ammonium concentration tests (Fig. S2 and Table S1).

Specific comments:

Line 16: The authors argued in the text that nitrifier-denitrification was the main N_2O producing pathway, remove heterotrophic denitrification if this is true

The ^{15}N -SP does not allow differentiating between nitrifier-denitrification and heterotrophic denitrification contribution, both involving the nitrite reduction pathway that produces N_2O . We checked our manuscript, nitrifier-denitrification was not indicated as the main N_2O producing pathway. We are not sure having correctly understood the remark of the reviewer.

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.

We agree that nitrite oxidation rate was not calculated. However, we inferred lower nitrite oxidation than ammonium oxidation rate from lower NO_x^- than oxidized ammonium budget. We clarified this throughout the manuscript. In addition to this, we changed sub-optimal oxygen levels to oxygen-depleted atmosphere.

Difference between oxidative and reductive rates of nitrite consumption was deduced from NO_2^- concentration and N_2O emission. Hence, nitrite oxidation rates seem to decrease as compared to ammonium oxidation rates at temperatures above 20 °C and under oxygen-depleted atmosphere, increasing N_2O production by the nitrite reduction pathway.

Line 19: You mentioned that heterotrophic denitrification could be present, if so, how do you know the N_2O was produced from NH_4^+ not from other substrates given that the inflow also comprised of NO_3^- ?

We hypothesized here that the NO_2^- produced from the oxidized NH_4^+ can be further reduced to N_2O either by nitrifier-denitrification or heterotrophic denitrification. However, the ^{15}N -SP of N_2O does not allow differentiating between the contributions from both processes. In addition to this, the range of $\delta^{15}\text{N}$ - N_2O estimates takes into account the isotope composition of inflow NO_2^- and NO_3^- .

Line 28: Is there a more recent estimate for N_2O emission? WMO?

We did not find more recent similar data in the WMO database (i.e. fraction of N_2O emitted from wastewater resource recovery facilities), and we do not think that it has changed by order of magnitude during the last 5 years. For this reason, the initial statement was not modified.

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 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).

We agree with the reviewer, we added sentences to better justify why we worked on a biofilm.

In order to achieve this, the nitrifying biomass of a submerged fixed-bed biofilm reactor was investigated. Among the wastewater treatment systems, the biofilm systems are adapted to large urban areas owing to their compactness, flexibility and reliability. An increase of their development can be thought in response to the additional 2.5 billion humans expected in urban areas by 2050 (United Nations Population Division, 2018). However, the biofilm systems have received much less attention than the suspended biomass systems and the relations between the N₂O producing/consuming pathways and controls remain largely unknown (Sabba et al., 2018; Todt and Dörsch, 2016).

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

It means that feeding solution flows through the reactor from the top to the bottom, while aeration being injected from the bottom of the reactor. We added a schematic of the reactor as new Figure S1 in the supplementary material. This should improve the clarity of the system description.

A lab-scale reactor with a working volume of 9.9 L (colonized Biostyrene[®] beads and interstitial volume) and a headspace of 1.4 L was operated in continuous down-flow counter-current mode for seven weeks (Fig. S1).

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?

As we used tap water to prepare the feeding solution, containing NO₂⁻ and NO₃⁻. We added in brackets the average NO₂⁻ and NO₃⁻ concentration in the tap water.

The feeding solution consisted of ammonium chloride (NH₄Cl) as substrate, monobasic potassium phosphate (KH₂PO₄) as phosphorus source for bacterial growth, and sodium hydrogen carbonate (NaHCO₃) as pH buffer and inorganic carbon source in 100 or 150 L of tap water (comprising in average 0.2 ± 0.4, 2.4 ± 1.1, and 2.5 ± 1.3 mg N L⁻¹ of NO₂⁻, NO₃⁻ and NO_x⁻, respectively).

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

Twenty-four experiments were performed. Two of them tested effects of both oxygenation and ammonium concentration. We added explanations in the text and in the footnote of Table S1.

The influence of environmental conditions on the ammonium oxidation rates and the N₂O emissions from various combinations of oxygenation levels, temperatures and ammonium concentrations were tested in twenty-four experiments (Tables 1). Note that two of them were used twice; as oxygenation and concentration tests.

Note that two experiments tested both oxygenation and ammonium concentration.

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.

The isotopic measurements were not conducted for all samples (please see above). However, concentration data were all presented to capture the whole dynamic constrained by oxygen, temperature and ammonia conditions. Noticeably, if we remove the first two conditions of the oxygenation tests as suggested by the referee, we will not have any isotope data at 21% O₂. Therefore, we kept these first two conditions. However, we agree with the referee that the additional effect of ammonium can mitigate the effect of oxygenation alone that is intended to be shown here. Therefore, the average isotope ratios at 21 % O₂ should be compared to the data solely measured for 23.8 NH₄⁺-N L⁻¹ at 16.8 % O₂. We specified it more clearly in the manuscript where these results are described (Lines 235-236 of original manuscript).

A similar marked change in nitrogen and oxygen isotope ratios of N₂O (decrease and increase, respectively) was observed when oxygenation increased from 16.8 to 21 % O₂ (Figs. 3e and f). Note that to observe the latter variations the effect of ammonium concentration was not included. One way to do so is to compare the isotope composition average at 21% O₂ with the isotope composition measured for 23.8 NH₄⁺-N L⁻¹ at 16.8 % O₂.

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

We reworded this sentence and added an explanation about the meaning of optimal oxygenation level for the next of the manuscript.

Atmospheric oxygenation level (i.e. 21 % O₂ in the gas mixture) was imposed for both tests (Figs. S1b and c). This gas mixture using compressed air with 21 % O₂ was considered hereafter as optimal as compared to the oxygen-depleted atmosphere used during the oxygenation tests. Noticeably, the atmospheric oxygenation level is the condition that represents the most optimal conditions of oxygenation applied in nitrification BAF of domestic WRRF.

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

We are grateful to the referee for raising this mistake. We rechecked our data and changed therefore the text and table in consistency with Table S1. Furthermore, Table S1 was used instead of Table 1 in the main text.

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.

We clarified and reorganized this paragraph as follow:

These methods consist in the conversion of the substrate (ammonium or nitrite or nitrate) into dissolved N₂O. The δ¹⁵N and δ¹⁸O for ammonium, nitrite, and nitrate were hence determined from a calibration curve created with a combination of nitrate or ammonium standards that have undergone the same chemical conversion as the samples (USGS-32, δ¹⁵N-NO₃⁻ = 180 ‰, δ¹⁸O-NO₃⁻ = 25.7 ‰; USGS-34, δ¹⁵N-NO₃⁻ = -1.8 ‰, δ¹⁸O-NO₃⁻ = -27.9 ‰ and USGS-35 δ¹⁵N-NO₃⁻ = 2.7 ‰, δ¹⁸O-NO₃⁻ = 57.5 ‰; or IAEA-N1, δ¹⁵N-NH₄⁺ = 0.4 ‰, IAEA-305A, δ¹⁵N-NH₄⁺ = 39.8 ‰, USGS-25, δ¹⁵N-NH₄⁺ = -30.4 ‰). The quality of calibration was controlled with additional international standards (IAEA-NO-3, δ¹⁵N-NO₃⁻ = 4.7 ‰, δ¹⁸O-NO₃⁻ = 25.6 ‰; or IAEA-N2, δ¹⁵N-NH₄⁺ = 20.3 ‰). Basically, an analytical sequence was comprised of triplicate standards for calibration and quality controls and duplicate samples. The average of the analytical replicates was then used for calibration, quality control and as result.

Since no international standards were available for N₂O isotopes, these were determined the same day as nitrate or ammonium standard analysis insuring correct functioning of the method and analysis. In addition to this, the internal N₂O standards were previously calibrated by exchange with the

laboratory of Naohiro Yoshida and Sakae Toyoda at the Tokyo Institute of Technology. All isotope measurements were determined using an isotope ratio mass spectrometer (IRMS, DeltaVplus; Thermo Scientific) in continuous-flow with a purge and trap system coupled with a Finnigan GasBench II system (Thermo Scientific). The precision was 0.8 ‰, 1.5 ‰ and 2.5 ‰ for $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $^{15}\text{N}\text{-SP}$, respectively.

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

The unit was corrected.

The ammonium oxidation rate (AOR) was estimated in each experiment for time ≥ 1 HRT from the difference between influent and effluent NH_4^+ concentrations multiplied by the liquid flow rate ($\text{kg NH}_4^+\text{-N d}^{-1}$).

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.

We did not calculate the nitrite oxidation rate. As part of the NO_2^- and NO_3^- produced from the oxidized ammonium could then react to other N compounds (NO , N_2O , N_2 , NO_3^- , NO_2^-), we were not able to estimate the nitrite oxidation rate. The discussion about this rate is speculative from the remaining NO_2^- concentration in the reactor outflow. We clarified this throughout the manuscript.

Further, we kept the introduction of nitrification efficiency as we use it at the beginning of the results and discussion section. However, we reworded the sentence consistently with parameters introduced in the method section.

Over the range of tested conditions, the ratio between ammonium oxidation rate and influent ammonium load ranged from 10 to 82 %, never exceeding 40 % for suboptimal nitrifying conditions imposed during oxygenation and temperature tests.

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

As suggested by the referee, we moved these sentences to the main text.

The experiments that tested the influence of ammonium concentrations on ammonium oxidation and nitrous oxide emissions also supported the nitrifying activity of the reactor. During these experiments, decreases in $[\text{NH}_4^+]$, increases in $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ were observed, while pH remaining below 8 prevented any relevant loss of ammonium by volatilization. For example, $[\text{NH}_4^+]$ decreased from 6.2 to 1.1, from 28.6 to 17 and from 62.1 to 49.1 mg N L⁻¹ by flowing through the nitrifying biomass. At the same time, $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ increased from 0 to 0.2-0.3 mg N L⁻¹ and from 1.4-1.8 to 5-10 mg N L⁻¹, respectively.

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.

We specified what we mean by suboptimal conditions between brackets.

Over the range of tested conditions, the ratio between ammonium oxidation rate and influent ammonium load ranged from 10 to 82 %, never exceeding 40 % for suboptimal nitrifying conditions imposed during oxygenation and temperature tests (i.e. oxygenation levels < 21 % O_2 , and temperatures < 20 °C).

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

We rephrased the sentence.

The ammonium concentration, oxygenation level and temperature affected the ammonium oxidation rates, as well N₂O emission rates and factors.

Line 191: The δ¹⁵N values of the outflowing NH₄⁺ and NO_x were estimated from equation? Were they not measured using the same method as the δ¹⁵N of the inflowing NH₄ and NO_x? If you did measure the outflowing δ¹⁵N of NH₄ and NO_x how did that compare to the ones estimated using the equations? Where did you get the "value from and why only 0 and 1 were used for f, given f should represent the fraction of NH₄⁺ or NO_x remained in the reactor.

The isotope ratios of N compounds in inflow and outflow were measured only for the three experiments shown in Fig.S2. Although these experiments allow calculating isotope enrichment factors (epsilons), these epsilons unlikely describe the whole range of operating conditions tested in our study. Therefore, we think that using isotope ratio estimates from the literature is more appropriate than values based on a limited number of experiments. We added text throughout the manuscript (in 2.5 and 3.1 sections) to better explain this and hence to improve its clarity. In addition, detailed data related to Figure S2 were added as new Table S1.

The isotope effect of a reaction step can be determined from the isotope composition of substrates or products. Although being performed on a few tests here, the obtained value can only be applied to a limited number of environmental conditions. The use of estimates from the literature seems therefore suitable.

The δ¹⁵N of inflow ammonium, nitrite and nitrate were $-3 \pm 0.1 \text{ ‰}$ (n = 3), $-15 \pm 0.1 \text{ ‰}$ (n = 2), $6.9 \pm 0.3 \text{ ‰}$ (n = 3) respectively during ammonium concentration experiments (Fig. S2). The δ¹⁵N of outflowing NH₄⁺ and NO_x⁻ were estimated from Eqs. (1-4) with f = 0 or 1, ε_{ao} = -30 ‰, the highest [NH₄⁺]_{in} (62.1 mg N L⁻¹) and the lowest [NO_x⁻]_{in} (1.4 mg N L⁻¹). They ranged from -3 to 27 ‰ and from -32 to 7 ‰, respectively, which encompasses a few isotope compositions measured in the outflow during ammonium concentration tests (Fig. S2 and Table S1).

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 δ¹⁵N and the availability of the substrate and do not necessarily associate with the total " from different part of the processes. Even if the δ¹⁵N 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 N₂O 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 N₂O) on the overall processing of NH₄⁺ 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?

Please see our previous answer regarding the net isotope effect estimate of ammonium oxidation to nitrite. Further, we did not added hydroxylamine directly into the feeding solution. Ammonium was added into the feeding solution. However, the production of N₂O from hydroxylamine oxidation during the oxidation of ammonium cannot be excluded. Therefore, we estimated the range of δ¹⁵N-N₂O

produced from hydroxylamine oxidation with ammonium as substrate. Thanks to existing literature, our method considered the broad range of values taken by both the $\delta^{15}\text{N}$ of hydroxylamine produced during ammonium oxidation and the fractionation effects related to both ammonium oxidation and hydroxylamine oxidation to N_2O . Further, we rechecked the values used from cited references. Correction involved minor changes (<1 ‰).

The net isotope effect of N_2O production by ammonium oxidation via hydroxylamine can be estimated by combining the isotope effects of ammonium oxidation and hydroxylamine oxidation to N_2O . The net isotope effect associated to the ammonium oxidation to nitrite ranges from -38.2 to -14.2 ‰ (Casciotti et al., 2003) and can approximate the nitrogen isotope ratio of hydroxylamine transitory produced. The isotope effect related to hydroxylamine oxidation to N_2O ranging from -26 to 5.7 ‰ from data in Sutka et al. (2003, 2006), the net isotope effect of N_2O production by ammonium oxidation via hydroxylamine can range from -64.2 ‰ (-26 + (-38.2)) to -8.5 ‰ (5.7 + (-14.2)). Considering the range of nitrogen isotope ratio of initial ammonium, this method provided a broad range of $\delta^{15}\text{N}$ values, from -68 to 19 ‰, for N_2O produced by hydroxylamine oxidation that encompassed the values proposed by others (-46.5 and -32.9 ‰; Sutka et al., 2006; Yamazaki et al., 2014).

Line 201: Which method are you referring to? And on what basis that the authors think that $\delta^{15}\text{N}$ - N_2O values here refer specifically to hydroxylamine oxidation? Were these $\delta^{15}\text{N}$ - N_2O values obtained from all the experiments? Or from a specific experiment (ammonium concentration or temperature or DO)?

In this paragraph, a general way to estimate ranges of nitrogen isotope ratio in N_2O is proposed. This relies on the method presented in 2.5 and the use in Eqs. (5) and (6) of fractionation estimates obtained from the literature. As stated above, we think that using isotope ratio estimates from the literature is more appropriate than measured values based on a limited number of experiments. Published isotope effects were then used. We assume that the range of published isotope effects enables taking into account a high diversity of environmental conditions and bacterial community involved in these isotope effects.

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?

We rephrased the sentence.

A higher net nitrogen isotope effect for nitrite reduction than hydroxylamine oxidation pathway was estimated for N_2O production.

Line 206: And again, where did you get these values from? Are the values in brackets represent the averages of the $\delta^{15}\text{N}$ of respective analytes? Please specify

These values come from the net isotope effect found in literature and mentioned in the introduction section. 'These values are imbricated between -52.8 and -6 ‰, the range of net isotope effects related to the N_2O production through nitrite reduction performed by nitrifiers or denitrifiers (Lewicka-Szczebak et al., 2014; Sutka et al., 2008).' (Lines 63-65 of original manuscript). However, we agree that a reminder is required here.

Prior to being reduced to N_2O through the nitrite reduction pathway, NO_x^- was mainly derived from ammonium oxidation in the nitrifying system (Eqs. (1-4)); the resulting $\delta^{15}\text{N}$ - NO_x^- ranging from -32 to 7 ‰. In addition to this, the net isotope effects related to the N_2O production through nitrite reduction performed by nitrifiers or denitrifiers ranges from -52.8 to -6 ‰, the (Lewicka-Szczebak et al., 2014;

Sutka et al., 2008). Consequently, the $\delta^{15}\text{N}$ of N_2O produced by nitrite reduction ranged from -85 ‰ (-53 + (-32)) to 1 ‰ (-6 + 7), according to Eq. (6).

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 $\delta^{15}\text{N}$ - N_2O derived from nitrite being similar for both bacteria? What if NO_3^- not NO_2^- was used as a substrate for denitrifier?

We mean that ammonium is oxidized to NO_2^- and NO_3^- in the nitrifying reactor. Both NO_2^- and NO_3^- can be reduced through either nitrifier-denitrification or heterotrophic denitrification or a combination of both reductive processes. However, the isotope composition of the substrate (NO_2^- - NO_3^-) is imposed by the prior oxidation of ammonium to $\text{NO}_2^-/\text{NO}_3^-$, whatever the reductive process at play (nitrifier-denitrification or heterotrophic denitrification).

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

We improved clarity of this sentence.

The oxygenation level had contrasting effects on ammonium oxidation rates, and N_2O emission rates and factors (Figs. 3a-c). Between an oxygenation of 0 to 10.5 % O_2 in the gas mixture, no clear trend in ammonium oxidation rates was observed although being rather low ($1.1 \pm 0.5 \text{ mg NH}_4^+\text{-N min}^{-1}$). In the same oxygenation levels interval, the N_2O emission rates and factors increased from $0.35 \cdot 10^{-3}$ to $1.6 \cdot 10^{-3} \text{ mg N min}^{-1}$ and from 0.05 to 0.16 %, respectively.

Line 230: To me, there was no increase in N_2O emission for the same NH_4 concentration for different DO condition. I think the authors should carefully consider the trend by comparing the data points for the same NH_4 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.

We agree that this section needs precisions. Note that we applied the same y-axis scale to be able to compare with each other the effects of the tested conditions had on the N_2O emissions (Figs. 3b, 4c and 4d). Consequently, the variations are less visible in response to oxygenation and temperature than ammonium concentration. Further, we do not want to apply a trend line to the whole dataset that includes different inflow ammonium concentrations. In addition to this, there is a limited number of data related to each inflow ammonium concentration in the interval 0-10.5 % O_2 (1 to 3 data point). Therefore, the application of a trend line to this limited number of data presents a limited interest. We described the results with more precision.

The oxygenation level had contrasting effects on ammonium oxidation rates, and N_2O emission rates and factors (Figs. 3a-c). Between an oxygenation of 0 to 10.5 % O_2 in the gas mixture, no clear trend in ammonium oxidation rates was observed although being rather low ($1.1 \pm 0.5 \text{ mg NH}_4^+\text{-N min}^{-1}$). In the same oxygenation levels interval, the N_2O emission rate increased for two of three inflow $[\text{NH}_4^+]$ tested. It increased from $0.35 \cdot 10^{-3}$ to $0.73 \cdot 10^{-3} \text{ mg N min}^{-1}$ between 0 and 10.5 % O_2 at $25.3 \text{ mg NH}_4^+\text{-N L}^{-1}$, and from $1.34 \cdot 10^{-3}$ to $1.4 \cdot 10^{-3} \text{ mg N min}^{-1}$ between 4.2 and 10.5 % O_2 at $23.8 \text{ mg NH}_4^+\text{-N L}^{-1}$; while it decreased from $2.86 \cdot 10^{-3}$ to $2.04 \cdot 10^{-3} \text{ mg N min}^{-1}$ between 4.2 and 10.5 % O_2 at $37.3 \text{ mg NH}_4^+\text{-N L}^{-1}$. Finally, the N_2O emission factor globally increased from 0.05 to 0.16 % in the 0-10.5 % O_2 interval.

Line 234: Why only 8 SP values are presented? But you have 13 data points for N_2O concentration. Justification is required.

The referee is correct. The isotopic measurements were not conducted for all samples. However, the concentration data were all presented to capture the whole dynamic constrained by environmental

conditions tested. We added sentences in the method section 2.2 and in the caption of Fig. 6 for improved clarity.

Note that gas sampling was lacking for 5 of 13 oxygenation tests.

Note that the isotopic measurements of gas samples taken at inflow ammonium concentration of 42.7 and 42.9 mg N L⁻¹ were averaged.

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

We mean nitrifier-denitrification and heterotrophic denitrification. We modified the sentence.

The ¹⁵N-SP values were close to the range of -11 to 0 ‰ reported for N₂O produced by nitrifying or denitrifying bacteria through nitrifier-denitrification and heterotrophic denitrification (Toyoda et al., 2017; Yamazaki et al., 2014).

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?

We agree that an explanation about the high ¹⁵N-SP observed at 21 % O₂ is lacking. We added a sentence.

If an increase in the hydroxylamine oxidation contribution to the N₂O emission might explain the higher ¹⁵N-SP observed at 21 % O₂ as compared to lower oxygenation levels, an additional mechanism can explain the variations observed for the experiments with oxygen-depleted atmosphere.

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'.

Independence of samples means that the samples are not temporally linked to each other. The N₂O sampled at 4.2 % O₂ is not a residual fraction of N₂O produced at 16.8 % O₂. We clarified these sentences.

The independence of samples taken during the oxygenation tests can explain this. The N₂O sampled at 4.2 % O₂ is not a residual fraction of N₂O produced at 16.8 % O₂ that would have undergone a partial reduction. The oxygenation level can alter the isotope fractionation factors through the control of reaction rates, as evidenced for the reduction of N₂O to N₂ by Vieten et al. (2007). These authors reported lower reaction rates and increased isotope fractionation factors with increasing oxygenation levels. In our case, a similar phenomenon might have influenced both oxidative and reductive processes leading to the production of N₂O and occurring before its ultimate reduction to N₂. However, knowledge, that the controls such as the oxygenation level have on the net isotope effect related to a sequence of non-exclusive oxidative and reductive processes is still lacking and require further investigations.

Line 261: Concentration of NO_x 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?

We are not referring to Fig.4c and d here, but unplotted data of cumulated concentration of NO₂⁻ and NO₃⁻. We clarified this sentence.

In the ammonium tests, the cumulated concentrations of NO_2^- and NO_3^- ($[\text{NO}_x^-]$) increased from 1.4-6.1 to 5.1-19.6 mg N L^{-1} between inflow and outflow (data not shown) and were composed by at least 74 and 91 % of NO_3^- , respectively.

Line 275: I agree that there was a stronger effect of temperature on N_2O emission compared to NH_4^+ oxidation rate but for the effect of NH_4^+ concentration, this could only be controlled by the very high NH_4^+ concentration – indicating a possible effect of NH_4^+ concentration on these points.

We agree that very high ammonium concentrations had an important effect, however, the highest NH_4^+ concentration is not the only one having an effect on the N_2O emission factor. We can see a progressive increase in N_2O emission factor with increasing NH_4^+ concentration from 6 to 58 mg N l^{-1} .

Line 276: Don't think the authors can draw conclusion on the N_2O processes based solely on the NH_4^+ oxidation and N_2O emission rates. Suggest discussing these processes after the discussion on the $\delta^{15}\text{N}$ values.

The reviewer is right, however no conclusions were drawn here. We discuss all the processes at play (hydroxylamine oxidation, nitrite reduction and N_2O reduction) and suggest from N_2O emission factor that increasing temperatures or ammonium concentrations unlikely increased N_2O reduction to N_2 . As suggested by the referee, the contributions of processes are discussed more in detail further, after presenting the isotope results (Lines 282-303).

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

As part of the NO_2^- and NO_3^- produced from the oxidized ammonium could then react to other N compounds (NO , N_2O , N_2 , NO_3^- , NO_2^-), we were not able to estimate the nitrite oxidation rate. The discussion about this rate is speculative from the remaining NO_2^- concentration in the reactor outflow. We clarified this throughout the manuscript when we discussed about the nitrite oxidation process.

Line 294: Optimal here means? Why was 21% O_2 considered optimal? Justifications/explanations are also required as to why 23.8 mg/L was chosen for the temperature experiment?

Please refer to a previous answer regarding the meaning of optimal O_2 . We clarified this in the method section.

Further, we chose for the temperature tests an inflow ammonium concentration close to the nominal load that receives the nitrifying unit ($0.7 \text{ kg N m}^{-3} \text{ d}^{-1}$). We added this precision in the method section.

Line 329: I assume you mean heterotrophic denitrification here? Why? You have just discussed that denitrifiers were sensitive to O_2 and can be excluded as an important process contributing N_2O at the high O_2 conditions.

The referee is correct, we mean heterotrophic denitrification, here. The last step of heterotrophic denitrification (i.e. N_2O reduction to N_2) is sensitive to O_2 , however we cannot exclude that nitrite reduction performed by heterotrophic denitrifiers can occur.

This explains most likely the increased contribution of the nitrite reduction pathway to N_2O emission, as more nitrite becomes available for nitrifier-denitrification and/or heterotrophic denitrification.

Figure 1: Very nice figure but references used for the ranges of the $\delta^{15}\text{N}$ $\delta^{18}\text{O}$ and SP should be included in the caption of the figure

We thank the reviewer for the suggestion and improvement of the figure, we added references related to d15N, d18O and 15N-SP ranges in the caption of the figure.

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.

The correlations and p values were mentioned in the results and method sections, respectively (3.3 and 2.4). We specified in the method section that replicates were sampled.

The feeding solutions were characterized from 1 to 5 replicates samples collected in the feeding tank. For each tested condition, the outflow was characterized within 5 days from 1 to 14 replicates samples immediately filtered through a 0.2 µm syringe filter and stored at 4 °C.

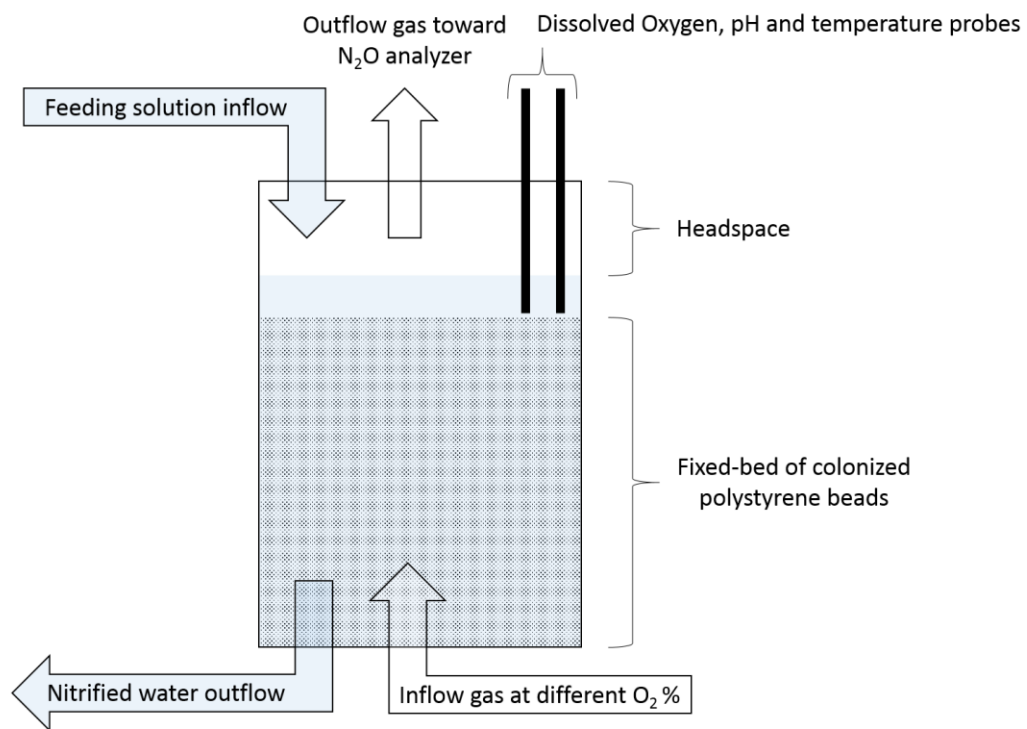
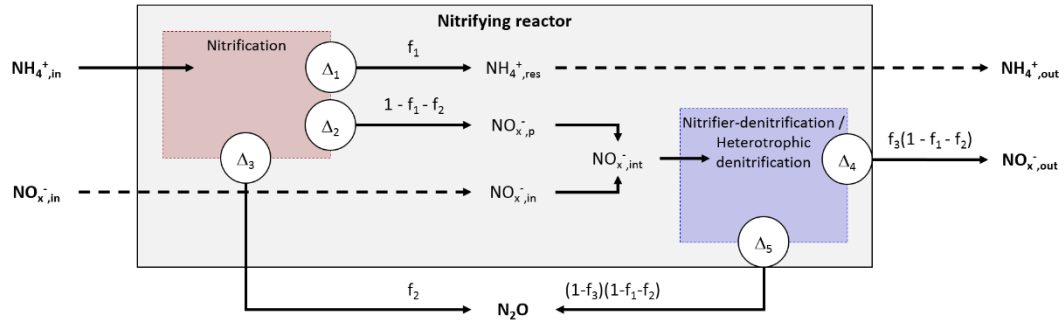


Figure S1. Schematic of the nitrifying reactor used in this study. Note that solution was down-flowing, while air was up-flowing.



Equations:

Nitrification:

$$\delta^{15}\text{N}-\text{NH}_4^+_{,\text{res}} \approx \delta^{15}\text{N}-\text{NH}_4^+_{,\text{in}} - \Delta_2(1 - f_1) + (\Delta_3 - \Delta_2)f_2$$

$$\delta^{15}\text{N}-\text{NO}_x^-_{,\text{p}} \approx \delta^{15}\text{N}-\text{NH}_4^+_{,\text{in}} + \Delta_2f_1 + (\Delta_3 - \Delta_2)f_2$$

$$\delta^{15}\text{N}-\text{NO}_x^-_{,\text{int}} \approx \frac{(\delta^{15}\text{N}-\text{NO}_x^-_{,\text{in}} \times ([\text{NO}_3^-]_{\text{in}} + [\text{NO}_2^-]_{\text{in}}) + \delta^{15}\text{N}-\text{NO}_x^-_{,\text{p}} \times (1 - f_1 - f_2) \times [\text{NH}_4^+]_{\text{in}})}{([\text{NO}_3^-]_{\text{in}} + [\text{NO}_2^-]_{\text{in}} + (1 - f_1 - f_2) \times [\text{NH}_4^+]_{\text{in}})}$$

$$\delta^{15}\text{N}-\text{N}_2\text{O} \approx \delta^{15}\text{N}-\text{NH}_4^+_{,\text{in}} - \Delta_2(1 - f_1 + f_2) + \Delta_3(1 + f_2)$$

**Nitrifier-denitrification /
Heterotrophic denitrification:**

$$\delta^{15}\text{N}-\text{NO}_x^-_{,\text{out}} \approx \delta^{15}\text{N}-\text{NO}_x^-_{,\text{int}}(1 - f_1 - f_2)^{-1} - \Delta_5(1 - f_3)$$

$$\delta^{15}\text{N}-\text{N}_2\text{O} \approx \delta^{15}\text{N}-\text{NO}_x^-_{,\text{int}}(1 - f_1 - f_2)^{-1} + f_3\Delta_5$$

Figure 2. Diagram and equations of the nitrifying reactor adapted from Fry (2006). It is considered as a sequence of two reactor boxes. (i) The nitrification of inflow ammonium ($\text{NH}_4^+_{,\text{in}}$) to a pool of nitrite and nitrate ($\text{NO}_x^-_{,\text{p}}$), residual ammonium ($\text{NH}_4^+_{,\text{res}}$), and nitrous oxide (N_2O) through the hydroxylamine oxidation pathway. (ii) The subsequent reduction of intermediate $\text{NO}_x^-_{,\text{int}}$; mixing of inflow $\text{NO}_x^-_{,\text{in}}$ and formed $\text{NO}_x^-_{,\text{p}}$ to nitrous oxide (N_2O) through the nitrite reduction pathway, and residual NO_x^- that exits the reactor ($\text{NO}_x^-_{,\text{out}}$). Note that residual substrates and formed product exit the reactor without further isotope fractionation. See text for details.