

We thank the reviewers for commenting on the manuscript, which helped to further improve the manuscript. We have responded to the reviewers' comments in blue below and applied changes accordingly in the manuscript.

The paper by Tang et al., with the title: "Variable contribution of wastewater treatment plant effluents to N₂O emission " has greatly improved in clarity and quality with the new changes. Given that there are very few studies worldwide on the impact of WWTPs on N₂O emissions in aquatic systems, as shown in the Supplementary figure 8 and in the Table 1 of the paper, I consider the article to be of great interest and that it will be widely disseminated. However, I have some suggestions:

General comments:

The title of the paper refers to the contribution of WWTPs to N₂O emissions. However, the manuscript hardly discusses water-atmosphere fluxes of N₂O, nor the contribution of WWTPs to water-atmosphere fluxes of N₂O in the Potomac River estuary in detail. So my suggestion is that just as the contribution of WWTPs to N₂O concentration is discussed, there should be more discussion of the effect of WWTPs on N₂O fluxes to the atmosphere in the system. Another option would be to change the title of the paper.

Following the suggestion from the reviewer, we added more discussion about the influence of WWTPs on N₂O emissions along with the discussion about N₂O concentrations (see detailed responses below). In addition, we changed the title to "Variable contribution of wastewater treatment plant effluents to downstream nitrous oxide concentrations and emissions".

Material and methods:

Ln 158: There is no mention of phosphate in the manuscript, so you should delete it. Were the samples taken in triplicate like the N₂O samples? Please indicate.

Since we used phosphorus in the correlation analysis of Figure 3, we decided to keep the description of phosphorus measurements. One sample was collected for phosphorus measurements. This has been updated in the manuscript.

Ln 167-168: Were obtained N₂O concentrations in the water from the measurements made in the headspace using the solubility proposed by Weiss and Price (1980)?

We were not measuring N₂O concentrations in the headspace. Instead, we measured the total amount of N₂O dissolved in the water by a mass spectrometer. The N₂O concentration was then calculated by dividing the amount of N₂O in the water by the water volume. This is detailed in lines 164-172.

Ln 168-170: The text: "Specifically, the monthly atmospheric N₂O concentrations were obtained from the nearby atmospheric station in Brentwood, Maryland (<https://gml.noaa.gov/>) (Andrews et al., 2023)." should be included in the N₂O flux calculation section, it could go on line 191 after (Weiss and Price, 1980).

Following the reviewer's suggestion, we moved this sentence after (Weiss and Price, 1980) to describe how equilibrium N₂O concentration was calculated in lines 177-179.

Ln 173: It should be included how you have calculated the saturation percentage. This parameter is discussed in the text and presented in figure 2a.

We have added the equation to calculate N₂O saturation: N₂O saturation (%) is calculated:

$$\frac{N_2O_{measured}}{N_2O_{equilibrium}} \times 100.$$

Ln 186: It should be indicated how the total N cited in lines 158-160 has been measured.

We have clarified how total N was estimated in lines 160-161: "Total N is the sum of total Kjeldahl nitrogen and nitrite plus nitrate."

Ln 191-194. The three gas transfer velocity (k) equations should be written in the same format:

- Or write the k proposed by Wanninkhof (2014) as k₆₆₀ as has been done for the other two parameterisations.:

$$k_{660} = 0.251 \times U^2$$

- Or write:

$$\text{Raymond and Cole (2001): } k = 1.91 \times e^{0.35xU} \times (Sc/600)^{-0.5}$$

$$\text{Jiang et al. (2008): } k = 0.314 \times U^2 - 0.436 \times U + 3.99 \times (Sc/600)^{-0.5}$$

$$\text{Wanninkhof (2014): } k = 0.251 \times U^2 \times (Sc/660)^{-0.5}$$

The three gas transfer velocity equations are now written in the same format based on the reviewer's suggestion.

Results and discussion

Ln 219-228. More should be commented on the water-atmosphere N₂O fluxes, practically only their range of variation in the whole system is presented. As with the N₂O concentrations, the water-atmosphere fluxes present seasonal variations (this if is commented in the abstract) and surely present spatial variations (you should comment on this). However, it is mentioned in the paper that the saturation percentage of N₂O is always higher than 100%, so the system behaves as a source of this gas, and that there is seasonal variation, but little is said about the fluxes to the atmosphere (Ln 218-220).

Following the reviewer's suggestion, we have added more discussion about N₂O fluxes/emissions: "N₂O fluxes ranged from 1 to 31.7 μmol N₂O m⁻² d⁻¹, generally decreasing from upstream to downstream (Figure 2d). N₂O fluxes showed a similar seasonal pattern to N₂O saturation: higher in summer and fall."

Ln 226-228: I do not believe that a maximum flux of N₂O to the atmosphere of 31.7 μmol m⁻² d⁻¹ in the Potomac River Estuary can be considered as an intense source of N₂O to the

atmosphere, as there are other estuaries with much more intense emissions. Perhaps it would be more accurate to put: Therefore, tributaries to the Chesapeake Bay (i.e., the Potomac River) are more intense sources of N₂O to the atmosphere than the Bay.

We have modified the sentence as the reviewer suggested: “Therefore, the tributaries (i.e., Potomac River) are more intense sources of N₂O to the atmosphere than mainstem of the bay”.

Ln 285-286: For better clarity and interpretation of the text, the values of your observed $\delta^{15}\text{N}$ of N₂O downstream of WWTPs and in the urban WWTPs should be included.

Since we have listed $\delta^{15}\text{N}$ of N₂O downstream of WWTPs and in the urban WWTPs in the text above, to avoid repeating, we modified this sentence to: “The $\delta^{15}\text{N}$ values of N₂O in these urban WWTPs were lower than those found in waters downstream of WWTPs in the Potomac River (median $\delta^{15}\text{N}$ at 13‰)”.

Ln 288: Do you know of any work where there is evidence of denitrification in WWTPs, in downstream creeks, or in sediments? If so, could you please cite it.

N₂O production from denitrification has been observed in WWTPs, creeks and sediments (e.g., Toyoda et al., 2011; Beaulieu et al., 2011). We hypothesized the partial N₂O reduction caused the elevated $\delta^{15}\text{N}$ of N₂O downstream of WWTPs. This partial N₂O reduction has been suggested to explain the occurrence of high $\delta^{15}\text{N}$ of N₂O in the core of marine oxygen minimum zones (Kelly et al., 2011; Bourbonnais et al., 2017). However, the effect of denitrification, especially the partial N₂O reduction to N₂, on N₂O isotopes in WWTPs, creeks and sediments remain to be evaluated.

Ln 343-345: Text in brackets is not in Times New Roman 12. Why is the number of data considered for the predictions so small? Especially for the stations without WWTPs, in the complete study there are 8 sampling x 8 stations (4 stations without WWTPs + 4 stations central channel) = 64 data compared to the 23 considered in the prediction.

The font in the equation (Cambria Math) is different from the font in the text (Times New Roman 12). The lower number of stations used for prediction is because not all the stations were sampled during each field campaign or not all the parameters of each station were measured. See the Dataset deposited in Zenodo repository for the sampled stations and measured parameters.

Ln 365. In the section: “Impact of wastewater treatment plants on N₂O concentrations and emissions” very little is mentioned about how N₂O fluxes to the atmosphere vary in the stations upstream and downstream of the WWTPs. However, there is much discussion of the effect of the WWTPs on N₂O concentrations. More should be said about these emissions, as the title of the paper says "Variable contribution of wastewater treatment plant effluents to N₂O emission". Furthermore, table 1 could present the N₂O fluxes as well as the concentrations.

Following the reviewer's suggestion, we have added more description of the N₂O fluxes/emissions in this section.

"Interestingly, the N₂O concentration and flux at the station downstream of Mooney WWTP in Neabsco Creek were lower than the N₂O concentration and flux at the station upstream of Mooney WWTP (15.0 nM vs 20.1 nM; 14.6 μmol m⁻² d⁻¹ vs 24.7 μmol m⁻² d⁻¹)."

"In contrast, we found a substantially higher N₂O concentration and flux downstream of the Noman Cole WWTP than the upstream station (30.8 nM vs 16.7 nM; 55 μmol m⁻² d⁻¹ vs 17.6 μmol m⁻² d⁻¹) in the Pohick Creek, which is less affected by the tidal cycle due to its semi-closed geography (salinity was 0.12)."

Table 1 shows either N₂O concentrations or fluxes or both depending on which one is available from previous studies. We have added N₂O fluxes from our study to the table.

Ln 366 - 369: Figure 4a should be mentioned, where the sampling stations considered in this study are shown in detail.

We have added Figure 4a to the end of this sentence.

Figures:

Figures 2 and 3 and Supplementary figures 3 and 5. It is not necessary to write the word "concentration" on the axes of the figures when referring to N₂O concentration (nM), just as you do not write NO_x- concentration.

Based on the reviewer's suggestion, we used N₂O (nM) when referring to N₂O concentration in the figure axes titles.

References

Ln 604-613: Rosentreter references should be put in chronological order.

Rosentreter references are now cited in chronological order in the reference list.

References:

Beaulieu, J. J., Tank, J. L., Hamilton, S. K., Wollheim, W. M., Hall, R. O., Jr., Mulholland, P. J., Peterson, B. J., Ashkenas, L. R., Cooper, L. W., Dahm, C. N., Dodds, W. K., Grimm, N. B., Johnson, S. L., McDowell, W. H., Poole, G. C., Valett, H. M., Arango, C. P., Bernot, M. J., Burgin, A. J., Crenshaw, C. L., Helton, A. M., Johnson, L. T., O'Brien, J. M., Potter, J. D., Sheibley, R. W., Sobota, D. J., and Thomas, S. M.: Nitrous oxide emission from denitrification in stream and river networks, *Proceedings of the National Academy of Sciences of the United States of America*, 108, 214-219, 10.1073/pnas.1011464108, 2011.

Bourbonnais, A., Letscher, R. T., Bange, H. W., Echevin, V., Larkum, J., Mohn, J., ... & Altabet, M. A.: N₂O production and consumption from stable isotopic and concentration data in the Peruvian coastal upwelling system, *Global Biogeochemical Cycles*, 31(4), 678-698, 2017.

Kelly, C. L., Travis, N. M., Baya, P. A., and Casciotti, K. L.: Quantifying Nitrous Oxide Cycling Regimes in the Eastern Tropical North Pacific Ocean With Isotopomer Analysis, *Global Biogeochemical Cycles*, 35, 10.1029/2020gb006637, 2021.

Toyoda, S., Suzuki, Y., Hattori, S., Yamada, K., Fujii, A., Yoshida, N., Kouno, R., Murayama, K., and Shiomi, H.: Isotopomer Analysis of Production and Consumption Mechanisms of N₂O and CH₄ in an Advanced Wastewater Treatment System, *Environmental Science & Technology*, 45, 917-922, 10.1021/es102985u, 2011.