Reviewer 2:
The manuscript "Variable contribution of wastewater treatment plan 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 WTTPs 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 WTTP 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 WTTP 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 their great comments on differences in the wastewater treatment processes among WWTPs and the dilution effect on N_2O concentrations by riverine discharge. The first reviewer also had the similar comments. We have responded to the reviewer's 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 were able to obtain the volume discharge and total N in treated water of each WWTP from Virginia Pollutant Discharge Elimination System and have included that 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). 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.

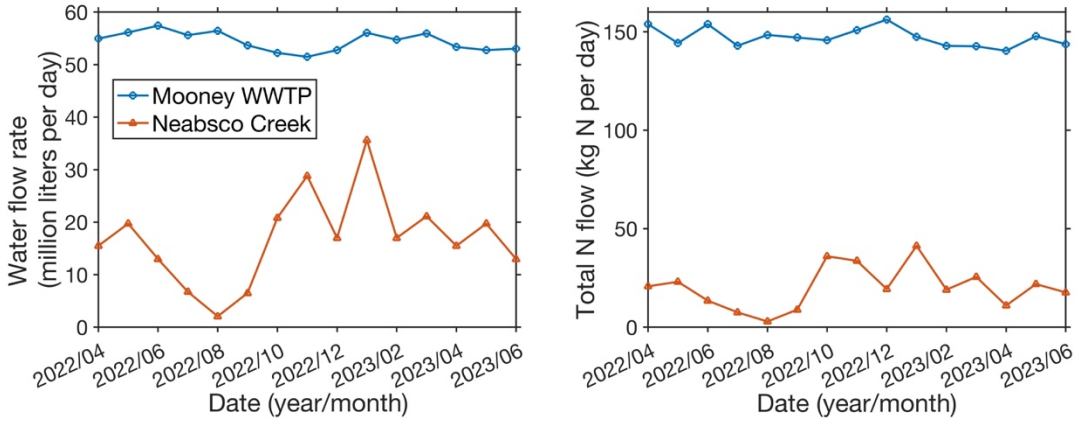

Figure R1. Comparison of water flows and total nitrogen inputs from Mooney WWTP effluent and Neabsco Creek.

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 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 N_2O 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.

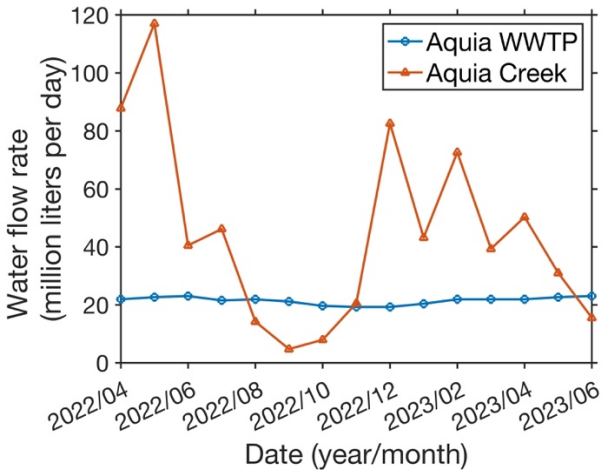

Figure R2. Comparison of water flows from Aquia WWTP effluent and Aquia Creek.

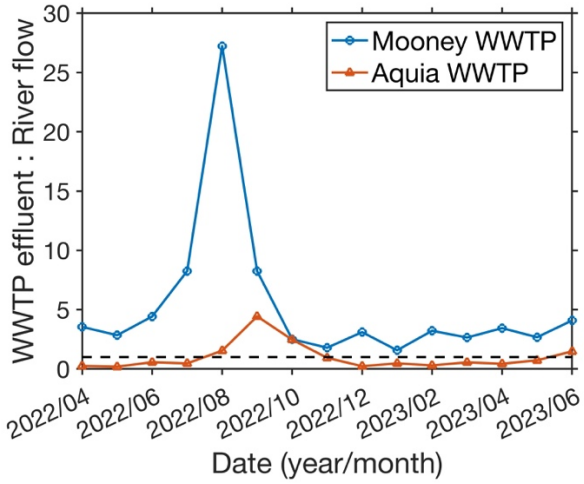

Figure R3. The ratio of WWTP effluent to river flow. The horizontal dashed line denotes a ratio of 1.

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 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 $m^3 s^{-1}$ with a large interannual variability (Jaworski et al., 2007). The annual total nitrogen loading is estimated to be around 27.7 $\times 10^6$ 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 sampled 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: "While estuarine N_2O concentrations could be affected by tides (Gonçalves et la., 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 N_2O 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 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).

L151: Did you measure replicates for N2O isotopes?

Yes, this is now clarified in the text.

L169-170: Why did you decide to use Wannikhof'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 N_2O fluxes. Average values of thee three estimates are presented in the manuscript and estimate of each parameterization is provided in the associated dataset.

$k_{600} = 1.91 \times e^{0.35 \times U}$ (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)

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 the text: 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 N2O 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.

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 reflects in seasonal changing δ15N-N2O values?

Yes, we saw a seasonal change in δ15N-N2O at stations downstream of WWTPs. We have added in the text: "δ^{15}N of N_2O 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 versus riverine discharge (Supplementary Figure 2c). For example, relatively larger WWTPs' effluents led to larger δ^{15}N of N_2O in summer when riverine flows were smaller. However, no clear seasonal pattern of δ^{15}N of N_2O 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 N_2O 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 N_2O 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 $\delta^{15}N$ of N_2O values. Thus, we focused on the spatiotemporal variation in $\delta^{15}N$ of N_2O.

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. N_2O close to WWTPs’ effluents had elevated $\delta^{15}N$ values compared to upstream stations (Figure 4b) suggested that at

least part of N_2O consumption occurred in the WWTPs. The $\delta^{15}N$ values of N_2O could be modified by N_2O 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?

NH_4^+ and NO_2^- concentrations were measured at a few selected stations. Their concentrations were much smaller than NO_3^- alone, mostly accounting for less than 10% of the DIN concentration. In addition, there was no clear correlations between NH_4^+ and N_2O or NO_2^- and N_2O (see Figure R4 below).

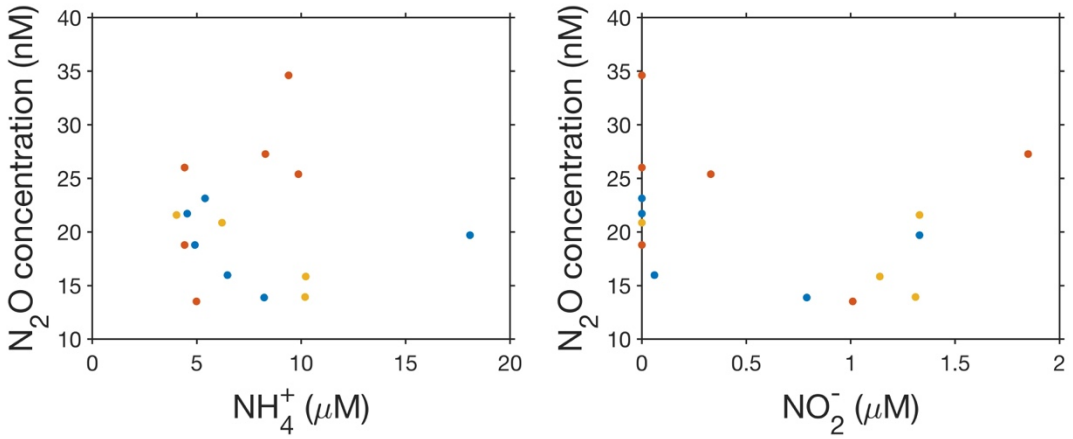

Figure R4. Relationship between N_2O and NH_4^+ concentrations, and between N_2O and NO_2^- concentrations.

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. (see Figures R5-R6 below).

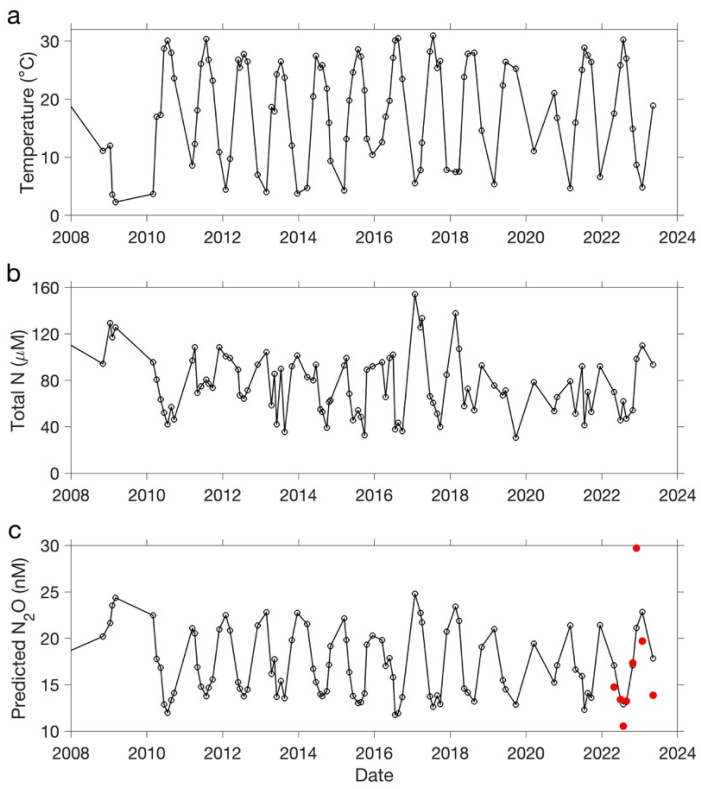

Figure R5. Historical measurements of temperature (a) and N concentration (b) at the Occoquan Bay sampling station without the influence of WWTPs. N_2O concentration (c) is predicted based on a multiple linear regression model developed for stations without the influence from WWTPs. The red points are the observed N_2O concentration.

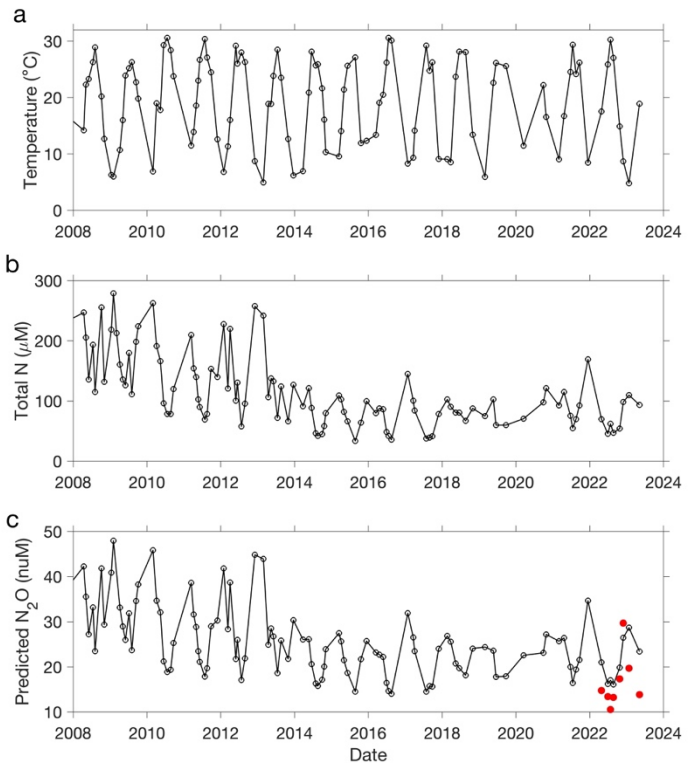

Figure R6. Historical measurements of temperature (a) and N concentration (b) at the Pohick Bay sampling station with the influence of Noman Cole WWTP. N_2O concentration (c) is predicted based on a multiple linear regression model developed for stations with the influence from WWTPs. The red points are the observed N_2O concentrations.

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

We clarified in the text: "While estuarine N_2O concentrations could be affected by tides (Gonçalves et la., 2015), sampling was not always conducted at the same tidal state due to logistic difficulties".

L334-335: Remove space between "NOx-" and ","

There was no space between NOx- 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 NOx concentration (a) and N2O concentration (b)"

Caption of this Supplementary Figure has been clarified: "The change in $\delta^{15}N$ of N_2O in relation to the changes in NO_x^- concentrations (a) and N_2O 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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