

We thank the editors and reviewers for their valuable and insightful comments! The main suggestions include the quantification of the dilution of WWTPs effluents by river flows and better estimates of N₂O emissions using multiple gas transfer coefficient parameterizations. We have responded to reviewers' comments below in blue font and made changes accordingly in the manuscript.

Reviewer 1:

GENERAL COMMENTS

Currently, there is considerable interest in understanding the production and emissions of greenhouse gases (GHGs) from wastewater treatment plants (WWTPs). Indeed, emissions of these gases have become a major concern in efforts to mitigate climate change and reduce global emissions. This renders the article by Tang et al. particularly significant as it investigates the impact of WWTPs on N₂O emissions in aquatic systems downstream of the Potomac River estuary by measuring nitrogenous nutrients and N₂O concentrations on a monthly resolution over the course of a year. The authors have identified spatially and temporally variable concentrations of N₂O and fluxes of N₂O, generally higher downstream of the WWTPs, highlighting the necessity for effective N₂O removal alongside nitrogen treatment at WWTPs.

The data are well presented and the discussion of the dataset is comprehensive and conclusive. However, from my point of view, I have some suggestions to render the work more attractive to readers. Therefore, I suggest its publication after major revisions.

It would be valuable for the study to clarify whether the three treatment plants (Noman Cole, Mooney, and Aquia) utilize identical wastewater treatment processes and treat similar volumes of water. Additionally, assessing whether the receiving channels into which the WWTPs discharge exhibit comparable water volumes is crucial for ensuring a consistent dilution effect of the gas in the water column. Moreover, understanding the depth of the water column is essential; in cases of shallow depths, the influence of gas emission from the sediment to the water column could be substantial.

It would be interesting for the study to elucidate whether the three treatment plants (Noman Cole, Mooney, and Aquia) employ the same wastewater treatment processes and the volume of water they treat. It is also important to determine if the receiving channels where the WWTPs discharge have similar water volumes, so that the dilution effect of the gas in the water column is similar. Similarly, it would be interesting to know the depth of the water column; if it is shallow, the influence of gas emission from the sediment to the water column could be significant.

We thank the reviewer for the comments. Although we contacted the WWTPs directly, we were not able to obtain detailed information about the treatment processes of the three treatment plants except they all implement tertiary treatment. We acknowledge that the different types of treatment affect the N₂O production yield in the WWTPs in the text (de Haas and Andrews, 2022; Zhao et al., 2024). However, we were able to obtain the information regarding the volume of treated water and total nitrogen concentrations of the three WWTPs from Virginia Pollutant Discharge Elimination System (VPDES) and have included that information in lines 109 – 114 in

the text: “We obtained volume discharge and total N in treated water of each WWTP from Virginia Pollutant Discharge Elimination System. Noman Cole WWTP discharges ~140.8 million liters of water and 370 kg N per day into Pohick Creek. Mooney WWTP discharges ~54.9 million liters of water and 147 kg N per day into the Neabsco Creek. Aquia WWTP discharges much less water and N into the Aquia Creek (~21.2 million liters per day and 35 kg N per day)”.

We were also able to obtain the river discharges at monitoring stations upstream of the Mooney WWTP (monitor station of Neabsco Creek at Dale City, Virginia) and Aquia WWTP (monitor station of Aquia Creek near Garrisonville, Virginia) from United States Geological Survey (USGS) and compared them to their WWTPs’ effluent volumes in order to evaluate the dilution effect on N₂O concentrations and emissions (Figures R1-3 and Supplementary Figure 2). In addition, total nitrogen concentrations were available from the monitoring station upstream of Mooney WWTP (Richmond Highway, Virginia). We then compared the total N flow between the Neabsco Creek flow and Mooney WWTP effluent.

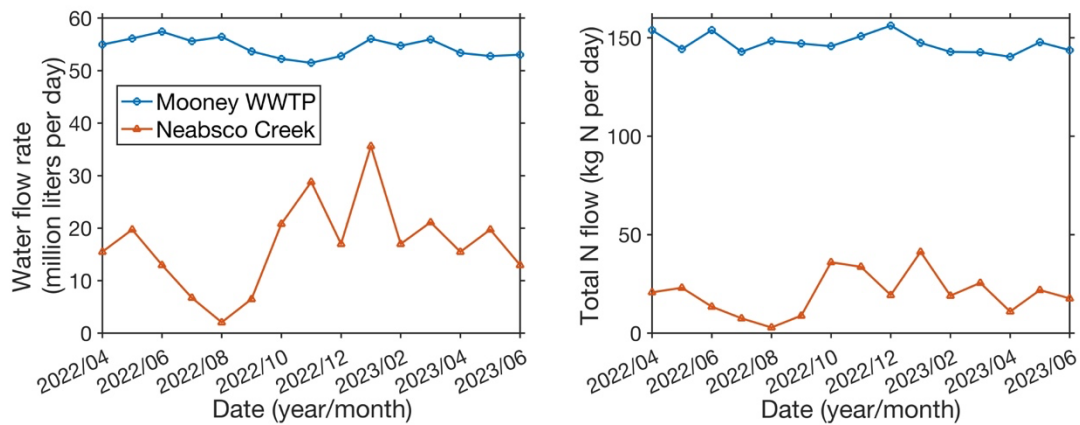


Figure R1. Comparison of water flows and total nitrogen inputs from Mooney WWTP effluent and Neabsco Creek. Climatological river flow rates were used for Neabsco Creek because river flow data were not available for years 2022 and 2023.

The volume and nitrogen discharge of Mooney WWTP effluent were always higher than the Neabsco Creek (Figure R1). Therefore, the dilution of N₂O in Mooney WWTP effluent by the river flow was small. In contrast, the volume of Aquia WWTP effluent was generally lower than the Aquia Creek flow rate (Figure R2). The high dilution by the river flow likely diminished the N₂O signal from Aquia WWTP. In addition, river flow rates were generally lower in summer while WWTPs’ effluent volumes were relatively constant throughout the year, leading to a larger ratio of WWTPs’ effluent to the river flow (less dilution) in the dry season. That’s probably one of reasons why the highest N₂O concentrations were observed downstream Mooney WWTP in August when the river flow was low.

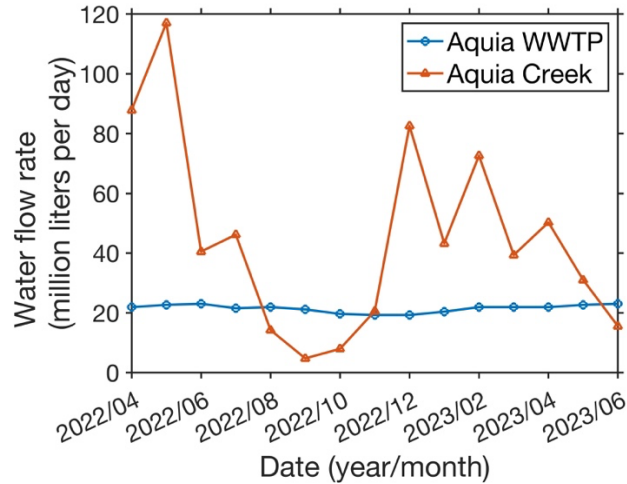


Figure R2. Comparison of water flows from Aquia WWTP effluent and Aquia Creek.

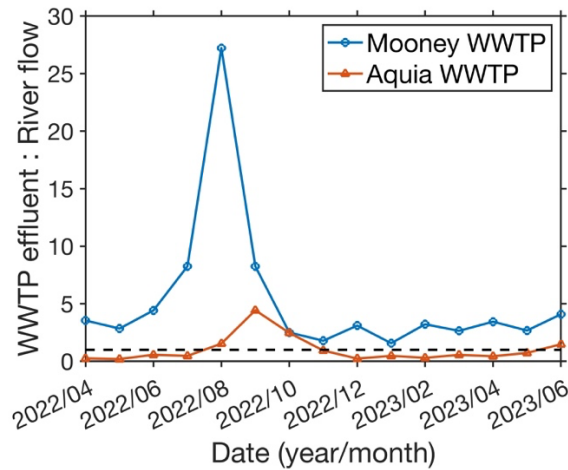


Figure R3. The ratio of WWTP effluent to river flow.

The water column depths of sampling stations are now added to the manuscript in lines 118 - 119: “The embayment stations were 2-3 meters deep while the average depth of central channel stations was around 8 meters”. Sedimentary N₂O production may supply N₂O to the water column and further N₂O emissions to the atmosphere. But we don’t have direct observations to support that, which deserves further observations.

The bibliographical references cited do not always follow the same criteria (chronological order or alphabetical order).

We have updated the reference order based on the journal’s requirement (chronologically in text and alphabetically at the end of the manuscript).

SPECIFIC COMMENTS

Ln 49. In a more recent article than those cited, Rosentreter et al., 2023 there are compiled N₂O emissions data from various estuaries, providing a wider range of emissions variation (0.2 – 5.7 Tg N₂O yr⁻¹). Specifically, the paper states: “Global estimates of estuarine N₂O emissions are highly uncertain, with large discrepancies for both observation-based (220–5,710 GgN₂O yr⁻¹) and modelling approaches (94–1,084 GgN₂O yr⁻¹).

We have updated the range of estimated estuarine N₂O emissions, citing Rosentreter et al., 2023.

Ln 65-66. References should be listed in ascending chronological order, consistent with the rest of the paper.

References are now cited chronologically in the text.

Ln 87. It should be indicated what type of treatment is given in the WWTPs (primary, secondary, tertiary, etc.) in order to understand if the nitrogen removal capacity of the three wastewater treatment plants is the same. At what distance from the WWTPs were the samples taken? Were the samples taken at approximately the same distance from the discharge point at all three WWTPs? Were the channels where the samples were taken similar? Did they have approximately the same water volume? An important factor when comparing the amount of N₂O in the receiving channels is dilution.

All the WWTPs involved in this study implement tertiary treatment. We have listed the distance between the sampling stations and WWTPs in lines 114 - 116: “The distances from the sampling stations to Noman Cole, Mooney, Aquia were approximately 4, 1.8 and 5.8 km, respectively”.

See the response to the general comments on the dilution effect.

Ln 99. Were the samples collected from a vessel? Please specify.

Samples were collected on vessel – “Grady White 208”, which has been added to the manuscript.

Ln 110-11. How was a 3 mL air headspace created in the 60 mL serum bottles? Did all samples have exactly the same volume of air headspace? Could this 3 mL of air in contact with the sample potentially interfere with the measurement? Was the N₂O content in the air also measured? Were the samples taken in duplicate?

Water N₂O concentration samples were collected in triplicate at each sampling sites. 3 mL air headspace was created by removing 3 mL water using a syringe. The monthly atmospheric N₂O concentrations were obtained from the nearby atmospheric station in Brentwood, Maryland (<https://gml.noaa.gov/dv/site/?stacode=BWD>) (Andrews et al., 2023). The amount of N₂O in 3 mL air headspace was generally less than 4% of the amount of N₂O dissolved in the 57 mL water samples. Thus, the effect of 3 mL air on N₂O measurements was minor and was accounted for the concentration calculations. The similar sampling method has previously been used (e.g., Kelly et al., 2020). See modified text in lines 167-171.

Ln 112. Leave a space between the 10 and the M.

Modified as suggested.

Ln 124-128. Figure 4a, depicting the sampling points of the four streams/ivers (Neabsco Creek (5 stations), Occoquan River (3 stations), Pohick Creek (4 stations), and Accotink Creek), should be included in the Materials and Methods section.

Rather than cite Figure 4 out of order in the text, or clutter up Figure 1 (we tried that, it makes the figure unreadable at the necessary scale), we now cite Figure 4 in the caption of Figure 1 for the locations of the additional creek sampling stations.

Ln 128. Where have the data on water discharge and nitrogen (kg) per day from the wastewater treatment plants been obtained? It would be interesting to include this information in the manuscript.

Data source has been added in lines 109-111: “We obtained volume discharge and total N in treated water of each WWTP from Discharge Monitoring Reporting required by Virginia Pollutant Discharge Elimination System permit”.

Ln 149. It is not reflected in the text how the 3 mL air headspace is taken from the serum bottles to estimate the amount of N₂O in the sample.

See the response to the related comment above.

Ln 167. “The equilibrium N₂O concentration was calculated based on the solubility of N₂O (Weiss and Price, 1980)...” Where did you obtain the value of N₂O in the atmosphere for the calculations? Which value did you consider, the daily, monthly...?

The monthly atmospheric N₂O concentrations were obtained from the nearby atmospheric station in Brentwood, Maryland (<https://gml.noaa.gov/dv/site/?stacode=BWD>) (Andrews et al., 2023).

Ln 170: What do the initials NCEP stand for? It would be more comprehensive to include the website from which the value of U was taken.

NCEP stands for National Centers for Environmental Prediction and the website of the data source has been added to the manuscript (<https://psl.noaa.gov/data/gridded/data.ncep.reanalysis.html>).

Ln 171. You should cite in the paper the expression from which Sc has been estimated, possibly from the proposed expression by Wanninkhof (2014). You should indicate whether for the calculation of Sc, you have considered the expression for salinity equal to zero, or if, on the contrary, the N₂O Schmidt number for each point has been scaled to the values proposed by Wanninkhof (2014) for salinities between 0 and 35, assuming that Sc varies linearly with salinity.

We have added in the text in lines 196-198: “Schmidt number was estimated as a function of temperature based on the equation from Wanninkhof (2014). Since our samples have salinity close to 0, we used the parameterization for freshwater”.

Ln 173. References should be listed in ascending chronological order, consistent with the rest of the paper.

References order has been updated.

Ln 170-176. I don't understand why they are using a gas transfer velocity parameterization (k) proposed for the ocean, such as the expression by Wanninkhof (2014), rather than a k for a coastal system. If they didn't have data on current velocity and depth of the system necessary to use the k by Borges et al. (2004) and Rosentreter et al. (2021), they could have used the expression proposed by Raymond and Cole (2001), which is based on a compilation of k proposed for different coastal systems, or that by Jiang et al. (2008), based on the compilation of Raymond and Cole (2001) as well as other studies conducted in estuaries. Furthermore, given the uncertainty associated with k , to minimize this, they could have estimated water-atmosphere fluxes considering two expressions of k (Raymond and Cole, 2001; Jiang et al., 2008; Wanninkhof, 2014), and taken the average value of the three fluxes obtained, as many other authors do in coastal systems (e.g., Call et al., 2015; Sánchez-Rodríguez et al., 2024).

This is a great suggestion. Following the reviewer's comment, we have now estimated N_2O fluxes based on three different parameterizations of k values (Raymond and Cole, 2001; Jiang et al., 2008; Wanninkhof, 2014). See lines 191-194.

$$k_{600} = 1.91 \times e^{0.35 \times U} \text{ (Raymond and Cole, 2001)}$$

$$k_{600} = 0.314 \times U^2 - 0.436 \times U + 3.99 \text{ (Jiang et al., 2008)}$$

$$k = k_{600} \times \left(\frac{Sc}{600}\right)^{-0.5}$$

$$k = 0.251 \times U^2 \times \left(\frac{Sc}{600}\right)^{-0.5} \text{ (Wanninkhof, 2014)}$$

Average values of the three estimates are presented in the manuscript and estimates of each parameterization are provided in the supplementary data.

Ln 233. I suggest wording it like this: ...vs 6‰ for stations of the central channel and without the influence by WWTPs

Modified the text as suggested.

Ln 242. In general, denitrification typically occurs in environments with low oxygen concentrations ($DO \leq 5 \mu\text{mol L}^{-1}$, Codispoti et al., 2001). As illustrated in Supplementary Figure 2, oxygen concentrations at the stations never reached low values. In fact, downstream stations of wastewater treatment plants exhibited dissolved oxygen levels ranging between 139.38 (25/07/2022) – 430.94 μM (7/02/2023). It is recognized that denitrification can also take place within oxygenated water columns containing suspended organic matter particles (Bange, 2008). Is there a substantial amount of suspended material in the studied system that could

induce denitrification in oxygenated water? On the other hand, it is well-established that coastal sediments provide optimal environments for denitrification due to continuous inputs of nutrients and organic matter from land. Could it be that some of the measured N₂O in the water originates from the sediment?

There was a substantial amount of suspended material in the study region: the total suspended particle concentration was 14.8±10 mg/L and the Secchi depth was generally below 1 m. We don't have direct evidence to show but acknowledge the possibility that denitrification could occur in the anoxic particles or in the sediments, supplying N₂O to the water column.

Ln 252. Correlations of 0.62 (r²=0.38) and 0.51 (r²=0.26), I do not consider them strong correlations, remove the word strong.

“Strong” was removed from the text.

Ln 252-256 and 263-264. In stations unaffected by WWTPs, there appears to be a good positive correlation between N₂O and DO and NO_x, which could indicate that nitrification is an important process in these N₂O production stations.

We have now added in lines 308-315: “Although previous studies have showed dissolved oxygen to be an important driver of N₂O concentrations or fluxes in rivers and estuaries (Rosamond et al., 2012; Wang et al., 2015; Zheng et al., 2022), we did not find a strong dependence of N₂O on oxygen concentrations in the Potomac River Estuary (Figure 3a). This lack of strong dependence is probably because of the overall oxygenated conditions (Supplementary Figure 1c) and opposite correlations found in stations without WWTPs (positive) or with WWTPs (negative) (Supplementary Figure 3), which could lead to different N₂O production pathways”.

Ln 262. References should be listed in ascending chronological order, consistent with the rest of the paper.

References order has been updated.

Ln 278-279. References should be listed in ascending chronological order, consistent with the rest of the paper.

References order has been updated.

Ln 292-294. Why does it not also present the predictive model of N₂O concentration based on total nitrogen and temperature for stations in the central channel of the Potomac Estuary? It could be interesting to have it to extrapolate to other areas of the estuary located in the channel. Perhaps you have included the data measured in the channel in the samples without wastewater treatment plants (WWTPs). If so, please indicate it. I believe you should have stated the number of stations/data considered in each prediction.

We have clarified in the text in lines 342-346: “Predictions were performed separately for stations with WWTPs ($N_2O \text{ concentration} = 0.115 \times \text{total } N - 0.241 \times \text{temperature} +$

17.185), (n=18, r=0.78; p<0.01) and without WWTPs including central channel stations (N_2O concentration = $0.049 \times total\ N - 0.298 \times temperature + 18.888$), (n=23, r=0.81, p<0.01)”).

Ln 298-300. Did you use the prediction model for stations without WWTPs? Please indicate it in the paper.

The embayment station in the Occoquan River was not in the downstream of WWTPs. N_2O concentrations were estimated using the predictive model built upon stations without WWTPs. For comparison, we have now made predictions for another station in the Pohick Creek that is downstream of Noman Cole WWTP, using the predictive model built upon stations with WWTPs. There was a decrease in N_2O concentrations following the decline of total N concentrations at the Pohick Bay sampling station (see Figures R4-R5 below and modified Supplementary Figures 6 and 7).

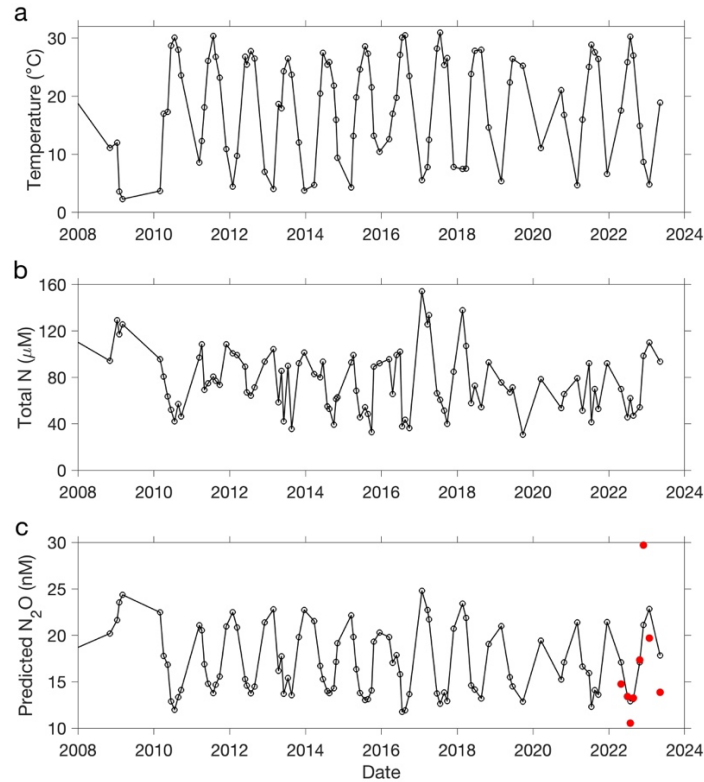


Figure R4. Historical measurements of temperature (a) and N concentration (b) at the Occoquan Bay sampling station without the influence of WWTPs. N_2O concentration (c) is predicted based on a multiple linear regression model developed for stations without the influence of WWTPs. The red points are the observed N_2O concentration.

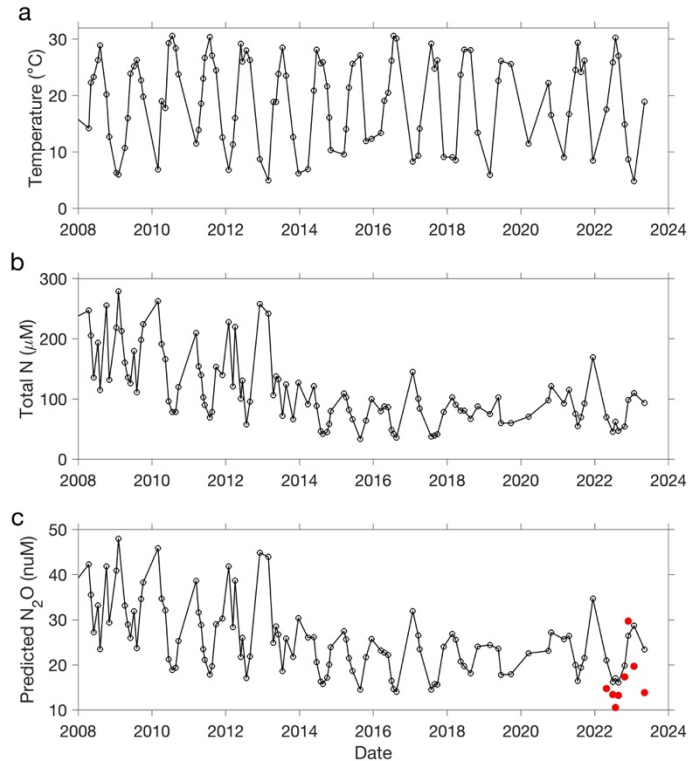


Figure R5. Historical measurements of temperature (a) and N concentration (b) at the Pohick Bay sampling station with the influence of Noman Cole WWTP. N₂O concentration (c) is predicted based on a multiple linear regression model developed for stations with the influence of WWTPs. The red points are the observed N₂O concentrations.

FIGURES

Figure 2 and Supplementary Figure 1. What does the "01" after the slash indicate on the x-axis of the central graphs? Wouldn't it be more intuitive for the reader to use "22" or "23" instead of "01," depending on the year the sampling was conducted?

We have now changed the axis tick labels to the format "year/month" as suggested.

Supplementary Figure 2. In the figure caption and in Figure a, a negative sign as a subscript is missing on NO_x- on the x-axis. In Figure b, on the x-axis, remove the space between N₂ and O.

Figure text and captions have been modified as suggested.

REFERENCES:

- Bange, H. W., 2008. Gaseous Nitrogen Compounds (NO, N₂O, N₂, NH₃) in the Ocean. In Nitrogen in the Marine Environment (pp. 51–94). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-372522-6.00002-5>
- Call, M., Maher, D. T., Santos, I. R., Ruiz-Halpern, S., Mangion, P., Sanders, C. J., ... & Eyre, B. D., 2015. Spatial and temporal variability of carbon dioxide and methane fluxes over semi-

diurnal and spring–neap–spring timescales in a mangrove creek. *Geochimica et Cosmochimica Acta*, 150, 211–225.

Codispoti, L.A., Brandes, J.A., Christensen, J.P., Devol, A.H., Naqvi, S.W.A., Paerl, H.W., Yoshinari, T., 2001. The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the anthropocene? *Sci. Mar.* 65 (S2), 85–105.

Jiang, L.Q., Cai, W.J., & Wang, Y. (2008). A comparative study of carbon dioxide degassing in river- and marine-dominated estuaries. *Limnology and Oceanography*, 53(6), 2603–2615.

Rosentreter, J. A., Laruelle, G. G., Bange, H. W., Bianchi, T. S., Busecke, J. J. M., Cai, W. J., Eyre, B. D., Forbrich, I., Kwon, E. Y., Maavara, T., Moosdorf, N., Najjar, R. G., Sarma, V. V. S. S., Van Dam, B., & Regnier, P., 2023. Coastal vegetation and estuaries are collectively a greenhouse gas sink. *Nature Climate Change*, 13(6), 579–587. <https://doi.org/10.1038/s41558-023-01682-9>

Sánchez-Rodríguez, J., Ortega, T., Sierra, A., Mestre, M., Ponce, R., Fernández-Puga, M. D. C., & Forja, J., 2024. Distribution, reactivity and vertical fluxes of methane in the Guadalquivir Estuary (SW Spain). *Science of The Total Environment*, 907, 167758.

Reviewer 2:

The manuscript “Variable contribution of wastewater treatment plant effluents to nitrous oxide emission” by Tang et al. studies the effects of wastewater treatment plants on the Potomac River estuary in the United States. For over one year, they took monthly samples for nitrous oxide, total nitrogen and dissolved inorganic nitrogen concentrations. Generally, the results showed spatial and seasonal variability in nitrous oxide concentrations with higher concentrations downstream of the WWTPs, highlighting the importance of WWTPs regarding estuarine N₂O emissions. Therefore, this manuscript will be of interest in the context of global N₂O emissions from estuaries and WWTPs. The data set is well presented and interpreted and the text well written and organized. However, major revisions are necessary to discuss effects of wastewater treatment processes and dilution effects.

General remarks:

The paper misses to discuss differences in wastewater treatments and dilution effects, which leads to some important unanswered questions:

- Do the WWTPs differ in type, removal strategy and treated water volume? Are differences visible in TN, DIN and N₂O effluents?
- How big are the water volumes of the WWTP effluents compared to the water volume in the estuary (especially in the tributaries)? I would recommend calculating a wastewater discharge fraction of stream flow.
- How big is the N load in the WWTP effluents compared to the N loads in the upstream river? How are the effluents diluted and are concentration increases expected/seen?
- Are there seasonal effects on the impact of wastewater effluents? For example, Murray et al. (2020) measured differences in N₂O concentrations affected by WWTPs between dry and wet season in an Australian estuary.

Thank the reviewer for these great comments on differences in the wastewater treatment processes among WWTPs and the dilution effect on N₂O concentrations by riverine discharge. The first reviewer also had the similar comments.

Although we contacted the WWTPs directly, we were not able to obtain detailed information about the treatment processes of the three treatment plants except they all implement tertiary treatment. We acknowledge that the different types of treatment affect the N₂O production yield in the WWTPs in the text (de Haas and Andrews. 2022; Zhao et al., 2024). However, we were able to obtain the information regarding the volume of treated water and total nitrogen concentrations of the three WWTPs from Virginia Pollutant Discharge Elimination System and have included that information in lines 109-114 in the text: “We obtained volume discharge and total N in treated water of each WWTP from Virginia Pollutant Discharge Elimination System. Noman Cole WWTP discharges ~140.8 million liters of water and 370 kg N per day into Pohick Creek. Mooney WWTP discharges ~54.9 million liters of water and 147 kg N per day into the

Neabsco Creek. Aquia WWTP discharges much less water and N into the Aquia Creek (~21.2 million liters per day and 35 kg N per day)”.

We were also able to obtain the river discharges at monitoring stations upstream of the Mooney WWTP (monitor station of Neabsco Creek at Dale City, Virginia) and Aquia WWTP (monitor station of Aquia Creek near Garrisonville, Virginia) from United States Geological Survey (USGS) and compared them to their WWTPs’ effluent volumes in order to evaluate the dilution effect on N₂O concentrations and emissions (Figure R6-8). In addition, total nitrogen concentrations were available from the monitoring station upstream of Mooney WWTP (Richmond Highway, Virginia). We then compared the total N flow between the Neabsco Creek flow and Mooney WWTP effluent.

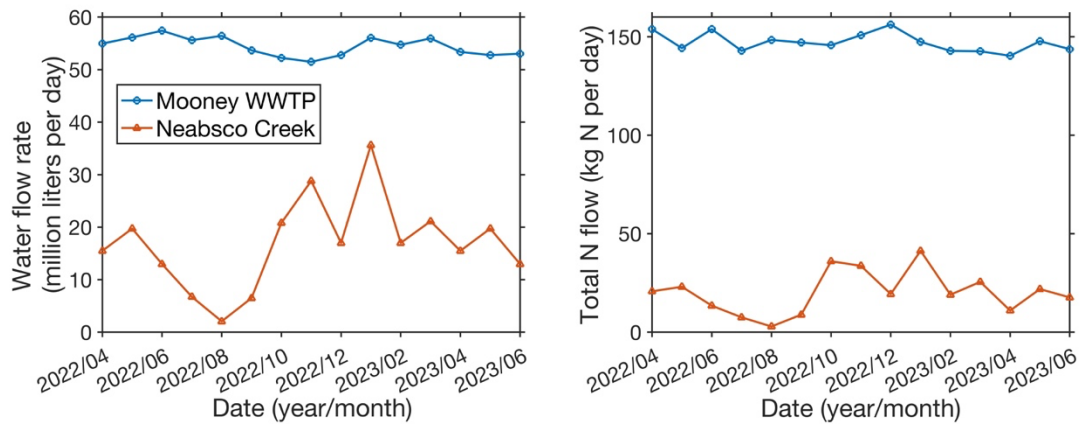


Figure R6. Comparison of water flows and total nitrogen inputs from Mooney WWTP effluent and Neabsco Creek.

The volume and nitrogen discharge of Mooney WWTP effluent were always higher than the Neabsco Creek (Figure R6). Therefore, the dilution of N₂O in Mooney WWTP effluent by the river flow was small. In contrast, the volume of Aquia WWTP effluent was generally lower than the Aquia Creek flow rate (Figure R7). The high dilution by the river flow likely diminished the N₂O signal from Aquia WWTP. In addition, river flow rates were generally lower in summer while WWTPs’ effluent volumes were relative constant throughout the year, leading to a larger contribution of WWTPs’ effluents to total river flow (less dilution) in the dry season. That’s probably one of reasons why the highest N₂O concentrations were observed downstream Mooney WWTP in August. This is similar to what Murry et al. (2020) found in an Australian estuary as the reviewer pointed out.

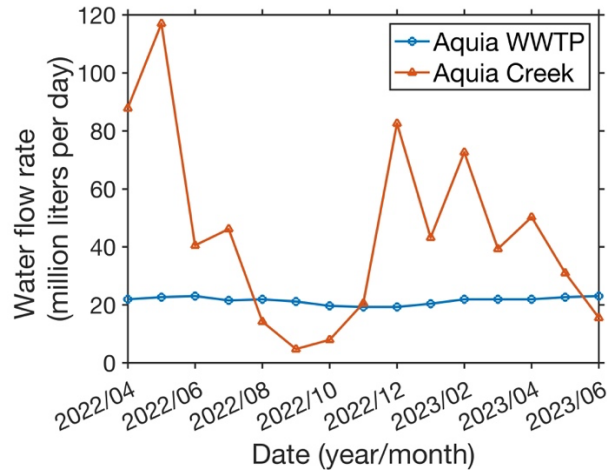


Figure R7. Comparison of water flows from Aquia WWTP effluent and Aquia Creek.

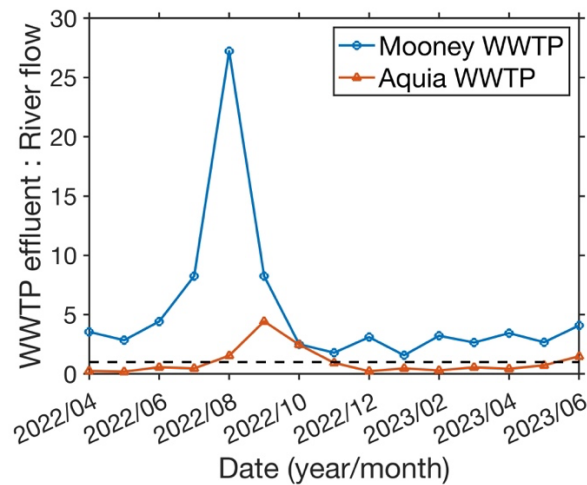


Figure R8. The ratio of WWTP effluent to river flow.

Specific remarks:

L63: “[...] are highly variable, and are normally [...]”

Text has been modified as suggested.

L75: What is the mean annual discharge entering the estuary from the upstream river? What are mean N loads?

We have added in lines 79-83 in the text: “Potomac River discharge has been measured by the USGS at Chain Bridge near Washington, DC. The annual mean discharge from 1895 to 2002 at Chain Bridge was $321 \text{ m}^3 \text{ s}^{-1}$ with a large interannual variability (Jaworski et al., 2007). The annual total nitrogen loading is estimated to be around $27.7 \times 10^6 \text{ kg N year}^{-1}$ in 2008-2009 (Bricker et al., 2014)”.

L84: “[...] nitrogen effluent concentration below 7.5 mg L^{-1} [...]”

Text has been clarified.

L108: At what tidal state was the sampling carried out? How does the tidal state affect the results? Did you always sample at the same tidal state to minimize effects?

Since the routine water quality sampling by the Department of Environmental Quality generally occurs in the morning, we were not able to collect samples at the same tidal state. We acknowledge this caveat in the text in lines 126-128: “While estuarine N₂O concentrations could be affected by tides (Gonçalves et al., 2015), sampling was not always conducted at the same tidal state due to logistic difficulties”.

L110: Did you take replicates?

Yes, triplicate DIN concentration and N₂O samples were collected.

L110-111: Did you measure N₂O concentrations in air headspace for correction? How did you estimate/measure atmospheric N₂O concentrations?

The monthly atmospheric N₂O concentrations were obtained from the nearby atmospheric station in Brentwood, Maryland (<https://gml.noaa.gov/dv/site/?stacode=BWD>) (Andrews et al., 2023).

L151: Did you measure replicates for N₂O isotopes?

Yes, this is now clarified in the text.

L169-170: Why did you decide to use Wanninkhof’s formula, which applies better to open oceans? There are formulas specifically designed for estuarine environments, e.g. Clark et al. (1995) and Raymond and Cole (2001).

Following the comments from both reviewers, we have used three different parametrizations (Raymond and Cole, 2001; Jiang et al., 2008; Wanninkhof, 2014) to calculate gas transfer coefficient to estimate N₂O fluxes. Average values of these three estimates are presented in the manuscript and estimate of each parameterization is provided in the supplementary data.

$$k_{600} = 1.91 \times e^{0.35 \times U} \text{ (Raymond and Cole, 2001)}$$

$$k_{600} = 0.314 \times U^2 - 0.436 \times U + 3.99 \text{ (Jiang et al., 2008)}$$

$$k = k_{600} \times \left(\frac{Sc}{600}\right)^{-0.5}$$

$$k = 0.251 \times U^2 \times \left(\frac{Sc}{660}\right)^{-0.5} \text{ (Wanninkhof, 2014)}$$

L128-131: How do these values (treated water volumes and N loads) compared to the riverine volume and N loads? See general comments above. Did you see changing impacts depending on the size of the WWTPs?

See the reply to the general comments above.

L149: How did you take the amount of N₂O in the 3 mL headspace into account?

We have clarified in line 130 and 167-171 in the text: “3 mL air headspace was created by removing 3 mL water using a syringe.”

“The monthly atmospheric N₂O concentrations were obtained from the nearby atmospheric station in Brentwood, Maryland (<https://gml.noaa.gov/dv/site/?stacode=BWD>) (Andrews et al., 2023). The amount of N₂O in 3 mL air headspace was generally less than 4% of the amount of N₂O dissolved in the 57 mL water samples. Thus, the effect of 3 mL air on N₂O measurements was minor and was accounted for the concentration calculations.”

L171: How did you calculate the Schmidt number?

We have added in the text: “Schmidt number was estimated as a function of temperature based on the equation from Wanninkhof (2014). Since our samples have salinity close to 0, we used the parameterizations for freshwater.”

L185: Do you also see these seasonal differences in the effect of the WWTPs? The effluent of WWTPs usually have a relatively constant N load throughout the entire year. Therefore, I could imagine that it makes a big difference whether the WWTPs discharge into an estuary with a high N concentration in winter or a low N concentration in summer. Further, riverine discharge is usually higher in winter, which leads to greater dilution and reduces the impact of WWTP effluents.

The reviewer is correct about the seasonal changes in the volume of WWTPs' effluent vs the riverine discharge. See response to the general comments above.

L190-191: Does this also reflect in seasonal changing $\delta^{15}\text{N}$ -N₂O values?

Yes, we saw a seasonal change in $\delta^{15}\text{N}$ - N₂O at stations downstream of WWTPs. We have added in the text in lines 275-281: “ $\delta^{15}\text{N}$ of N₂O for stations with the influence of WWTPs showed a clear seasonal variation: higher values in summer than winter (Figure 2e). This seasonal difference may be related to the seasonal change in the relative importance of WWTPs' effluents vs riverine discharge (Supplementary Figure 2c). For example, relatively larger WWTPs' effluents led to larger $\delta^{15}\text{N}$ of N₂O in summer when riverine flows were smaller. However, no clear seasonal pattern of $\delta^{15}\text{N}$ of N₂O was seen for stations without the influence of WWTPs”.

L218: Calculating a wastewater discharge fraction of stream flow would help to estimate the different dilution effects for each WWTP.

See response to the general comments above.

L220: Can you estimate the wastewater discharge fraction of stream flow considering the water volume of the estuary and water volume and N load from the WWTP?

See response to the general comments above.

L224: “High-resolution spatial and temporal sampling” – I don’t agree that the conducted sampling campaign has a high spatial and temporal resolution considering the existence of laser-based measurements that allow resolution by the second. Sampling was conducted once or twice a month at eleven stations or once at 14 stations. I would suggest rephrasing this statement.

We have modified the text to: “Repeated spatial and temporal sampling allowed us to capture these N₂O hotspots”.

L233: Do you observe seasonal changes?

Yes, see the response to the related comments above.

L238: What kind of treatments are performed at the WWTPs discharging into the Potomac River estuary? There are different ways of operating N removal within WWTP (biological, chemical, and physical methods) (e.g. Winkler and Straka, 2019; Zhou et al., 2023). Further, biological removal strategies, for example, can also differ significantly: (1) denitrification followed by nitrification, where a part of the treated water is fed back into the denitrification after nitrification, (2) nitrification is followed by denitrification with organic carbon being added to the denitrification chamber (e.g. part of the untreated water before nitrification), (3) intermittent denitrification, in which longer phases with aerobic nitrification and anoxic denitrification alternate in the same tank, (4) simultaneous denitrification due to the discontinuous or punctual supply of oxygen, (5) cascade denitrification, in which the wastewater passes through several tanks with alternating denitrification and nitrification, or (6) alternating denitrification, consisting of two aeration tanks that are alternately fed with wastewater and aerated. N₂O production and N₂O production pathway may differ significantly depending on the treatment strategy. Therefore, it would be very valuable to discuss treatment strategies considering possible isotope changes. Do the WWTP even use biological treatments or other physical/chemical ones?

We agree with the reviewer that the type of treatment affects the nitrogen removal efficiency and N₂O production yield in the WWTPs (de Haas and Andrews, 2022; Zhao et al., 2024). However, the lack of information about the types of treatment process of WWTPs in this study prevented us from comparing their $\delta^{15}\text{N}$ of N₂O values (we were not able to obtain these information from WWTPs). Thus, we focused on the spatiotemporal variation in $\delta^{15}\text{N}$ of N₂O.

L242: Oxygen concentration during your measurements (supplementary material Fig. 1, L264) were always above the threshold for denitrification (< 6.25 μM ; Seitzinger, 1988).

Denitrification can occur in anoxic microsites close to particles (Liu et al., 2013; Zhu et al., 2018; Schulz et al., 2022) or in anoxic sediments. Where do you suggest denitrification occurs? Is it an artefact of denitrification in the WWTP?

We are not certain about the locations of the denitrification. As the reviewer pointed out, denitrification could occur in anoxic zones in particles or sediments. N₂O close to WWTPs’ effluents had elevated $\delta^{15}\text{N}$ values compared to upstream stations (Figure 4b) suggested that at least part of N₂O consumption occurred in the WWTPs. The $\delta^{15}\text{N}$ values of N₂O could be

modified by N₂O cycling processes downstream WWTPs including denitrification in anoxic particles and sediments.

L250: Not a strong ($r = 0.51$), but a significant correlation ($p < 0.01$) – Thus, I would rephrase “N₂O concentrations showed a significant positive correlation [...]”

Text has been modified.

L254: Did you observe correlations between NH₄⁺ and/or NO₂⁻ concentrations with N₂O?

NH₄⁺ and NO₂⁻ concentrations were measured at a few selected stations. Their concentrations were much smaller than NO₃⁻ alone, mostly accounting for less than 10% of the DIN concentration. In addition, there was no clear correlations between NH₄⁺ and N₂O or NO₂⁻ and N₂O (see the Figure R9 below).

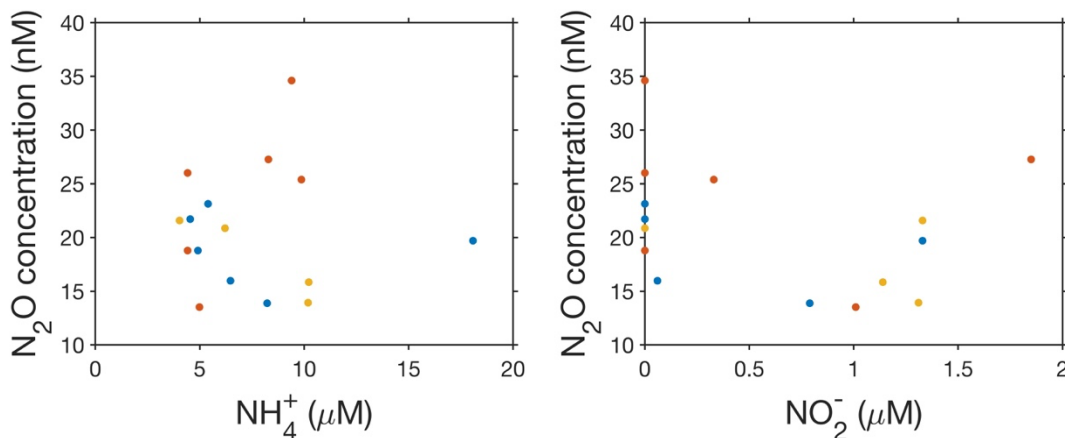


Figure R9. Relationship between N₂O and NH₄⁺ concentrations, and between N₂O and NO₂⁻ concentrations.

Figure 3: Why is Chlorophyll a in brackets?

We have changed to use the full name of Chlorophyll a in the figure.

L292: “WWTPs”

Modified.

L299: Did you use the prediction with or without WWTPs?

The embayment station in the Occoquan River was not in the downstream of WWTPs. Thus, we used the predictive model built upon stations without WWTPs. For comparison, we have now made predictions for another station in the Pohick Creek that is downstream of Noman Cole WWTP, using the predictive model built upon stations with WWTPs. There was a decrease in N₂O concentrations following the decline of total N concentrations at the Pohick Bay sampling station (see Figures R10-11 below and modified Supplementary Figures 6 and 7).

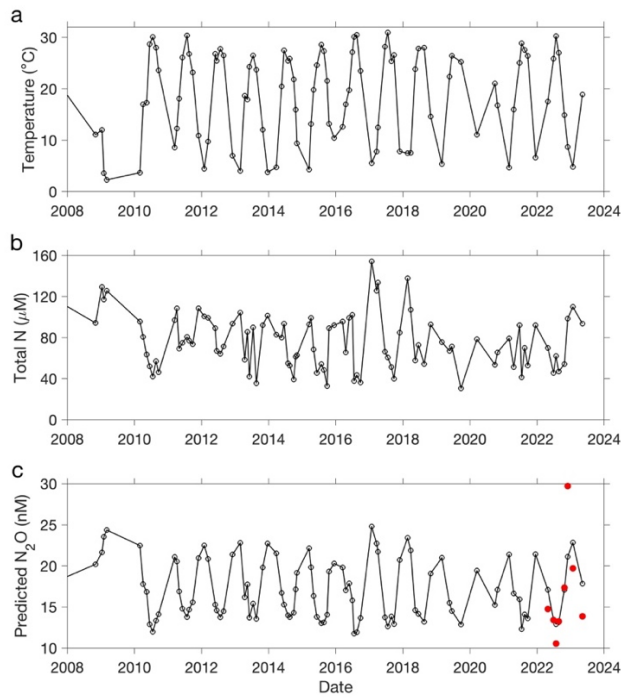


Figure R10. Historical measurements of temperature (a) and N concentration (b) at the Occoquan Bay sampling station without the influence of WWTPs. N₂O concentration (c) is predicted based on a multiple linear regression model developed for stations without the influence of WWTPs. The red points are the observed N₂O concentration.

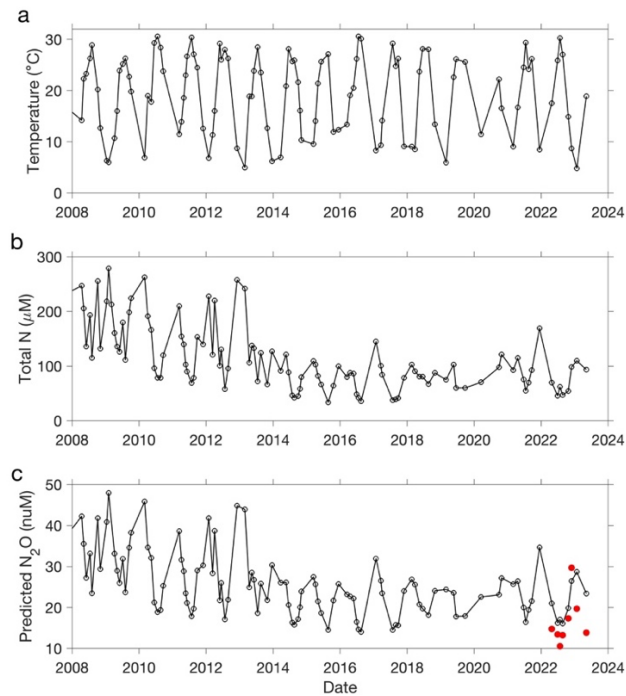


Figure R11. Historical measurements of temperature (a) and N concentration (b) at the Pohick Bay sampling station with the influence of Noman Cole WWTP. N₂O concentration (c) is

predicted based on a multiple linear regression model developed for stations with the influence of WWTPs. The red points are the observed N₂O concentrations.

L317: Did you consider tidal state during your sampling (e.g. always sampled at same tidal state)?

We clarified in lines 126-128: “While estuarine N₂O concentrations could be affected by tides (Gonçalves et al., 2015), sampling was not always conducted at the same tidal state due to logistic difficulties”.

L334-335: Remove space between “NO_x” and “;”

There was no space between NO_x and “;”. It was because of the different lines.

L357-359: Brown et al. (2022) also found estuarine type, mixing regime and stratification important factors controlling N₂O emissions.

We have added these factors and cited Brown et al. (2022) in the text.

Supplementary Material S24: “δ¹⁵N of NO_x concentration (a) and N₂O concentration (b)”

Caption of this Supplementary Figure has been clarified: “The change in δ¹⁵N of N₂O in relation to the changes in NO_x⁻ concentrations (a) and N₂O concentrations (b).”

Supplementary Material Fig. 3: Why is Chlorophyll a in brackets?

Full name of Chlorophyll a is now shown in the figure.

Supplementary Material L33: “[...] the influence of WWTPs [...]”

Text was modified as suggested.

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