



Variable contribution of wastewater treatment plant effluents to downstream nitrous oxide concentrations and emissions

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Abstract. Nitrous oxide (N_2O), a potent greenhouse gas and ozone-destroying agent, is produced during nitrogen transformations in both natural and human-constructed environments. Wastewater treatment plants (WWTPs) produce and emit N_2O into the atmosphere during the nitrogen removal process. However, the impact of WWTPs on N_2O emissions in downstream aquatic systems remains poorly constrained. By measuring N_2O concentrations at a monthly resolution over a year in the Potomac River estuary, a tributary of the Chesapeake Bay in the eastern United States, we found a strong seasonal variation in N_2O concentrations and fluxes: N_2O concentrations were larger in fall and winter, but the flux was larger in summer and fall. Observations at multiple stations across the Potomac River estuary revealed hotspots of N_2O emissions downstream of WWTPs. N_2O concentrations were higher at stations downstream of WWTPs compared to other stations (median: 21.2 nM vs. 16.2 nM) despite the similar concentration of dissolved inorganic nitrogen, suggesting the direct discharge of N_2O from WWTPs into the aquatic system or a higher N_2O production yield in waters influenced by WWTPs. Meta-analysis of N_2O measurements associated with WWTPs globally revealed variable influence of WWTPs on downstream N_2O concentrations and emissions. Since wastewater production has increased substantially with the growing population and is projected to continue to rise, accurately accounting for N_2O emissions downstream of WWTPs is important for constraining and predicting future global N_2O emissions. Efficient N_2O removal, in addition to dissolved nitrogen removal, should be an essential part of water quality control in WWTPs.

1 Introduction

Nitrogen (N) enters the aquatic environment from agricultural and urban runoff, atmospheric deposition, and wastewater treatment plants (WWTPs), potentially leading to eutrophication, especially in densely populated regions (Galloway et al., 2008; Morée et al., 2013). During microbial transformations of N in aquatic systems (e.g., nitrification and denitrification), a powerful greenhouse gas and ozone depleting agent – N_2O – is produced (Quick et al., 2019). Aquatic systems are large but highly variable sources of N_2O to the atmosphere (Wang et al., 2023). For example, on a global basis, 0.04–0.291 and 0.04–3.6 Tg N yr^{−1} of N_2O are estimated to outgas from rivers and estuaries, respectively (Murray et al., 2015; Maavara et al., 2019; Yao et al., 2019; Rosentreter et al., 2023). The high end of the estimates in these inland and coastal waters approaches the scale of the global marine N_2O emissions (2.5–4.3 Tg N yr^{−1} in Tian et al., 2020). The large uncertainty in the estimate of aquatic N_2O emission is partly due to high spatial and temporal variabilities of N_2O flux within/across rivers and estuaries and the lack of observations to capture such variability. Therefore, sampling and measurements of N_2O concentration at high spatial and temporal resolutions would be desirable to constrain aquatic N_2O emission.

The major factors that appear to correlate with N_2O concentration are dissolved inorganic nitrogen (DIN) and oxygen status (Hu et al., 2016; Zheng et al., 2022). Waste and wastewater release large amounts of DIN into the aquatic environment. In addition, waste and wastewater emit ~ 0.3 Tg N yr^{−1} of N_2O (estimated from 2007–2016) into the atmosphere globally, an amount that is continuously increasing at a rate of 0.04 ± 0.01 Tg N yr^{−1} per decade (Tian et al.,

2020). N₂O emission from WWTPs accounts for $\sim 5.2\%$ of total N₂O emission in 2021 in the United States (US EPA, 2023). N₂O emissions from different WWTPs are highly variable and are normally calculated as a function of DIN loading into the WWTPs, using an N₂O emission factor (Kampschreur et al., 2009). N₂O emission factors range from 0.16 % to 4.5 % (N₂O emitted/DIN loading) (Eggleston et al., 2006; de Haas and Andrews, 2022). In addition to direct emission from the WWTPs, N₂O can be discharged via WWTP effluent and produced due to DIN release from WWTP effluent into the creeks, rivers, and other downstream aquatic systems (McElroy et al., 1978; Beaulieu et al., 2010; Masuda et al., 2018). However, the impact of WWTPs on downstream N₂O concentration is less studied, and the downstream N₂O emission remains poorly constrained. Here we specifically compared the N₂O concentration upstream and downstream of the WWTPs in order to assess the impact of WWTPs on N₂O emission, which could help to constrain the emission factor associated with the WWTP effluents.

The Potomac River is a major tributary of the Chesapeake Bay – the largest estuary in the United States. The Potomac River estuary is located in a highly populated area, mainly surrounded by Washington, DC, and the states of Virginia and Maryland in the eastern United States. The annual mean discharge of the Potomac River from 1895 to 2002 measured at the Chain Bridge near Washington, DC, was $321 \text{ m}^3 \text{ s}^{-1}$ with a large interannual variability (Jaworski et al., 2007). The annual total nitrogen loading was estimated to be around $27.7 \times 10^6 \text{ kg N yr}^{-1}$ in 2008–2009 (Bricker et al., 2014). The Potomac River estuary has experienced ecological degradation for decades partly due to excess nutrient inputs including from the effluents of WWTPs (Bricker et al., 2014; Jaworski et al., 2007). For example, the Blue Plains Advanced WWTP in Washington, DC, is one of largest WWTPs in the world, treating an average of $\sim 1.4 \times 10^9 \text{ L}$ of water per day. Pioneering work in 1978 showed that Blue Plains WWTP was a large source of nitrogen to the Potomac River estuary, triggering high N₂O production and concentration downstream (McElroy et al., 1978). Thanks to higher standards mandated by governmental agencies (nitrogen concentration in effluents below 7.5 mg L^{-1}) starting in the 1980s and the technical improvements in N removal from the wastewater, the nitrogen concentration in effluents of WWTPs in the Potomac River has decreased substantially (Pennino et al., 2016). However, the concurrent effect on N₂O concentration is largely unknown. The Department of Environmental Quality (DEQ) of Virginia maintains an approximately monthly routine monitoring program for water quality (e.g., nitrogen concentration, phosphorus concentration, chlorophyll concentration) and physical properties (e.g., temperature, salinity, pH, and dissolved oxygen concentration) in the Potomac River estuary but not for N₂O. Therefore, we collaborated with the DEQ of Virginia to measure the spatial and temporal variation of N₂O concentrations in the Potomac River estuary.

2 Materials and methods

2.1 Sample collection for N₂O and nutrients

Surface waters at $\sim 0.5 \text{ m}$ depth at 11 stations in the tidal Potomac River estuary were sampled monthly or bimonthly (depending on the weather) on a vessel (Grady-White 208) for the analysis of DIN concentration and both concentration and nitrogen isotopes of N₂O from April 2022 to May 2023 (Fig. 1). The 11 stations are characterized into 3 groups: embayment downstream of WWTPs, embayment not associated with WWTPs, and the central channel of the Potomac River. Three embayment stations downstream of WWTPs are associated with three different WWTPs: Noman Cole, Mooney, and Aquia, all of which implement tertiary treatment of the wastewater. We obtained the volume discharge and total N in treated water of each WWTP from the discharge monitoring report required by the Virginia Pollutant Discharge Elimination System permit. Noman Cole WWTP discharges $\sim 1.4 \times 10^8 \text{ L}$ of water and 370 kg N d^{-1} into the Pohick Creek. Mooney WWTP discharges $\sim 5.5 \times 10^7 \text{ L}$ of water and 147 kg N d^{-1} into the Neabsco Creek. Aquia WWTP discharges much less water and N into the Aquia Creek ($\sim 2.1 \times 10^7 \text{ L}$ per day and 35 kg N d^{-1}). The distances from the embayment stations downstream of WWTPs to Noman Cole, Mooney, and Aquia WWTPs were approximately 4, 1.8, and 5.8 km, respectively.

The embayment stations were 2–3 m deep, while the average depth of central channel stations was around 8 m. The embayment stations have been routinely sampled for water quality analyses by the DEQ of Virginia since the early 1970s. The central channel stations were added for this study. The purposes of this sampling design are to evaluate the impact of WWTPs on downstream distribution of DIN and N₂O and to compare DIN and N₂O concentrations between the edge and central channel of the river. The central channel is likely affected both by the Potomac mainstem flow and by the input from tributaries, while the embayment stations may be mainly affected by water flow from tributaries but also influenced by the tidal cycle (see the salinity change in Fig. S1b in the Supplement). 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. Triplicate water samples for N₂O concentrations and isotopes were collected via a submersible pump into 60 mL serum bottles after overflowing 3 times the bottle's volume. After removing 3 mL water to create a 3 mL air headspace via a syringe, the serum bottles were immediately sealed with butyl stoppers and aluminum crimps and preserved with 0.5 mL of 10 M NaOH solution to stop biological activities. NaOH has been shown to be an effective and less environmentally hazardous preservative for N₂O and nutrient analysis (Frame et al., 2016; Wong et al., 2017).

In addition to the routine sampling in the Potomac River estuary, we also sampled its tributaries, some of which were

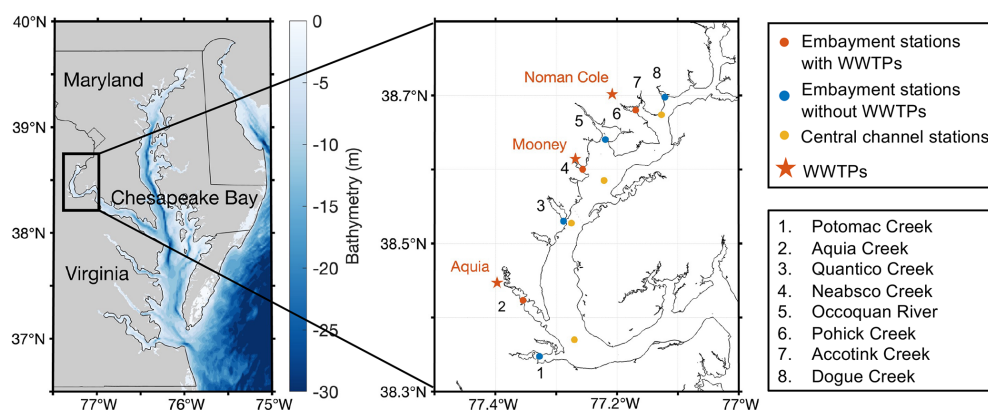


Figure 1. Sampling stations in the Potomac River estuary including embayment stations with WWTPs (red circles) and without WWTPs (blue circles) and central channel stations (yellow circles). Locations of WWTPs (Noman Cole, Mooney, and Aquia) are shown in red stars. Creeks/ivers with sampling stations are numbered in the map with names shown in the legend. Stream sampling sites upstream and downstream of WWTPs in creeks 4–7 are shown in Fig. 4 below.

associated with the WWTPs, on 18 May 2023 (Fig. 1) to specifically evaluate the impact of WWTPs on downstream N₂O concentrations. Four creeks/ivers were sampled, including the Neabsco Creek (five stations: two stations upstream and three stations downstream of Mooney WWTP), the Occoquan River (three stations, no WWTP), the Pohick Creek (four stations: two stations upstream and two stations downstream of Noman Cole WWTP), and the Accotink Creek (two stations, no WWTP). Because Aquia WWTP discharges much less water and N into the Aquia Creek, its impact was not specifically investigated. Since water depths of these creeks/ivers were shallow, the water samples were collected by directly submerging 60 mL serum bottles into the surface water (~ 0.1 m) and preserving them as described above.

Besides N₂O sampling, temperature, salinity, and dissolved O₂ concentrations were recorded via a YSI EXO1 sonde. Chlorophyll-*a* samples (300 mL) were filtered onto GF/F filters and kept on ice in a cooler. The filters were then kept frozen at -20°C in the lab until analysis within 3 months (Arar and Collins, 1997). One additional sample for total nitrogen and phosphorus (both particulate and dissolved) was collected into 250 mL HDPE bottles and kept on ice in a cooler until analysis within 48 h on land (Rice et al., 2012; US EPA, 1974). Total nitrogen is the sum of total Kjeldahl nitrogen and nitrite plus nitrate.

2.2 Measurement of N₂O and nutrient concentrations

N₂O in the serum bottles was stripped by helium carrier gas into a DELTA V Plus mass spectrometer (Thermo Fisher Scientific) for the analyses of N₂O concentration and isotope ratio ($m/z = 44, 45, 46$) (Tang et al., 2022). The total amount of N₂O in the serum bottles was determined using a standard curve of N₂O peak area with N₂O standards containing a known amount of N₂O reference gas (0, 0.207, 0.415, 0.623,

0.831, 1.247 nmol N₂O). The total amount of N₂O dissolved in the water was calculated after subtracting the amount of N₂O in a 3 mL air headspace. The amount of N₂O in a 3 mL air headspace was generally less than 4 % of the amount of N₂O dissolved in the 57 mL water samples. The N₂O concentration in samples was then calculated from the total amount of N₂O dissolved in the water divided by the volume of water in the serum bottles. The detection limit and precision of N₂O concentration measurement were 1.29 and 0.33 nM, respectively. We used N₂O produced from nitrate isotope standards (USGS34 = -1.8‰ and IAEA = 4.7‰) to calibrate for $\delta^{15}\text{N}$ of N₂O samples. We then estimated N₂O saturation (%): $\frac{\text{N}_2\text{O}_{\text{measured}}}{\text{N}_2\text{O}_{\text{equilibrium}}} \times 100$. The equilibrium N₂O concentration (N₂O_{equilibrium}) was calculated based on the solubility of N₂O and atmospheric N₂O concentrations (Weiss and Price, 1980). The monthly atmospheric N₂O concentrations were obtained from the nearby atmospheric station in Brentwood, Maryland (<https://gml.noaa.gov/>, last access: 8 May 2024) (Andrews et al., 2023).

After analyzing N₂O concentration, samples were neutralized to a pH of ~ 7 by adding 10 % hydrochloric acid. NO₂[−] + NO₃[−] (NO_x[−]) concentration in these samples was measured using the vanadium (III) reduction method by converting NO_x[−] to NO, which was then quantified by a chemiluminescence analyzer (Braman and Hendrix, 1989). The detection limit of NO_x[−] concentration was 0.15 μM . NH₄⁺ and NO₂[−] concentrations were measured at a few selected stations using the fluorometric *ortho*-phthalaldehyde method (Holmes et al., 1999) and the colorimetric method (Hansen and Koroleff, 1999), respectively. Their concentrations were much smaller than NO₃[−] alone, mostly accounting for less than 10 % of the DIN concentration. Therefore, we only present NO_x[−] data in this study.

2.3 N₂O flux calculation

Surface N₂O flux was calculated using the following equation: $\text{Flux} = k \times (\text{N}_2\text{O}_{\text{measured}} - \text{N}_2\text{O}_{\text{equilibrium}})$. The gas transfer velocity (k) was estimated based on three different parameterizations: $k = 1.91 \times e^{0.35 \times U} \times \left(\frac{Sc}{660}\right)^{-0.5}$ (Raymond and Cole, 2001), $k = (0.314 \times U^2 - 0.436 \times U + 3.99) \times \left(\frac{Sc}{660}\right)^{-0.5}$ (Jiang et al., 2008), and $k = 0.251 \times U^2 \times \left(\frac{Sc}{660}\right)^{-0.5}$ (Wanninkhof, 2014). U is the wind speed at the 10 m height obtained from the National Centers for Environmental Prediction (NCEP) reanalysis (Kalnay et al., 1996; <https://psl.noaa.gov/data/gridded/data.ncep.reanalysis.html>, last access: 8 May 2024). Sc is the Schmidt number that could be estimated as a function of temperature (Wanninkhof, 2014). Since our samples have a salinity close to 0, we used the parameterization of Sc for freshwater. Average values of the three N₂O flux estimates are presented in the paper, and N₂O fluxes estimated by different parameterizations are provided in the associated dataset. We acknowledge large variations in estimating k values in the riverine and estuarine systems by using different empirical models (Raymond and Cole, 2001; Borges et al., 2004; Rosentreter et al., 2021). For instance, the effect of water velocity and water depth on gas transfer velocity was not considered in the parameterizations above. Therefore, we focus on evaluating the spatiotemporal variations in N₂O fluxes and their driving factors instead of their absolute magnitude.

3 Results and discussion

3.1 Spatial and temporal variations of N₂O concentrations in the Potomac River estuary

Along the roughly 50 km sampling transect in the Potomac River estuary, NO_x[−] concentration decreased from 98 to < 1 μM from upstream to downstream (Fig. 2a). NO_x[−] concentration showed a clear seasonal pattern: higher in winter and spring while lower in summer and fall. The spatial and temporal patterns were likely attributable to the distribution of nutrient sources into the Potomac River, DIN uptake, and other removal processes along the river (Glibert et al., 1995; Carstensen et al., 2015). For example, the maximum N loading into the Chesapeake Bay occurs in winter and spring (Da et al., 2018). Meanwhile, N₂O concentration decreased from approximately 40 to 10 nM along the sampling transect and was higher in the fall and winter (Fig. 2b). Since temperature decreased from ~ 31°C in summer to 4°C in winter (Fig. S1a), the increase in N₂O solubility in colder water during winter partly explained the seasonal change. In contrast, N₂O saturation had higher values in summer and fall (Fig. 2c), suggesting a higher N₂O production in summer and fall. It is worth noting that N₂O saturation was above

100 % at all sampling stations with a maximum reaching 500 %, indicating the Potomac River estuary was a consistent and strong source of N₂O to the atmosphere. N₂O fluxes ranged from 1 to 31.7 μmol N₂O m^{−2} d^{−1}, generally decreasing from upstream to downstream (Fig. 2d). N₂O fluxes showed a similar seasonal pattern to N₂O saturation: higher in summer and fall. N₂O concentrations (median: 18.2 nM) and fluxes (median: 5.6 μmol N₂O m^{−2} d^{−1}) in the Potomac River estuary were substantially higher than in the mainstem of the Chesapeake Bay (2.6 to 20.9 nM N₂O with a median value at 10.6 nM and −0.3 to 4.3 μmol N₂O m^{−2} d^{−1} with a median at 0.5 μmol N₂O m^{−2} d^{−1}; Tang et al., 2022; Laperriere et al., 2019). Therefore, the tributaries (i.e., the Potomac River) are more intense sources of N₂O to the atmosphere than mainstem of the bay.

Stations close to each other had similar NO_x[−] concentrations (e.g., upstream stations > downstream stations), regardless of station category (i.e., with WWTP, without WWTP, central channel of the Potomac River). In contrast, N₂O concentrations and fluxes varied within locations according to the station category: N₂O concentrations and fluxes were substantially higher at stations downstream of WWTPs ($p < 0.01$, t test). N₂O concentrations and fluxes were similar between stations in embayments without WWTPs and the central channel (Fig. 2). This suggests these WWTPs are efficient in removing DIN from sewage and other sources, but WWTPs may discharge N₂O directly into the effluent or enhance downstream N₂O production (e.g., higher N₂O production yield from the same amount of DIN). This effect extended to our sampling stations ~ 1.8–4 km downstream of the WWTPs. However, the effect of WWTPs on downstream N₂O varied among stations. For example, elevated N₂O concentrations were observed downstream of Noman Cole and Mooney WWTPs but not downstream of Aquia WWTP. This difference may be related to the different N removal processes of WWTPs that produce N₂O at different yields (de Haas and Andrews, 2022; Zhao et al., 2024). However, we do not have detailed information about the three WWTPs other than that they all implement tertiary treatment. In addition, the different dilution factors by riverine discharges also matter. For example, the volume of effluent from Mooney WWTP was higher than the discharge of the Neabsco Creek, while the volume of effluent from Aquia WWTP was generally lower than the discharge of the Aquia Creek (Fig. S2a and b). Particularly, the highest N₂O concentration of up to 40 nM was found at two stations downstream of the Noman Cole and Mooney WWTPs on 23 August 2022 when the river discharge was low (Fig. S2). Thus, the effect of WWTPs on downstream N₂O concentrations also varies seasonally (Schulz et al., 2023; Murray et al., 2020), with a relatively more important role in the dry season. Repeated spatial and temporal sampling allowed us to capture these N₂O hotspots. Previous studies have shown the impact of WWTPs on downstream N₂O concentrations and emissions in aquatic environments. For example, the highest N₂O con-

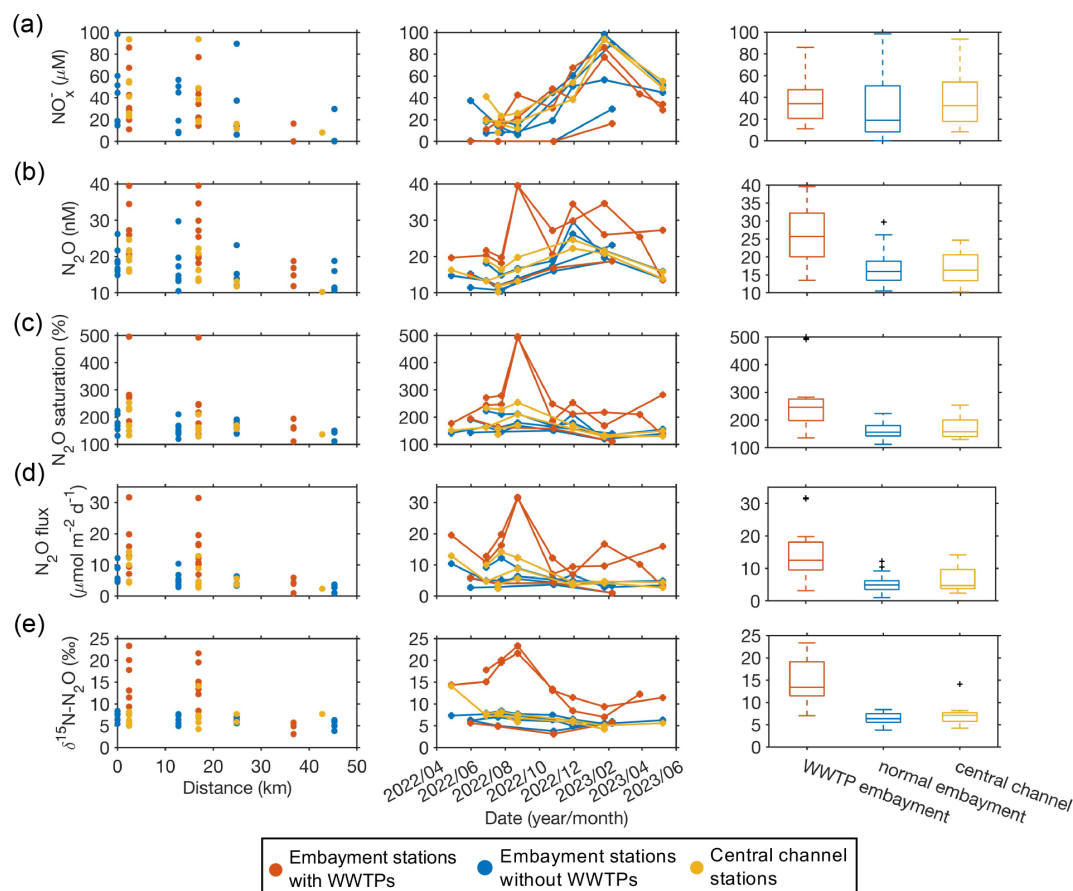


Figure 2. Spatial and temporal variations of NO_x^- concentration (a), N_2O concentration (b), N_2O saturation (c), N_2O flux (d), and $\delta^{15}\text{N}$ of N_2O (e). The distance shows, from upstream to downstream, stations in the Potomac River. Embayment stations with WWTPs (red circles and lines) and without WWTPs (blue circles and lines) and central channel stations (yellow circles and lines). For the boxplots, the red line in each box is the median. The bottom and top of each box are the 25th and 75th percentiles of the observations, respectively. The error bars represent 1.5 times the interquartile range away from the bottom or top of the box, with black + signs showing outliers beyond that range. Embayment stations associated with WWTPs had significantly higher N_2O concentration, N_2O saturation, N_2O flux, and $\delta^{15}\text{N}$ values compared to other stations ($p < 0.01$, t test) but not a significantly different NO_x^- concentration.

centration of ~ 675 nM in the Potomac River was measured near the discharge of the Blue Plains WWTP in 1977 (McElroy et al., 1978). The highest N_2O emissions in the Ohio River near Cincinnati were attributed to direct input of N_2O from WWTP effluents (Beaulieu et al., 2010).

In addition, a higher nitrogen isotopic signature ($\delta^{15}\text{N}$) of N_2O associated with WWTPs (median $\delta^{15}\text{N}$ at 13 ‰) also suggests the distinct sources or cycling processes of N_2O compared to stations of the central channel and without the influence of WWTPs (median $\delta^{15}\text{N}$ of N_2O at 6 ‰; Fig. 2e) in the Potomac River estuary. In comparison, the average $\delta^{15}\text{N}$ of N_2O in the tropospheric air is around 6.55 ‰ (Snider et al., 2015). $\delta^{15}\text{N}$ of N_2O for stations with the influence of WWTPs showed a clear seasonal variation: higher in summer than in winter (Fig. 2e). This seasonal difference may be related to the seasonal change in the relative importance of WWTP effluents versus riverine discharge (Fig. S2c). For example, a relatively larger WWTP effluent volume com-

pared to the riverine discharge led to a larger $\delta^{15}\text{N}$ of N_2O in summer. However, no clear seasonal pattern of $\delta^{15}\text{N}$ of N_2O was seen for stations without the influence of WWTPs. $\delta^{15}\text{N}$ of N_2O produced in WWTPs depends on the treatment stages and aeration conditions (Toyoda et al., 2011; Tumendelger et al., 2014). For example, the average $\delta^{15}\text{N}$ values were reported to be -24.5 ‰ and 0 ‰, respectively, for N_2O produced from nitrification during oxic treatment versus N_2O produced from anaerobic denitrification in a California WWTP (Townsend-Small et al., 2011). The $\delta^{15}\text{N}$ values of N_2O 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 ‰). One of the reasons for the increased $\delta^{15}\text{N}$ of N_2O may be partial N_2O reduction via denitrification in the WWTPs, in downstream creeks, or in sediments; this denitrification effect has been seen in the marine oxygen minimum zones (Kelly et al., 2021). Denitrification as the cause of the elevated $\delta^{15}\text{N}$ is partly supported by the

higher $\delta^{15}\text{N}$ of N₂O when NO_x^- was reduced to less than 40 μM , suggesting the occurrence of N₂O reduction when the concentration of other denitrification substrates became low (Fig. S3). However, we do not know the exact locations where denitrification occurred (e.g., WWTPs, anoxic niches in suspended particles, sediments), which deserves further investigation. The influence of denitrification on unique isotopic signatures of N₂O produced from WWTPs has also been observed in the Tama River in Japan (Toyoda et al., 2009).

3.2 Environmental controls on N₂O concentrations

N₂O concentrations showed positive correlations with total N ($r = 0.62$, $p < 0.01$) and NO_x^- concentrations ($r = 0.51$, $p < 0.01$) (Fig. 3a). Correlation analyses done separately for stations with or without WWTPs had similar patterns (Fig. S4). A better correlation between the N₂O concentration and total N may indicate the contribution of other N sources besides NO_x^- to N₂O production. N₂O could be produced from nitrification in the process of oxidizing NH_4^+ to NO_x^- in the oxic environment as previously shown in the oxygenated mainstem of the Chesapeake Bay (Tang et al., 2022). However, we cannot exclude the possibility of N₂O production from denitrification associated with anaerobic microsites in particles or in sediment (Beaulieu et al., 2011; Wan et al., 2023). Future investigations with ^{15}N tracers should be conducted to differentiate N₂O production pathways around the WWTPs. Furthermore, N₂O concentration was negatively correlated with temperature since higher temperature reduced the N₂O solubility. 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 (Fig. 3a). This lack of strong dependence is probably because of the overall oxygenated conditions (Fig. S1c) and opposite correlations found in stations without WWTPs (positive) versus in stations with WWTPs (negative) (Fig. S4), which may be influenced by the different N₂O production pathways.

The significant positive relationship between N₂O and NO_x^- concentration existed for samples collected at stations from all three different categories (Fig. 3b). N₂O concentrations at stations downstream of WWTPs were notably higher than at other stations not associated with WWTPs even in the similar range of NO_x^- concentration. The larger slope of N₂O concentration versus NO_x^- concentration at stations downstream of WWTPs may be related to the direct input of N₂O from WWTPs into the downstream waters or different N₂O production pathways and production yields that deserve further investigation. The DIN concentration has been found to be a good predictor of N₂O concentration and emission in many other rivers and estuaries (Murray et al., 2015; Reading et al., 2020; Zheng et al., 2022). However, the correla-

tion varied spatially, which may be affected by the variable N₂O emission factors from DIN cycling. The emission factors are affected by temperature, concentration, and forms of N, oxygen, and organic carbon concentration, among other factors (Hu et al., 2016). The external N₂O input (e.g., input from WWTPs) could also affect the relationship between N₂O and DIN concentrations (Dong et al., 2023). Compared to DIN (~ 28 to $71 \mu\text{M}$) and N₂O concentrations (~ 16 to 61 nM) measured approximately 45 years ago in the same section of the Potomac River (McElroy et al., 1978), current DIN and N₂O concentrations have slightly decreased. Thus, an additional benefit of nutrient regulation is the reduction of greenhouse gas – N₂O – emissions, beyond improving water quality.

Since N₂O concentrations had the strongest correlation with total N concentrations (reflecting the N₂O production potential) and temperature (affecting N₂O solubility), we developed a predictive model of N₂O concentration based on total N and temperature. Predictions were performed separately for stations with WWTPs (N₂O 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 (N₂O concentration = $0.049 \times \text{total N} - 0.298 \times \text{temperature} + 18.888$, $n = 23$, $r = 0.81$, $p < 0.01$). The observed N₂O variability was generally captured by these simple linear models (Fig. S5), but there were variabilities in the observations remaining to be explained. The addition of other predictors did not significantly improve the model performance, so we chose a simple predictive model that is mechanistically understandable. We then applied the two predictive models separately to estimate N₂O concentrations at the embayment station in the Pohick Bay (with WWTP) and the embayment station in the Occoquan Bay (without WWTP) using the total N concentration and temperature that were measured since 2008 by the DEQ of Virginia monitoring program (Figs. S6 and S7). Predicted N₂O concentrations showed a clear seasonality: higher in winter and lower in summer. N₂O concentrations in the Pohick Bay decreased substantially (-0.9 nM yr^{-1}) possibly due to the nutrient reduction (total N concentration decreasing at $8.8 \mu\text{M yr}^{-1}$) over the last 14 years (Fig. S6). However, N₂O concentrations in the Occoquan Bay only decreased slightly (-0.1 nM yr^{-1} , not statistically significant) along with the minor nutrient reduction (total N concentration decreasing at non-statistically significant rate of $0.5 \mu\text{M yr}^{-1}$) (Fig. S7). Continuation of environmental monitoring in the Potomac River (e.g., N nutrients and temperature), which is much easier than sampling and measuring N₂O gas, could be used to indirectly estimate the changes in N₂O concentrations in the future. These predictors are likely to be important in other estuaries, but the weighting would vary among locations.

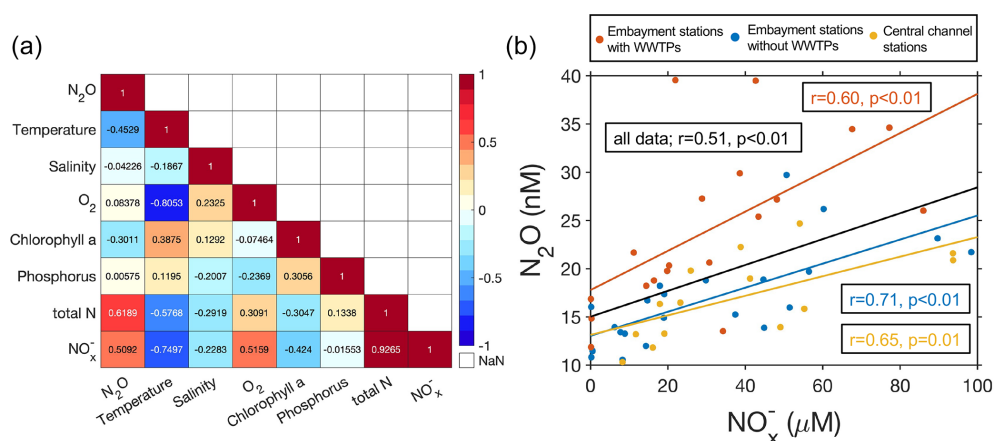


Figure 3. (a) Correlation coefficients among different environmental factors and N_2O concentrations. (b) Relationship between N_2O and NO_x^- concentrations at different categories of sampling stations.

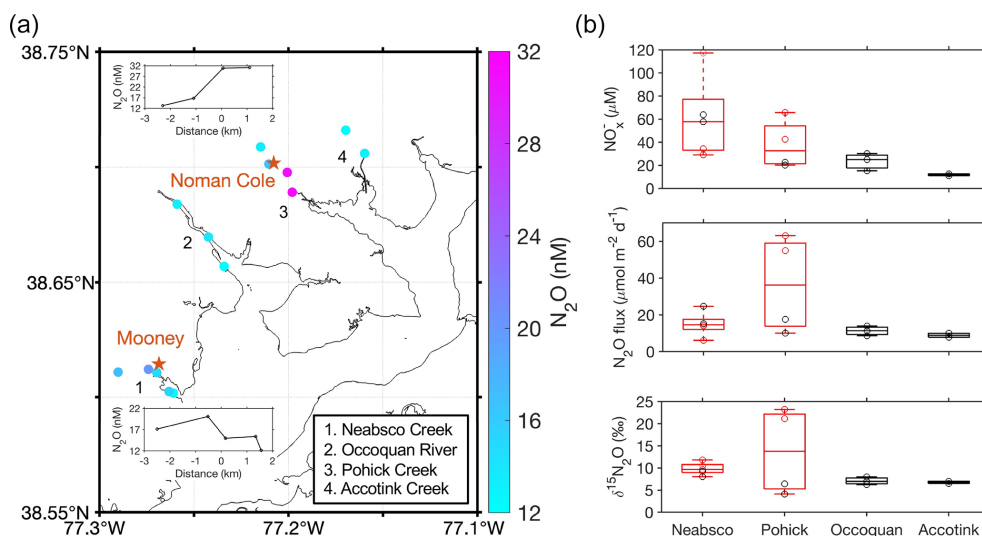


Figure 4. (a) Color-coded N_2O concentration at creek sampling stations on 18 May 2023. WWTPs (Mooney and Noman Cole) are shown in red stars. The inset figures show the change in N_2O concentrations as a function of distance upstream or downstream of the WWTPs. Creeks/river with sampling stations are numbered in the map with names shown in the legend. (b) Boxplots of NO_x^- , N_2O flux, and $\delta^{15}\text{N}$ of N_2O comparing four creeks. Neabsco and Pohick Creeks with WWTPs are displayed with red color boxes. Red circles in the boxplots show data points of stations downstream of WWTPs, and black circles show data points of stations upstream of WWTPs or without the influence from WWTPs. NO_x^- , N_2O flux, and $\delta^{15}\text{N}$ of N_2O were clearly higher at stations downstream of the WWTP in the Pohick Creek.

3.3 Impact of wastewater treatment plants on N_2O concentrations and emissions

To further evaluate how WWTPs affect the N_2O distribution in the Potomac River, we measured N_2O concentrations upstream and downstream of the two WWTP effluents (Mooney and Noman Cole in the Neabsco Creek and the Pohick Creek, respectively) and compared them to N_2O concentrations measured in two creeks that do not have WWTPs (Fig. 4a). Interestingly, the N_2O concentration and flux at the station downstream of Mooney WWTP in the Neabsco Creek were lower than the N_2O concentration and flux at the

station upstream of Mooney WWTP (15.0 nM vs. 20.1 nM; $14.6 \mu\text{mol m}^{-2} \text{d}^{-1}$ vs. $24.7 \mu\text{mol m}^{-2} \text{d}^{-1}$). The exact mechanisms were not clear, but one of the potential reasons could be the influence of tidal cycles: high tide during the sampling time (salinity was 0.17 instead of 0) may have reversed the water flow and diluted the WWTP effluent with low N_2O concentration Potomac water (12.1 nM at the outflow of the Neabsco Creek into the Potomac River estuary). In contrast, we found a substantially higher N_2O concentration and flux downstream of the Noman Cole WWTP than in the upstream station (30.8 nM vs. 16.7 nM; $55 \mu\text{mol m}^{-2} \text{d}^{-1}$ vs. $17.6 \mu\text{mol m}^{-2} \text{d}^{-1}$) in the Pohick Creek, which is less

Table 1. Global N₂O observations in aquatic systems associated with wastewater treatment plants. N₂O data are presented in concentration (nM), saturation (%), or flux ($\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$) according to how they are reported in different studies.

River/location	WWTP	N ₂ O upstream or in tributaries without WWTPs	N ₂ O in WWTP effluents	N ₂ O downstream or in tributaries with WWTPs	Average fold change*	Reference
Potomac River/ Washington, DC, USA	Blue Plains WWTP	11–34 nM		147–318 nM	9.3	McElroy et al. (1978)
Assabet River/ Massachusetts, USA	Westborough WWTP	~ 10 nM	1045 nM	163 nM	16.3	Hemond and Duran (1989)
Tama River/ Tokyo, Japan	Plant 1 Plant 2	350.7 % 219.3 %	12411.4 % 3326.2 %	3454.8 % 1029.6 %	9.8 4.7	Toyoda et al. (2009)
Ohio River/ Cincinnati, USA		27.9 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$		1068 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$	38.2	Beaulieu et al. (2010)
Grand River/ Ontario, Canada	e.g., Kitchener WWTP	4–12 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$		9–113 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$	9.4	Rosamond et al. (2012)
Wascana Creek/ Saskatchewan, Canada	Regina WWTP	–32.5 to 109 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$	227 to 72 800 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$	398 to 40 800 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$	374	Dylla (2019)
Han River/ Seoul, South Korea	JNW	39.7 nM	602.1 nM	441.6 nM	11.1	Chun et al. (2020)
A-river	A-WWTP	61 nM	493 nM	180 nM	3	Masuda et al. (2021, 2018)
B-river	B-WWTP	95 nM	246 nM	286 nM	3	
C-river/Miyagi, Japan	C-WWTP	100 nM	319 nM	145 nM	1.45	
Potomac River estuary/ Virginia, USA	Noman Cole Mooney Aquia	10.8–29.7 nM 1–12.2 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$		11.87–39.5 nM 0.95–31.7 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$	1.6 2.2	This study
Neabsco Creek/ Virginia, USA	Mooney	20.1 nM 24.7 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$		15.0 nM 14.6 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$	0.75 0.59	This study
Pohick Creek/ Virginia, USA	Noman Cole	16.7 nM 17.6 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$		30.8 nM 55 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$	1.84 3.12	This study

* Downstream vs. upstream.

affected by the tidal cycle due to its semi-closed geography (salinity was 0.12). The high downstream N₂O concentration and flux may suggest the direct addition of N₂O from WWTP effluent to the downstream environment. Furthermore, $\delta^{15}\text{N}$ values of N₂O in stations downstream of WWTPs were generally higher than in the other two creeks that do not have

WWTPs (Fig. 4b), confirming the distinct source of N₂O production by WWTPs found in the Potomac River estuary. Overall, the influence of WWTP effluents on downstream distribution of N₂O is variable and could be affected by the physical movement of water.

Dong et al. (2023) evaluated the potential impact of wastewater nitrogen discharge on estuarine N₂O emissions globally. Here we compiled data from previous studies with direct N₂O measurements in aquatic systems associated with WWTPs (not included in Dong et al., 2023) to assess the global impact of WWTPs on aquatic N₂O concentrations or emissions (McElroy et al., 1978; Hemond and Duran, 1989; Toyoda et al., 2009; Beaulieu et al., 2010; Rosamond et al., 2012; Chun et al., 2020; Masuda et al., 2021, 2018; Dylla, 2019). WWTP effluents and water downstream of the WWTPs contain some of the highest N₂O concentrations and fluxes observed in the aquatic system (Table 1 and Fig. S8). For example, up to 12 411.4 % saturation of N₂O was measured in the effluent of WWTPs in the Tama River in Japan (Toyoda et al., 2009). In addition, N₂O flux up to 40 800 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$ was found downstream of the Regina WWTP in the Wascana Creek in Canada (Dylla, 2019). The downstream N₂O flux was > 300 times higher than the N₂O flux upstream of the Regina WWTP. In comparison, the maximum N₂O saturation and flux previously reported in a global riverine N₂O dataset were around 2500 % and 12 754 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{d}^{-1}$ (Hu et al., 2016). Across the sites listed in Table 1, N₂O concentration/saturation/flux downstream of the WWTPs was 1.45 to 374-fold of the upstream waters. The only exception was our observed decrease in N₂O concentrations downstream of Mooney WWTP on 18 May 2023, which was likely influenced by the tidal cycle. The wide range of apparent WWTP effect is related to many factors including the variable N₂O emission factors in the WWTPs, the ratio of WWTP effluent volume to riverine discharge, the distance from the WWTPs where measurements were conducted, and the direction of water flow (e.g., tidal cycle). In addition, the estuarine type, mixing regime, and stratification are also important factors controlling N₂O emissions (Brown et al., 2022). Overall, failing to account for N₂O emissions downstream of the WWTPs and their variability would substantially bias estimates of aquatic N₂O emissions. This uncertainty is increased by the fact that only a few observations are available (all in the Northern Hemisphere) (Fig. S8) compared to > 58 000 WWTPs present globally (Ehalt Macedo et al., 2022). It is also important to restrict the N₂O emission via efficient N₂O reduction in the WWTPs considering the projected increase in future wastewater production (Qadir et al., 2020).

4 Conclusions

Taking advantage of the routine water monitoring program by the DEQ of Virginia, we detected strong spatial and temporal variabilities of N₂O concentrations and emissions in the Potomac River estuary, a major tributary of the Chesapeake Bay. Observations across the Potomac River estuary also allowed us to identify hotspots of N₂O emissions associated with WWTP effluents. Higher N₂O concentrations

downstream of WWTPs compared to regions with similar nitrogen nutrient concentrations suggested the direct discharge of dissolved N₂O from WWTPs and/or intense N₂O production. The influence of WWTPs on downstream N₂O concentrations and emissions is largely affected by volumes of river discharge versus WWTP effluents. A survey of globally available data shows N₂O concentrations or emissions are consistently elevated in waters downstream of WWTPs. Future ¹⁵N tracer incubations would help to explain the high N₂O concentration downstream of WWTPs by disentangling the N₂O production pathways. In addition, concurrent measurements of the N flux and N₂O concentration downstream of WWTPs will help to constrain overall N₂O emission factors associated with WWTPs. Our work could encourage potential collaborations between the scientific community, governmental agencies, and the public to better observe environmental pollution or water quality, e.g., increasing the frequency and resolution of observations of N₂O and other greenhouse gases along with many regularly monitored environmental factors like temperature and nutrients. Such efforts may identify previously overlooked sources of N₂O emissions and help to better estimate N₂O emissions from aquatic systems.

Data availability. Data presented in this study have been deposited in the Zenodo repository: <https://doi.org/10.5281/zenodo.11492525> (Tang et al., 2024).

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Competing interests. The contact author has declared that none of the authors has any competing interests.

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Supplement of

Variable contribution of wastewater treatment plant effluents to downstream nitrous oxide concentrations and emissions

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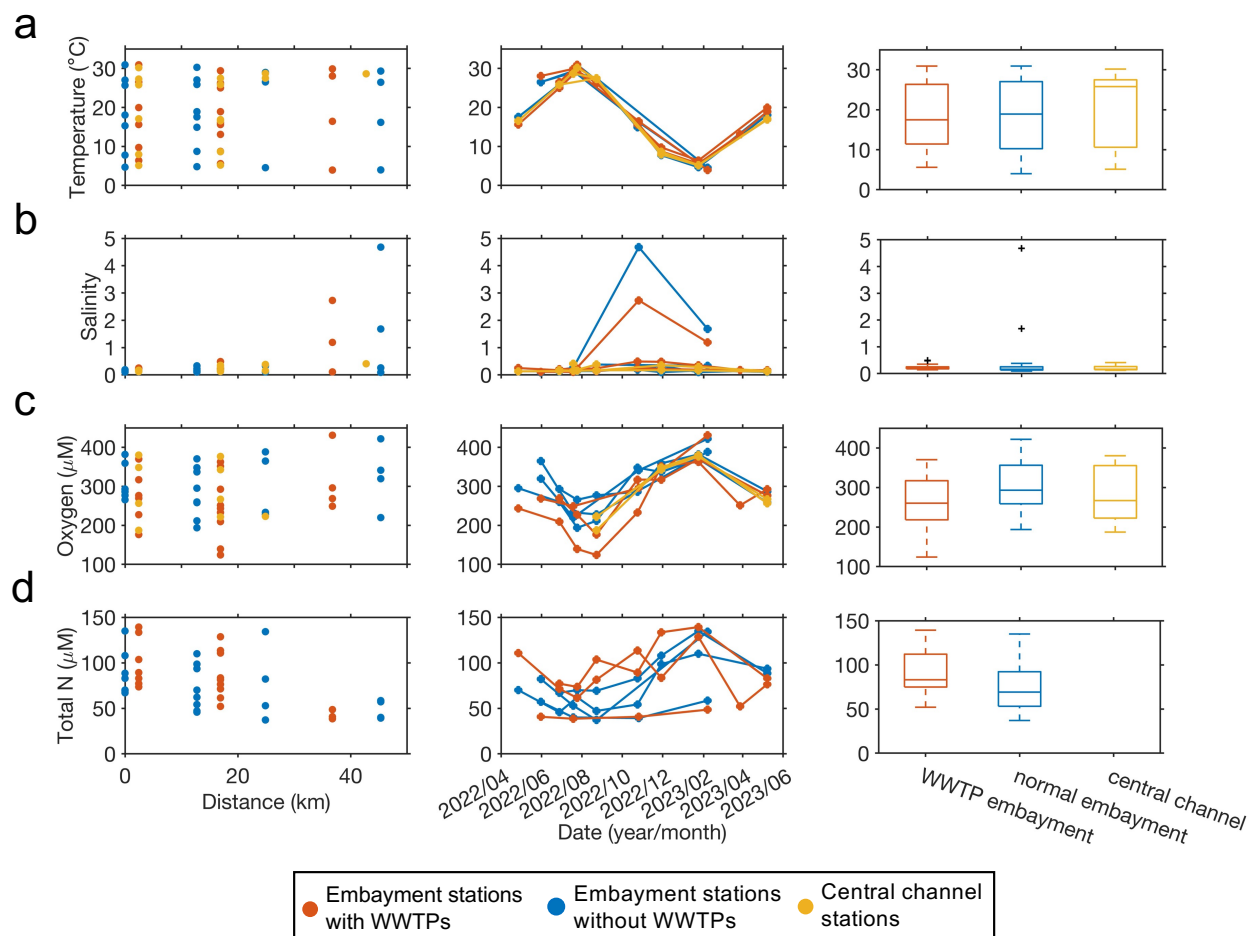


Fig. S1. Spatial and temporal variations of temperature (a), salinity (b), oxygen (c) and total nitrogen concentration (d). The distance shows from upstream to downstream stations in the Potomac River. Embayment stations associated with wastewater treatment plants (WWTPs, red circles and lines) and without WWTPs (blue circles and lines), and central channel stations (yellow circles and lines). Total N concentration was not measured at central channel stations. For the boxplots, the red line in each box is the median. The bottom and top of each box are the 25th and 75th percentiles of the observations, respectively. The error bars represent 1.5 times the interquartile range away from the bottom or top of the box, with red + signs showing outliers beyond that range.

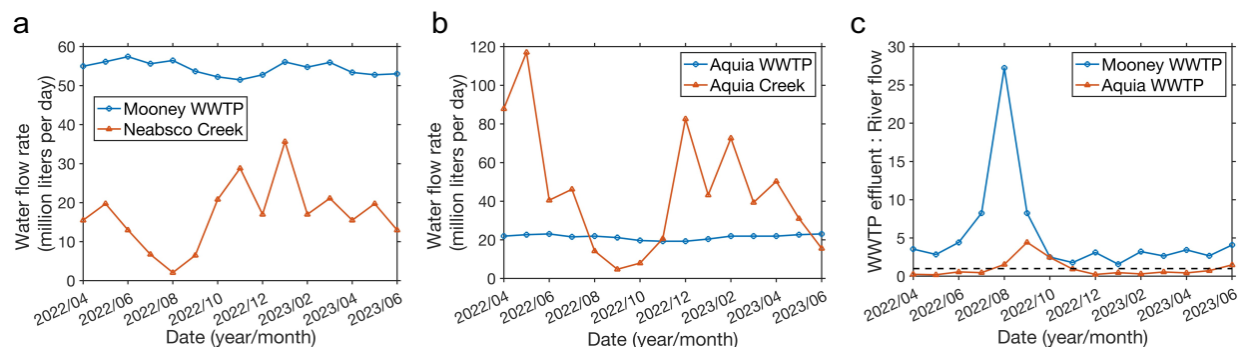


Fig. S2. Comparison between wastewater treatment plants effluents and riverine discharge. (a) Mooney WWTP vs Neabsco Creek. (b) Aquia WWTP vs Aquia Creek. (c) the ratio of wastewater treatment plants effluents to riverine discharge. The horizontal dashed line denotes a ratio of 1. Riverine discharges at monitoring stations upstream of the Mooney WWTP (monitoring station of Neabsco Creek at Dale City, Virginia) and Aquia WWTP (monitoring station of Aquia Creek near Garrisonville, Virginia) were obtained from United States Geological Survey (USGS). Climatological riverine discharge was used for Neabsco Creek because data were not available for years 2022 and 2023.

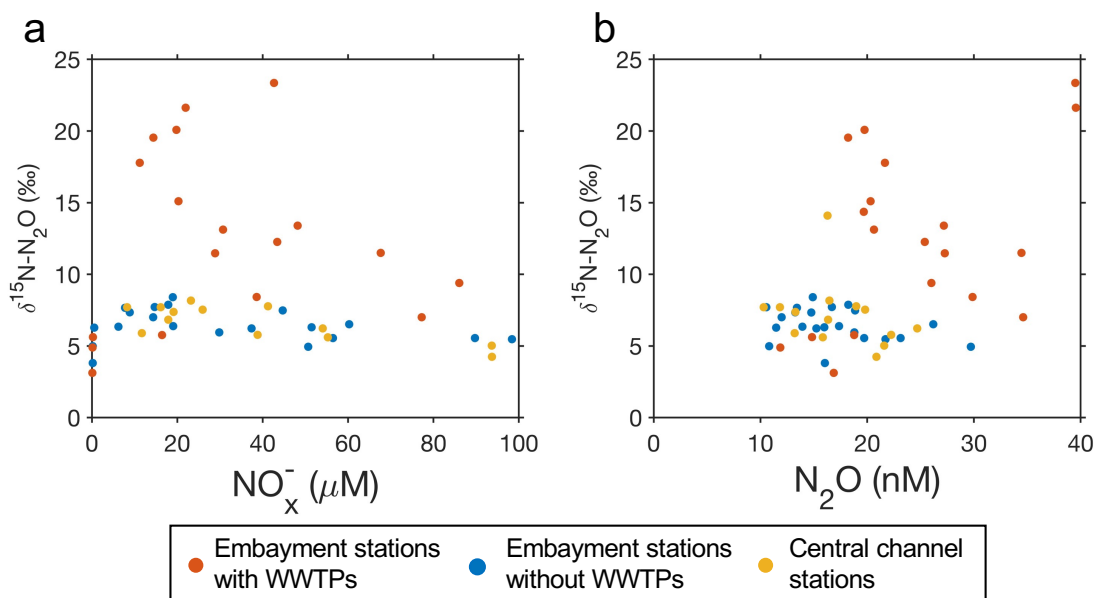


Fig. S3. The change in $\delta^{15}\text{N}$ of N_2O in relation to the changes in NO_x^- concentrations (a) and N_2O concentrations (b).

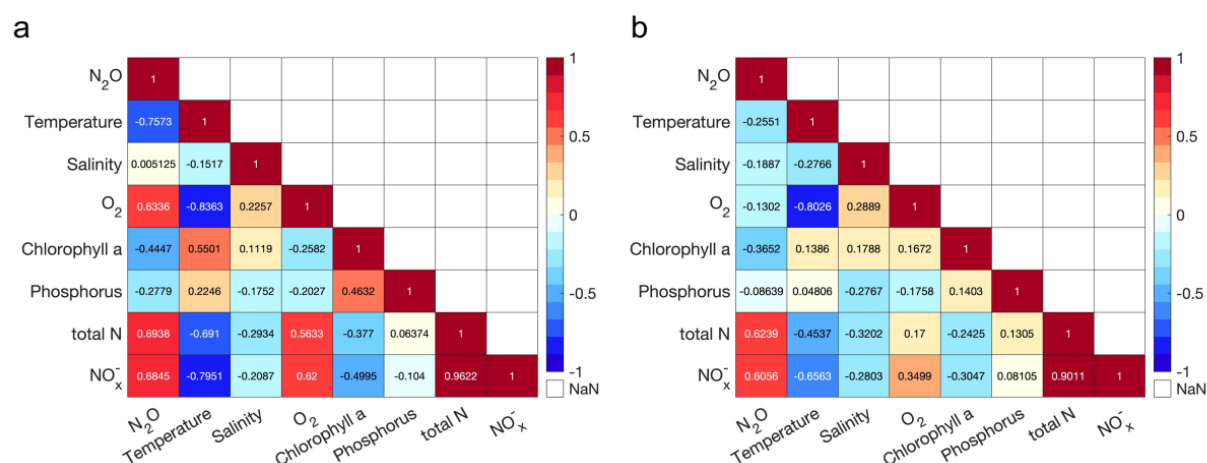


Fig. S4. Correlation coefficients among different environmental factors and N₂O concentration for stations without (a) or with (b) the influence of WWTPs.

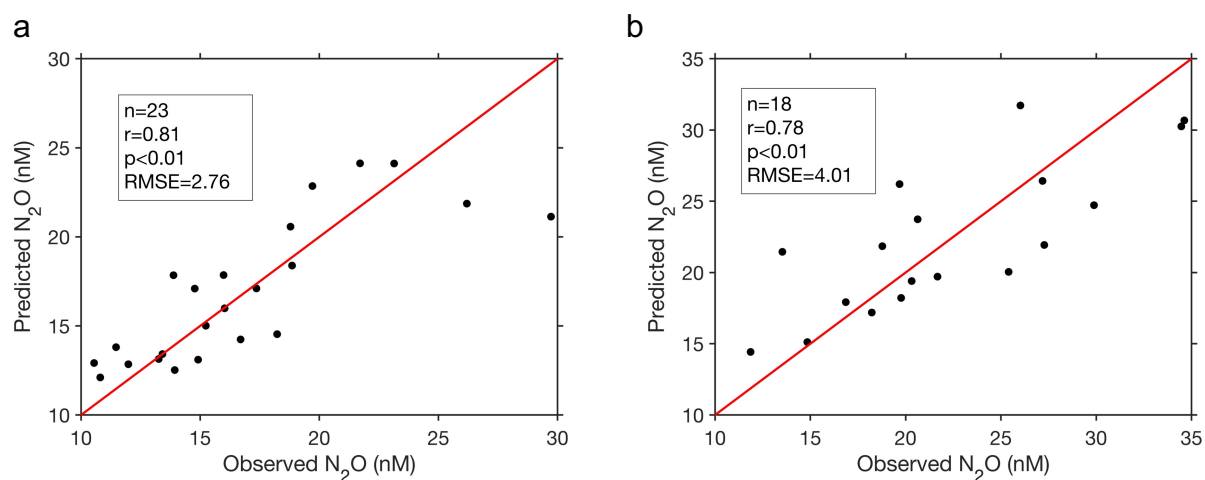


Fig. S5. Predicted versus observed N₂O concentration based on a multiple linear regression model for stations without (a) or with (b) the influence of WWTPs. The number of data points (n), correlation coefficient (r), p value and root mean square error (RMSE) are presented in the legend.

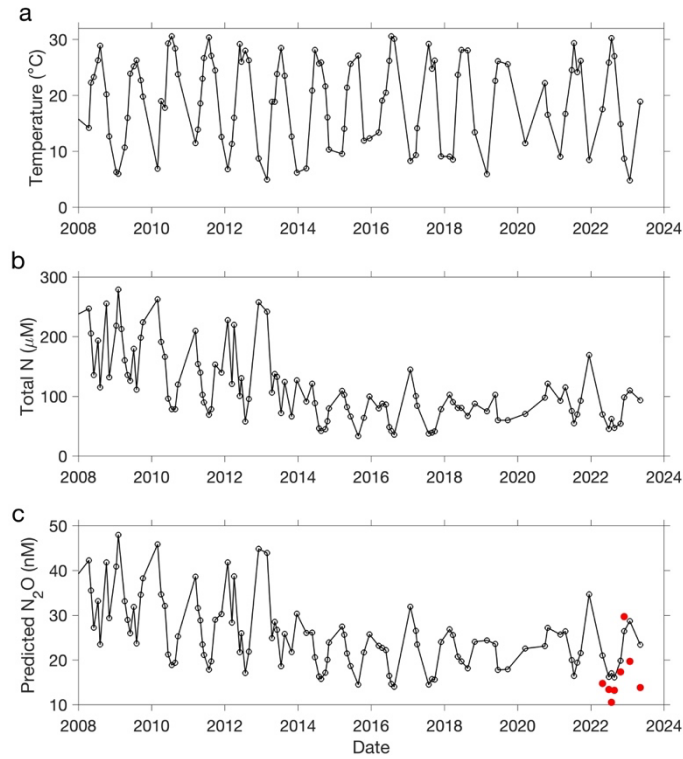


Fig. S6. Historical measurements of temperature (a) and N concentration (b) at the Pohick Bay sampling station with the influence of Noman Cole WWTP. N₂O concentration (c) is predicted based on a multiple linear regression model developed for stations with the influence from WWTPs. The red points are the observed N₂O concentration.

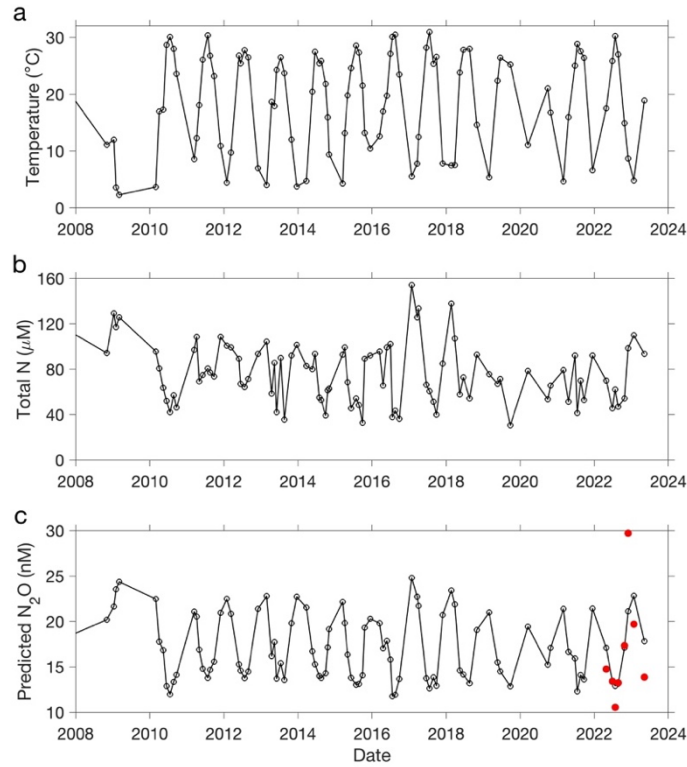


Fig. S7. Historical measurements of temperature (a) and N concentration (b) at the Occoquan Bay sampling station without the influence of WWTPs. N₂O concentration (c) is predicted based on a multiple linear regression model developed for stations without the influence from WWTPs. The red points are the observed N₂O concentration.

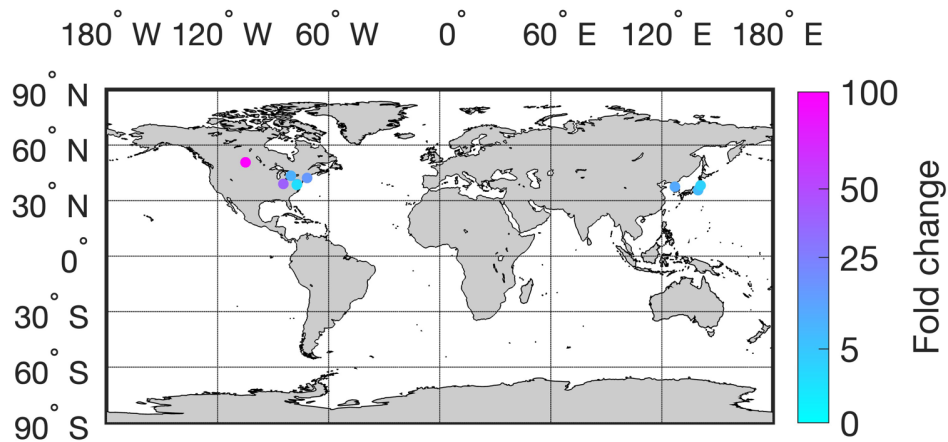


Fig. S8. N₂O observations associated with WWTPs globally, color-coded by the fold change in N₂O concentration, saturation or flux comparing downstream and upstream of WWTPs (see Table 1 for details).