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 N_2O emissions. Therefore, this manuscript will be of interest in the context of global N_2O 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 N_2O 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 N_2O concentrations affected by WWTPS between dry and wet season in an Australian estuary.

Specific remarks:

L63: "[…] are highly variable, and are normally […]"

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

L84: "[…] nitrogen effluent concentration below 7.5 mg L^{-1} […]"

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?

L110: Did you take replicates?

L110-111: Did you measure N_2O concentrations in air headspace for correction? How did you estimate/measure atmospheric N_2O concentrations?

L151: Did you measure replicates for N_2O isotopes?

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).

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?

L149: How did you take the amount of N_2O in the 3 mL headspace into account?

L171: How did you calculate the Schmidt number?

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.

L190-191: Does this also reflects in seasonal changing $\delta^{15}N\text{-}N_2O$ values?

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

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?

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.

L233: Do you observe seasonal changes?

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. N_2O production and N_2O 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?

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?

L250: Not a strong ($r = 0.51$), but a significant correlation ($p<0.01$) – Thus, I would rephrase "N_2O concentrations showed a significant positive correlation […]"

L254: Did you observe correlations between NH_4^+ and/or NO_2^- concentrations with N_2O?

Figure 3: Why is Chlorophyll a in brackets?

L292: "WWTPs"

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

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

L334-335: Remove space between "NO_x^-" and ","

L357-359: Brown et al. (2022) also found estuarine type, mixing regime and stratification important factors controlling N_2O emissions.

Supplementary Material S24: "$\delta^{15}N$ of NO_x concentration (a) and N_2O concentration (b)"

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

Supplementary Material L33: "[…] the influence of WWTPs […]"

References

Brown, A. M., Bass, A. M., and Pickard, A. E.: Anthropogenic-estuarine interactions cause disproportionate greenhouse gas production: A review of the evidence base, Mar. Pollut. Bull., 174, 113240, https://doi.org/10.1016/j.marpolbul.2021.113240, 2022.

Clark, J. F., Schlosser, P., Simpson, H. J., Stute, M., Wanninkhof, R., and Ho, D. T.: Relationship between gas transfer velocities and wind speeds in the tidal Hudson River determined by the dual tracer technique, in: Air-Water Gas Transfer, edited by: Jähne, B. and Monahan, E. C., AEON Verlag, Hanau, 785–800, 1995.

Liu, T., Xia, X., Liu, S., Mou, X., and Qiu, Y.: Acceleration of denitrification in turbid rivers due to denitrification occurring on suspended sediment in oxic waters, Environ. Sci. Technol., 47, 4053–4061, https://doi.org/10.1021/es304504m, 2013.

Murray, R. H., Erler, D. V., Rosentreter, J., Wells, N. S., and Eyre, B. D.: Seasonal and spatial controls on N2O concentrations and emissions in low-nitrogen estuaries: Evidence from three tropical systems, Mar. Chem., 221, 103779, https://doi.org/10.1016/j.marchem.2020.103779, 2020.

Raymond, P. A. and Cole, J. J.: Gas exchange in rivers and estuaries: Choosing a gas transfer velocity, Estuaries, 24, 312–317, https://doi.org/10.2307/1352954, 2001.

Schulz, G., Sanders, T., van Beusekom, J. E. E., Voynova, Y. G., Schöl, A., and Dähnke, K.: Suspended particulate matter drives the spatial segregation of nitrogen turnover along the hyper-turbid Ems estuary, Biogeosciences, 19, 2007–2024, https://doi.org/10.5194/bg-19-2007-2022, 2022.

Seitzinger, S. P.: Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance, Limnol. Oceanogr., 33, 702–724, https://doi.org/10.4319/lo.1988.33.4part2.0702, 1988.

Winkler, M. K. H. and Straka, L.: New directions in biological nitrogen removal and recovery from wastewater, Curr. Opin. Biotechnol., 57, 50–55, https://doi.org/10.1016/j.copbio.2018.12.007, 2019.

Zhou, Y., Zhu, Y., Aber, J., Li, C., and Chen, G.: A Comprehensive Review on Wastewater Nitrogen Removal and Its Recovery Processes, Int. J. Environ. Res. Public. Health, 20, 3429, https://doi.org/10.3390/ijerph20043429, 2023.

Zhu, W., Wang, C., Hill, J., He, Y., Tao, B., Mao, Z., and Wu, W.: A missing link in the estuarine nitrogen cycle?: Coupled nitrification-denitrification mediated by suspended particulate matter, Sci. Rep., 8, 2282, https://doi.org/10.1038/s41598-018-20688-4, 2018.