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 their valuable and insightful comments! The main suggestions include the quantification of the dilution of WWTPs effluents by river flows and better estimates of N_2O emissions using multiple gas transfer coefficient parameterizations. We have responded to reviewers' comments below in blue font and made changes accordingly in the manuscript.

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 acknowledged that the different types of treatment affect the N_2O production yield in the WWTPs in the text (de Haas and Andrews. 2022; Zhao et al., 2024).

For evaluating the dilution effect, we obtained volume discharge and total N in treated water of each WWTP from Virginia Pollutant Discharge Elimination System and we have included these information in the revised manuscript: 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 N_2O concentrations and emissions (Figures R1-R3 below). In addition, total nitrogen concentrations were available from the monitor station upstream of Mooney WWTP (Richmond Highway, Virginia). We then compared the total N flow between the Neabsco Creek flow and Mooney WWTP effluent.

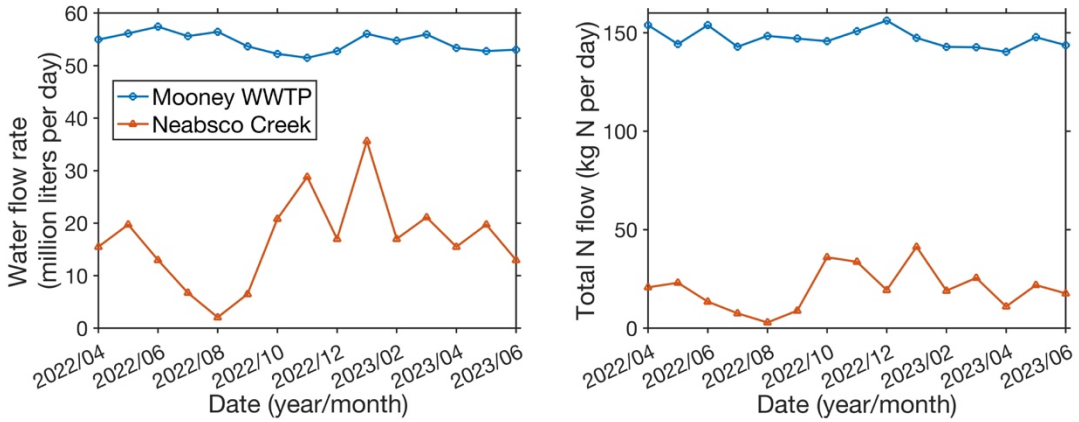

Figure R1. Comparison of water flows and total nitrogen inputs from Mooney WWTP effluent and Neabsco Creek. Climatological river flow rates were used for Neabsco Creek because river flow data were not available for years 2022 and 2023.

The volume and nitrogen discharge of Mooney WWTP effluent were always higher than the Neabsco Creek (Figure R1 above). Therefore, the dilution of N_2O 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 below). The high dilution by the river flow likely diminished the N_2O 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 N_2O concentrations were observed downstream Mooney WWTP in August when the river flow was low.

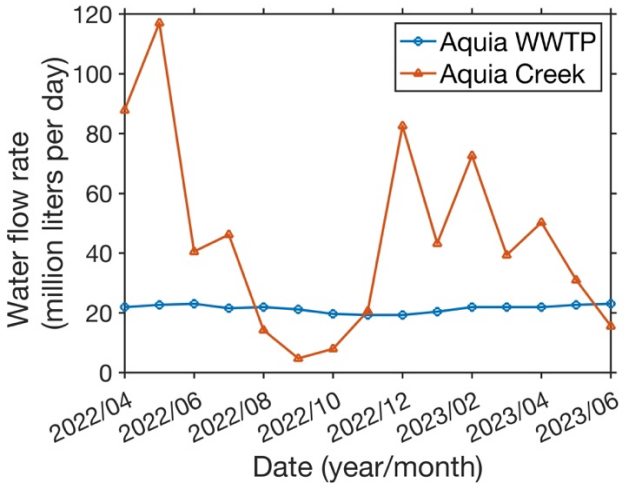

Figure R2. Comparison of water flows from Aquia WWTP effluent and Aquia Creek.

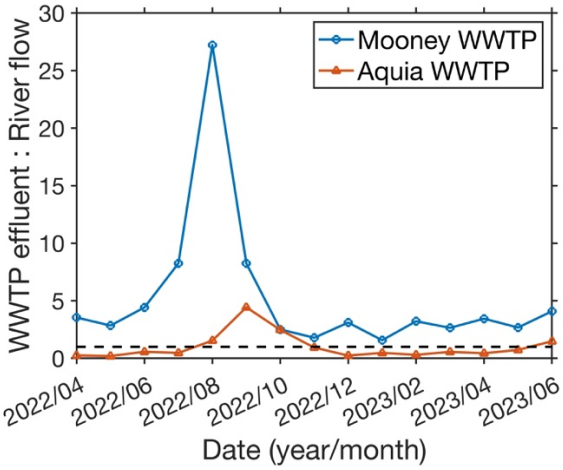

Figure R3. The ratio of WWTP effluent to river flow.

The water column depths of sampling stations have been added to the manuscript: "The embayment stations were 2-3 meters deep while the average depth of central channel stations was around 8 meters". Sedimentary N_2O production may supply N_2O to the water column and further N_2O 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 N_2O 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: "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 N_2O 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 N_2O 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 N_2O in 3 mL air headspace was generally less than 4% of the amount of N_2O dissolved in the 57 mL water samples. Thus, the effect of 3 mL air on N_2O measurements was minor and was accounted for the concentration calculations. The similar sampling method has previously been used (e.g., Kelly et al., 2020). We have added this description in the manuscript.

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/rivers (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: "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 N_2O 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: “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-Rodriguez 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).

$k_{600} = 1.91 \times e^{0.35 \times U}$ (Raymond and Cole, 2001)
$k_{600} = 0.314 \times U^2 - 0.436 \times U + 3.99$ (Jiang et al., 2008)
$k = k_{600} \times \left(\frac{Sc}{600}\right)^{-0.5}$
$k = 0.251 \times U^2 \times \left(\frac{Sc}{660}\right)^{-0.5}$ (Wanninkhof, 2014)

Average values of the three estimates are presented in the manuscript and estimates of each parameterization are provided in the associated dataset.

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 ≤ 5 µmol 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 N_2O 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 NOx, which could indicate that nitrification is an important process in these N2O production stations.

We have now added in the text: "Although previous studies have showed dissolved oxygen to be an important driver of N_2O 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 N_2O 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 N_2O 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: "Predictions were performed separately for stations with WWTPs ($N_2O\ concentration = 0.115 \times total\ N - 0.241 \times temperature + 17.185$, 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. (see Figures R4-R5 below).

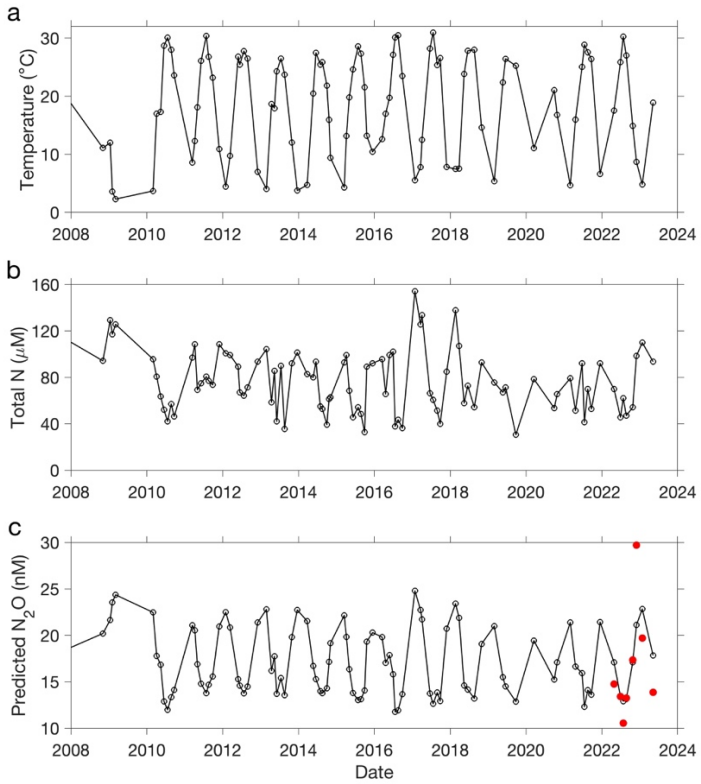

Figure R4. Historical measurements of temperature (a) and N concentration (b) at the Occoquan Bay sampling station without the influence of WWTPs. N_2O concentration (c) is predicted based on a multiple linear regression model developed for stations without the influence from WWTPs. The red points are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

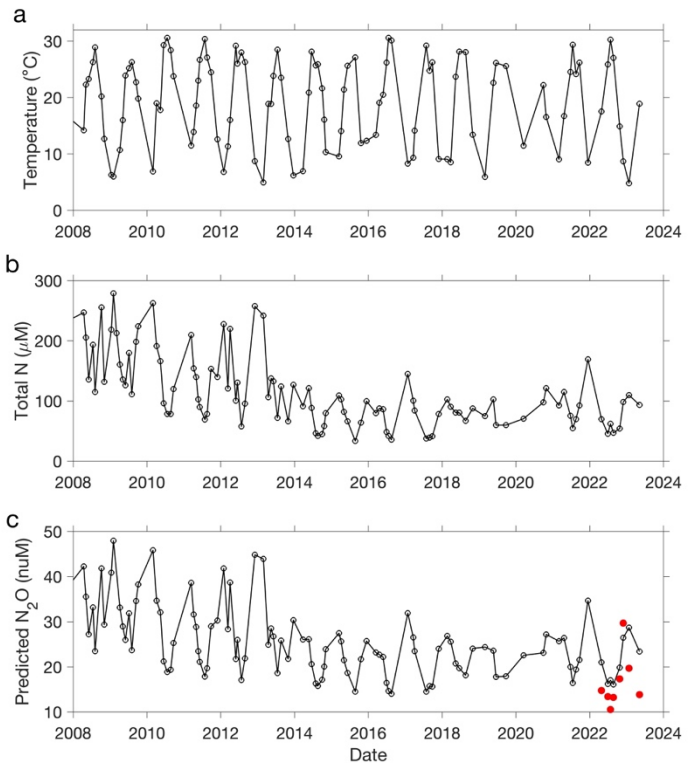

Figure R5. Historical measurements of temperature (a) and N concentration (b) at the Pohick Bay sampling station with the influence of Noman Cole WWTP. N_2O concentration (c) is predicted based on a multiple linear regression model developed for stations with the influence from WWTPs. The red points are the observed N_2O 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 NOx- 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.

References:

Andrews, A., Crotwell, A., Crotwell, M., Handley, P., Higgs, J., Kofler, J., Lan, X., Legard, T., Madronich, M., McKain, K., Miller, J., Moglia, E., Mund, J., Neff, D., Newberger, T., Petron, G., Turnbull, J., Vimont, I., Wolter, S., & NOAA Global Monitoring Laboratory. (2023). NOAA Global Greenhouse Gas Reference Network Flask-Air PFP Sample Measurements of N2O at Tall Tower and other Continental Sites, 2005-Present [Data set]. NOAA GML. https://doi.org/10.15138/C11N-KD82 Version: 2023-08-23.

de Haas, D., & Andrews, J. (2022). Nitrous oxide emissions from wastewater treatment-Revisiting the IPCC 2019 refinement guidelines. *Environmental Challenges*, *8*, 100557.

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.

Raymond, P. A., & Cole, J. J. (2001). Gas exchange in rivers and estuaries: Choosing a gas transfer velocity. *Estuaries*, *24*(2), 312-317.

Rosentreter, J. A., Laruelle, G. G., Bange, H. W., Bianchi, T. S., Busecke, J. J., Cai, W. J., ... & Regnier, P. (2023). Coastal vegetation and estuaries are collectively a greenhouse gas sink. *Nature Climate Change*, *13*(6), 579-587.

Wanninkhof, R.: Relationship between wind speed and gas exchange over the ocean revisited, Limnol. Oceanogr. Methods, 12, 351-362, 2014.

Zhao, Y. W., Du, L. L., Hu, B., Lin, H. Y., Liang, B., Song, Y. P., ... & Wang, H. C. (2024). Impact of influent characteristics and operational parameters on nitrous oxide emissions in wastewater treatment: Strategies for mitigation and microbial insights. *Current Research in Biotechnology*, 100207.