Variable contribution of wastewater treatment plant effluents to nitrous oxide emissions

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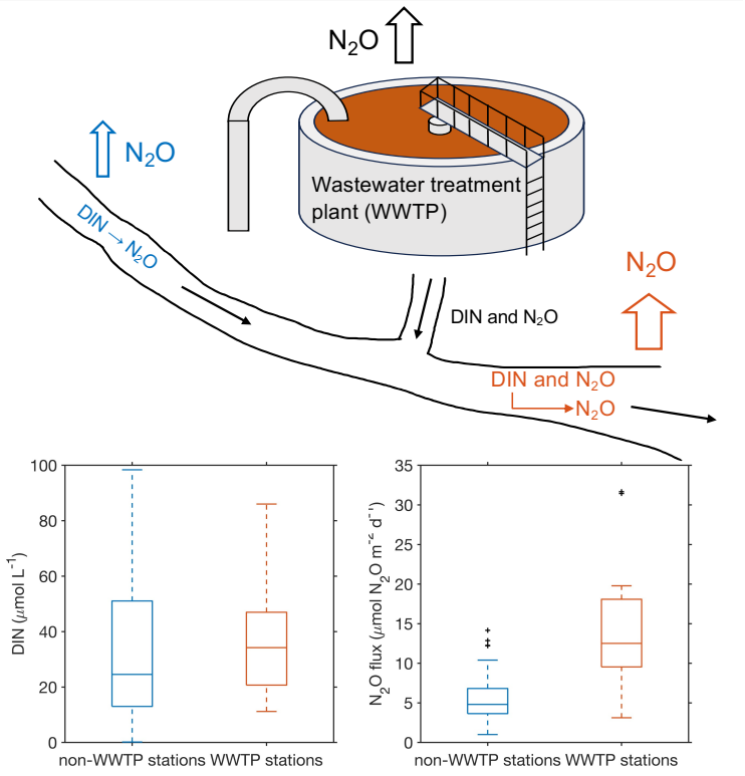
Abstract

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 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. 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 the 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.

Key words: nitrous oxide, greenhouse gas emission, nitrogen pollution, wastewater treatment plants, spatial and seasonal variation

Summary: Wastewater treatment plants (WWTPs) are known to be hotspots of greenhouse gas emissions. However, the impact of WWTPs on the emission of the greenhouse gas N_2O in downstream aquatic environments is less constrained. We found spatially and temporally variable but overall higher N_2O concentrations and fluxes in waters downstream of WWTPs, pointing to the need for efficient N_2O removal in addition to treating nitrogen in WWTPs.

Graphical abstract



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 Tg N yr^{-1} and 0.04 - 3.6 Tg N yr^{-1} of N_2O is 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). N_2O emission from WWTPs accounts for ~5.2% of total N_2O emission in 2021 in the United States (EPA, 2023). N_2O emissions from different WWTPs are highly variable, and are normally calculated as a function of DIN loading into the WWTPs, using an N_2O emission factor (Kampschreur et al., 2009). N_2O emission factors range from 0.16% to 4.5% (N_2O emitted/DIN loading) (Eggleston et al., 2006; De Haas and Andrews, 2022). In addition to direct emission from the WWTPs, N_2O 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 N_2O concentration is less studied and the downstream N_2O emission remains poorly constrained.

Here we specifically compared the N_2O concentration upstream and downstream of the WWTPs in order to assess the impact of WWTPs on N_2O emission, which could help to constrain the emission factor associated with the WWTPs 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, D.C., and the states of Virginia and Maryland in the eastern United States. The annual mean discharge of Potomac River from 1895 to 2002 measured at Chain Bridge near Washington, DC was 321 $m^3\ s^{-1}$ with a large interannual variability (Jaworski et al., 2007). The annual total nitrogen loading was estimated to be around 27.7 $\times 10^6$ kg N $year^{-1}$ 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, D.C. is one of largest WWTPs in the world, treating an average of ~1454 million liters 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 N_2O 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 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 N_2O 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 N_2O. Therefore, we collaborated with DEQ of Virginia to measure the spatial and temporal variation of N_2O concentrations in the Potomac River Estuary.

Materials and Methods

Sample collection for N_2O and nutrients

Surface waters at ~0.5 m depth at eleven 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 N_2O from April 2022 to May 2023 (Figure 1). The eleven 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 Discharge Monitoring Reporting required by Virginia Pollutant Discharge Elimination System permit. 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). The distances from the embayment stations downstream of WWTPs to Noman Cole, Mooney, Aquia WWTPs were approximately 4, 1.8 and 5.8 km, respectively.

The embayment stations were 2-3 meters deep while the average depth of central channel stations was around 8 meters. The embayment stations have been routinely sampled for water quality analyses by the DEQ of Virginia since the early 1970's. 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 N_2O, and to compare DIN and N_2O concentrations between 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 Supplementary Figure 1b). 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. Triplicate water samples for N_2O concentrations and isotopes were collected via a submersible pump into 60 mL serum bottles after overflowing three 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 N_2O and nutrient analysis (Frame et al., 2016; Wong et al., 2017).

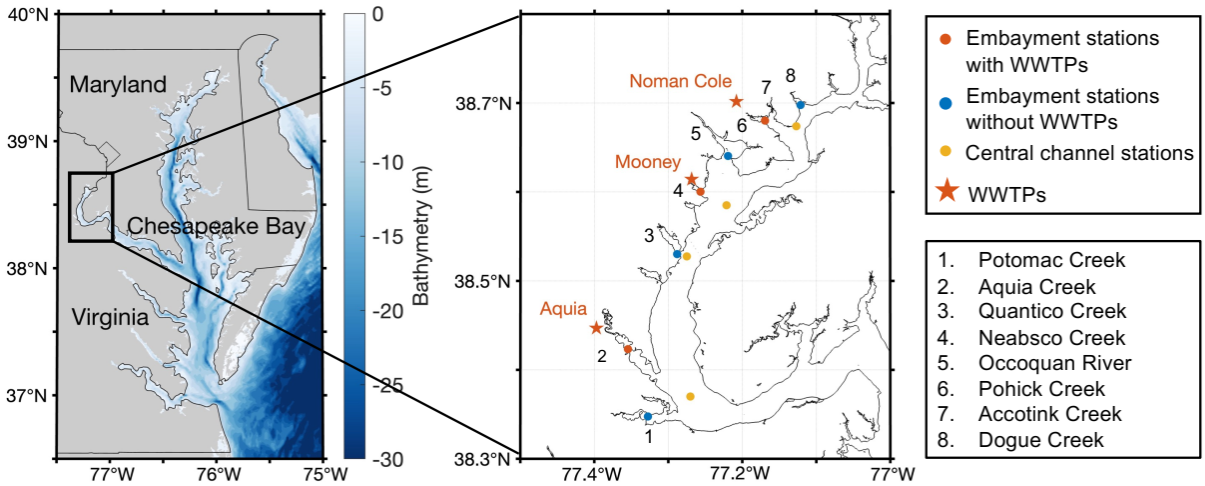


Figure 1. Sampling stations in the Potomac River Estuary including embayment stations associated with WWTPs (red circles) and without WWTPs (blue circles), and central channel stations (yellow circles). Locations of WWTPs (Noman Cole, Mooney and Aquia) are shown in red stars. Creeks/rivers with sampling 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 Figure 4 below.

In addition to the routine sampling in the Potomac River Estuary, we also sampled its tributaries, some of which were associated with the WWTPs, on May 18, 2023 (Figure 1) to specifically evaluate the impact of WWTPs on downstream N_2O concentrations. Four creeks/rivers were sampled including Neabsco Creek (5 stations: 2 stations upstream and 3 stations downstream of Mooney WWTP), Occoquan River (3 stations, no WWTP), Pohick Creek (4 stations: 2 stations upstream and 2 stations downstream of Noman Cole WWTP), and Accotink Creek (2 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/rivers 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 N_2O sampling, temperature, salinity, and dissolved O_2 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). Samples of total nitrogen and phosphorus (both particulate and dissolved) were collected into 250 mL HDPE bottles and kept in ice in a cooler until analysis within 48 hours on land (Rice et al., 2012; EPA, 1983).

Measurement of N_2O and nutrient concentrations

N_2O in the serum bottles was stripped by helium carrier gas into a Delta V Plus mass spectrometer (Thermo) for the analyses of N_2O concentration and isotope ratio (m/z = 44, 45, 46) (Tang et al., 2022). The total amount of N_2O in the serum bottles was determined using a standard curve of N_2O peak area with N_2O standards containing a known amount of N_2O reference gas (0, 0.207, 0.415, 0.623, 0.831, 1.247 nmol N_2O). The total amount of N_2O dissolved in the water was calculated after subtracting the amount of N_2O in 3 mL air headspace. Specifically, the monthly atmospheric N_2O concentrations were obtained from the nearby atmospheric station in Brentwood, Maryland (https://gml.noaa.gov/) (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. The N_2O concentration in samples was then calculated from the total amount of N_2O dissolved in the water divided by the volume of water in the serum bottles. The detection limit and precision of N_2O concentration measurement were 1.29 and 0.33 nM, respectively. We used N_2O produced from nitrate isotope standards (USGS34 = -1.8‰ and IAEA = 4.7‰) to calibrate for $\delta^{15}N$ of N_2O samples.

After analyzing N_2O concentration, samples were neutralized to pH ~7 by adding 10% hydrochloric acid. NO_2^- + NO_3^- (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 chemiluminescence analyzer (Braman and Hendrix, 1989). The detection limit of NO_x^- concentration was 0.15 μM. NH_4^+ and NO_2^- concentrations were measured at a few selected stations using the fluorometric orthophthalaldehyde method (Holmes et al., 1999) and the colorimetric method (Hansen and Koroleff, 1999), respectively. Their concentrations were much

smaller than NO_3^- alone, mostly accounting for less than 10% of the DIN concentration. Therefore, we only present NO_x^- data in this study.

N_2O flux calculation

Surface N_2O flux was calculated using the following equation: $Flux = k \times (N_2O_{measured} - N_2O_{equlibrium})$. The equilibrium N_2O concentration ($N_2O_{equlibrium}$) was calculated based on the solubility of N_2O (Weiss and Price, 1980). The gas transfer velocity (k) was estimated based on three different parameterizations: $k_{600} = 1.91 \times e^{0.35 \times U}$ (Raymond and Cole, 2001); $k_{600} = 0.314 \times U^2 - 0.436 \times U + 3.99$ (Jiang et al., 2008); $k = 0.251 \times U^2 \times (\frac{Sc}{660})^{-0.5}$ (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). Sc is the Schmidt number that could be estimated as a function of temperature (Wanninkhof, 2014). Since our samples have salinity close to 0, we used the parameterization of Sc for freshwater. Average values of the three N_2O flux estimates are presented in the paper and N_2O 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 N_2O fluxes and their driving factors instead of their absolute magnitude.

Results and discussion

Spatial and temporal variations of N_2O 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 (Figure 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, N_2O concentration decreased from approximately 40 to 10 nM along the sampling transect and was higher in the fall and winter (Figure 2b). Since temperature decreased from ~31°C in summer to 4°C in winter (Supplementary Figure 1a), the increase in N_2O solubility in colder water during winter partly explained the seasonal change. In contrast, N_2O saturation had higher values in summer and fall (Figure 2c), suggesting a higher N_2O production in summer and fall. It is worth noting that N_2O 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 N_2O to the atmosphere. N_2O flux ranged from 1 to 31.7 μmol N_2O m^{-2} d^{-1} (Figure 2d). N_2O concentration (median: 18.2 nM) and flux (median: 5.6 μmol N_2O 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 N_2O with a median value at 10.6 nM and -0.3 to 4.3 μmol N_2O m^{-2} d^{-1} with a median at 0.5 μmol N_2O m^{-2} d^{-1} (Tang et al., 2022; Laperriere et al., 2019)). Therefore, the tributaries of the Chesapeake Bay (i.e., Potomac River) are intense sources of N_2O to the atmosphere.

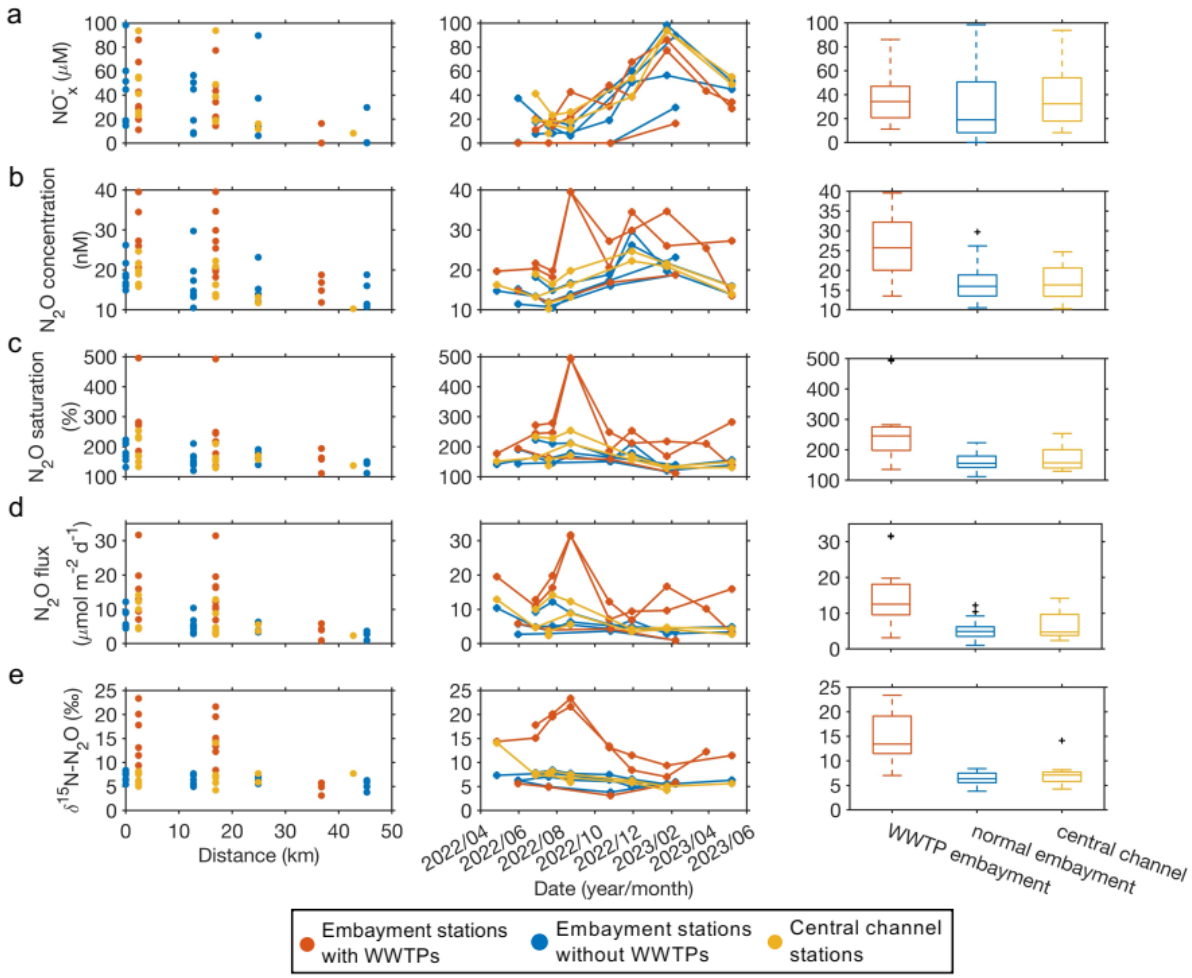


Figure 2. Spatial and temporal variations of NO_x^- concentration (a), N_2O concentration (b), N_2O saturation (c), N_2O flux (d) and $\delta^{15}N$ of N_2O (e). The distance shows from upstream to downstream stations in the Potomac River. Embayment stations associated with WWTPs (red circles and lines) 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 $\delta^{15}N$ values compared to other stations ($p<0.01$, *t*-test) but not significantly different NO_x^- concentration.

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, N_2O concentrations varied within locations according to the station category: N_2O concentrations were substantially higher at stations downstream of WWTPs ($p<0.01$, *t*-test). N_2O concentrations were similar between stations in embayments without WWTPs and the central channel (Figure 2). This suggests these WWTPs are efficient in removing DIN from sewage and other sources but WWTPs may discharge N_2O directly into the effluent or enhance downstream N_2O production (e.g., higher N_2O 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 N_2O varied among stations. For example, elevated N_2O concentrations were observed downstream from Noman Cole and Mooney WWTPs but not downstream from Aquia WWTP. This difference may be related to the different N removal processes of WWTPs that produce N_2O at different yields (de Haas and Andrews. 2022; Zhao et al., 2024). However, we don't 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 Neabsco Creek while the volume of effluent from Aquia WWTP were generally lower than the discharge of Aquia Creek (Supplementary Figure 2a-b). Particularly, the highest N_2O concentration of up to 40 nM was found at two stations downstream of the Noman Cole and Mooney WWTPs on August 23, 2022 when the river discharge was low (Supplementary Figure 2). Thus, the effect of WWTPs on downstream N_2O concentrations also varies seasonally (Schult 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 N_2O hotspots. Previous studies have shown the impact of WWTPs on downstream N_2O concentration in aquatic environments. For example, the highest N_2O concentration ~675 nM in the Potomac River was measured near the discharge of the Blue Plains WWTP in 1977 (McElroy et al., 1978). Highest N_2O emissions in the Ohio River near Cincinnati were attributed to direct input of N_2O from WWTPs' effluents (Beaulieu et al., 2010).

In addition, a higher nitrogen isotopic signature ($\delta^{15}N$) of N_2O associated with WWTPs (median $\delta^{15}N$ 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 $\delta^{15}N$ of N_2O at 6‰, Figure 2e) in the Potomac River Estuary. In comparison, the average $\delta^{15}N$ of N_2O in the tropospheric air

is around 6.55‰ (Snider et al., 2015). $\delta^{15}N$ of N_2O for stations with the influence of WWTPs showed a clear seasonal variation: higher 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, a relatively larger WWTPs' effluent volume compared to the riverine discharge led to a larger $\delta^{15}N$ of N_2O in summer. However, no clear seasonal pattern of $\delta^{15}N$ of N_2O was seen for stations without the influence of WWTPs. $\delta^{15}N$ 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 $\delta^{15}N$ 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). Our observed $\delta^{15}N$ of N_2O downstream of WWTPs was higher than the values found in these urban WWTPs. One of the reasons for the increased $\delta^{15}N$ 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 $\delta^{15}N$ is partly supported by the higher $\delta^{15}N$ of N_2O when NO_x^- was reduced to less than 40 μM, suggesting the occurrence of N_2O reduction when the concentration of other denitrification substrates became low (Supplementary Figure 3). However, we do not know the exact locations where denitrification occurred (e.g., WWTPs, anoxic niches in suspended particles, sediments), which deserves further investigations. The influence of denitrification on unique isotopic signatures of N_2O produced from WWTPs has also been observed in Tama River in Japan (Toyoda et al., 2009).

Environmental controls on N_2O concentrations

N_2O concentrations showed positive correlations with total N ($r=0.62$, $p<0.01$) and NO_x^- concentrations ($r=0.51$, $p<0.01$) (Figure 3a). Correlation analyses done separately for stations with or without WWTPs had similar patterns (Supplementary Figure 4). A better correlation between the N_2O concentration and total N may indicate the contribution of other N sources besides NO_x^- to N_2O production. N_2O 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 can't exclude the possibility of N_2O 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 N_2O production pathways around the WWTPs. Furthermore, N_2O concentration was negatively correlated with temperature since higher temperature reduced the N_2O solubility. 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) versus in stations with WWTPs (negative) (Supplementary Figure 4), which may be influenced by the different N_2O production pathways.

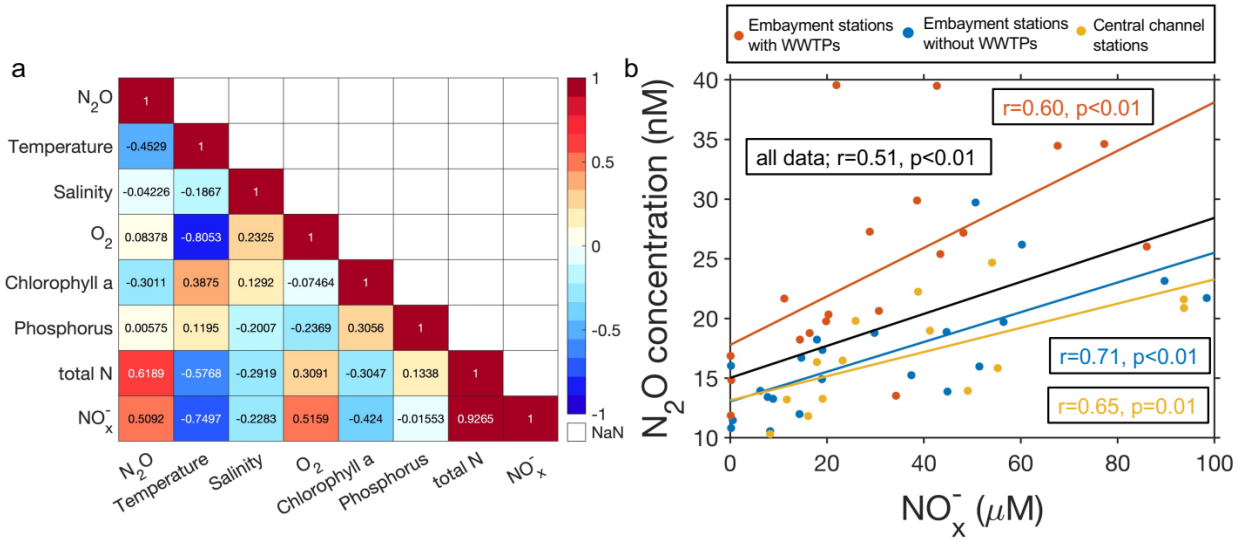


Figure 3. (a) Correlation coefficients among different environmental factors and N_2O concentrations. (b) Relationship between N_2O and NO_x^- concentrations at different categories of sampling stations.

The significant positive relationship between N_2O and NO_x^- concentration existed for samples collected at stations from all three different categories (Figure 3b). N_2O concentrations at stations downstream of WWTPs were notably higher than at other stations not associated with WWTPs even under the similar range of NO_x^- concentration. The larger slope of N_2O concentration versus NO_x^- concentration at stations downstream of WWTPs may be related to the direct input of N_2O from WWTPs into the downstream waters or different N_2O production pathways and production

yields that deserve further investigations. The DIN concentration has been found to be a good predictor of N_2O concentration and emission in many other rivers and estuaries (Murray et al., 2015; Reading et al., 2020; Zheng et al., 2022;). However, the correlation varied spatially, which may be affected by the variable N_2O emission factors from DIN cycling. The emission factors are affected by temperature, concentration and forms of N, oxygen, organic carbon concentration and many other factors (Hu et al., 2016). The external N_2O input (e.g., input from WWTPs) could also affect the relationship between N_2O and DIN concentrations (Dong et al., 2023). Compared to DIN (~28 to 71 μM) and N_2O 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 N_2O concentrations have slightly decreased. Thus, an additional benefit of nutrient regulation is the reduction of greenhouse gas - N_2O - emissions, beyond improving water quality.

Since N_2O concentrations had the strongest correlation with total N concentrations (reflecting the N_2O production potential) and temperature (affecting N_2O solubility), we developed a predictive model of N_2O concentration based on total N and temperature. 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). The observed N_2O variability was generally captured by these simple linear models (Supplementary Figure 5) but there were variabilities in the observations remaining to be explained. Addition of other predictors did not significantly improve the model performance, so we chose the simple predictive model that is mechanistically understandable. We then applied the two predictive models separately to estimate N_2O concentrations at the embayment station in the Pohick Bay (with WWTP) and the embayment station in the Occoquan Bay (without WWTP) using total N concentration and temperature that were measured since 2008 by the DEQ of Virginia monitoring program (Supplementary Figures 6 and 7). Predicted N_2O concentrations showed a clear seasonality: higher in winter and lower in summer. N_2O concentrations in the Pohick Bay decreased substantially (-0.9 nM/year) possibly due to the nutrient reduction (total N concentration decreasing at 8.8 μM/year) over the last 14 years (Supplementary Figure 6). However, N_2O concentrations in the Occoquan Bay only decreased slightly (-0.1 nM /year, not statistically significant) along with the minor nutrient reduction (total N concentration decreasing at non-

statistically significant rate of 0.5 μM/year) (Supplementary Figure 7). Continuation of environmental monitoring in the Potomac River (e.g., N nutrients and temperature), which is much easier than sampling and measuring N_2O gas, could be used to indirectly estimate the changes in N_2O concentrations in the future. These predictors are likely to be important in other estuaries, but the weighting would vary among locations.

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 Neabsco Creek and Pohick Creek, respectively) and compared them to N_2O concentrations measured in two creeks that do not have WWTPs. Interestingly, the N_2O concentration at the station upstream of Mooney WWTP in Neabsco Creek was higher than the N_2O concentration at the station downstream of Mooney WWTP (20.1 vs 15.0 nM) (Figure 4a). The exact mechanisms were not clear but one of the potential reasons could be the influence by 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 Neabsco Creek into the Potomac River Estuary). In contrast, we found substantially higher N_2O concentration downstream of the Noman Cole WWTP (30.8 nM downstream vs 16.7 nM upstream) in the Pohick Creek, which is less affected by the tidal cycle due to its semi-closed geography (salinity was 0.12). The high downstream N_2O concentration may suggest the direct addition of N_2O from WWTP effluent to the downstream environment. Furthermore, $\delta^{15}N$ of N_2O in stations downstream of WWTPs were generally higher than the other two creeks that do not have WWTPs (Figure 4b), confirming the distinct source of N_2O production by WWTPs found in the Potomac River Estuary. Overall, the influence of WWTP effluents on downstream distribution of N_2O is variable, and could be affected by the physical movement of water.

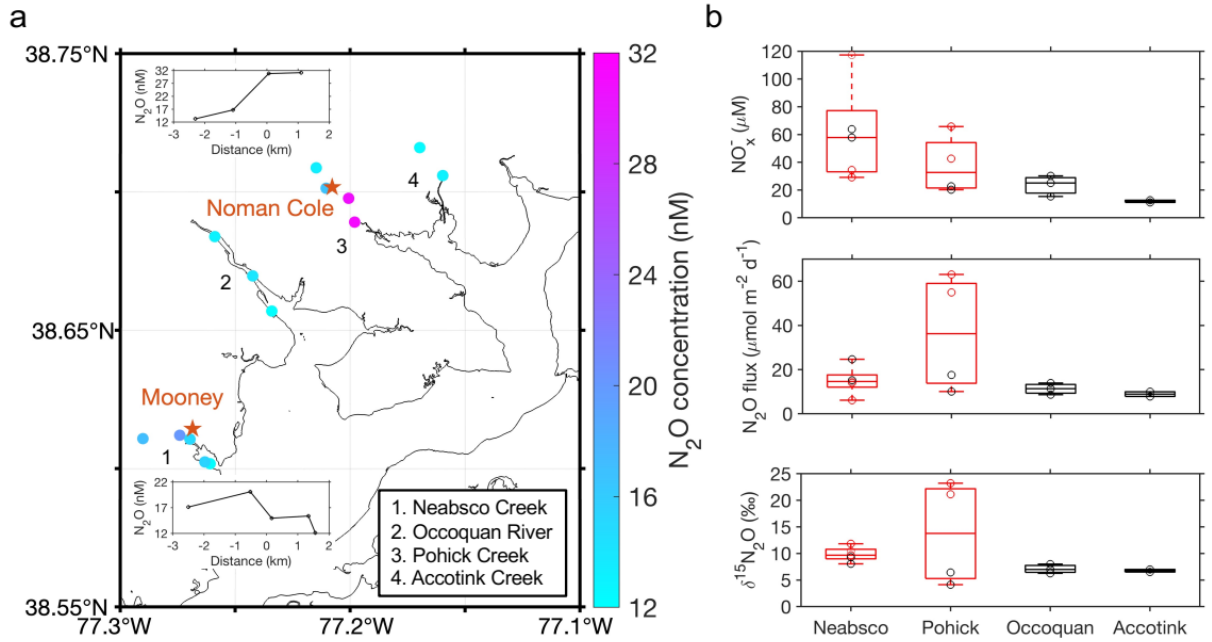


Figure 4. (a) Color-coded N_2O concentration at creek sampling stations on May 18, 2023. WWTPs (Mooney and Noman Cole) are shown in red stars. The insert figures show the change in N_2O concentrations as a function of distance up or down stream from the WWTPs. Creeks/rivers with sampling stations are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

Dong et al. (2023) evaluated the potential impact of wastewater nitrogen discharge on estuarine N_2O emissions globally. Here we compiled data from previous studies with direct N_2O measurements in aquatic systems associated with WWTPs (not included in Dong et al., 2023) to assess the global impact of WWTPs on aquatic N_2O 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; Masuda et al., 2018; Dylla, 2019). WWTP effluents or water downstream of the WWTPs contain some of the highest N_2O concentrations and fluxes observed in the aquatic system (Table 1 and Supplementary Figure 8). For example, up to 12,411.4% saturation of N_2O was measured in the effluent of WWTPs in the Tama River in Japan

(Toyoda et al., 2009). In addition, N_2O flux up to 40,800 μmol N_2O-N m^{-2} d^{-1} was found downstream of the Regina WWTP in the Wascana Creek in Canada (Dylla, 2019). The downstream N_2O flux was >300 times higher than the N_2O flux upstream of the Regina WWTP. In comparison, the maximum N_2O saturation and flux previously reported in a global riverine N_2O dataset were around 2,500% and 12,754 N_2O-N m^{-2} d^{-1} (Hu et al., 2016). Across the sites listed in Table 1, N_2O 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 N_2O concentrations downstream of Mooney WWTP on May 18, 2023, which was likely influenced by the tidal cycle. The wide range of apparent WWTP effect is related to many factors including the variable N_2O 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 N_2O emissions (Brown et al., 2022). Overall, failing to account for N_2O emissions downstream of the WWTPs and their variability would substantially bias estimates of aquatic N_2O emissions. This uncertainty is increased by the fact that only a few observations are available (all in the northern hemisphere) (Supplementary Figure 8) compared to >58 000 WWTPs present globally (Ehalt Macedo et al., 2022). It is also important to restrict the N_2O emission via efficient N_2O reduction in the WWTPs considering the projected increase in future wastewater production (Qadir et al., 2020).

Table 1. Global N_2O observations in aquatic systems associated with wastewater treatment plants. N_2O data are presented in concentration (nM), saturation (%) or flux (μmol N_2O-N m^{-2} d^{-1}) according to how they are reported in different studies.

River/location	WWTP	N_2O upstream or in tributaries without WWTP	N_2O in WWTP effluent	N_2O downstream or in tributaries with WWTP	Average fold change (downstream vs upstream)	Reference
Potomac River/ Washington, D.C., 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% saturation 219.3%	12411.4% saturation 3326.2%	3454.8% saturation 1029.6%	9.8 4.7	Toyoda et al., 2009

Ohio River/ Cincinnati, USA		27.9 μmol N_2O-N m^{-2} d^{-1}		1068 μmol N_2O-N m^{-2} d^{-1}	38.2	Beaulieu et al., 2010
Grand River/ Ontario, Canada	e.g., Kitchener WWTP	4-12 μmol N_2O-N m^{-2} d^{-1}		9-113 μmol N_2O-N m^{-2} d^{-1}	9.4	Rosamond et al., 2012
Wascana Creek/ Saskatchewan, Canada	Regina WWTP	–32.5 to 109 μmol N_2O-N m^{-2} d^{-1}	227 to 72800 μmol N_2O-N m^{-2} d^{-1}	398 to 40800 μmol N_2O-N m^{-2} d^{-1}	374	Dylla. 2019
Han River/ Seoul, Korea	JNW	39.7 nM	602.1 nM	441.6 nM	11.1	Chun et al., 2020
A-river	A-WWPT	61 nM	493 nM	180 nM	3	Masuda et al., 2021
B-river	B-WWTP	95	246	286	3	Masuda et al., 2018
C-river/Miyagi, Japan	C-WWTP	100	319	145	1.45	
Potomac River Estuary /Virginia, USA	Noman Cole Mooney Aquia	10.8-29.7 nM		11.87-39.5 nM	1.6	This study
Neabsco Creek/ Virginia, USA	Mooney	20.1 nM		15.0 nM	0.75	This study
Pohick Creek/ Virginia, USA	Noman Cole	16.7 nM		30.8 nM	1.84	This study

Conclusion

Taking advantage of the routine water monitoring program by the DEQ of Virginia, we detected strong spatial and temporal variabilities of N_2O concentrations and emissions in the Potomac River Estuary, a major tributary of Chesapeake Bay. Observations across the Potomac River Estuary also allowed us to identify hotspots of N_2O emissions associated with WWTPs effluents. Higher N_2O concentrations downstream of WWTPs compared to regions with similar nitrogen nutrient concentrations suggested the direct discharge of dissolved N_2O from WWTPs and/or intense N_2O production. A survey of globally available data shows N_2O concentrations or emissions are consistently elevated in waters downstream from WWTPs. Future ^{15}N tracer incubations would help to explain the high N_2O concentration downstream of WWTPs by disentangling the N_2O production pathways. In addition, concurrent measurements of the N flux and N_2O concentration downstream of WWTPs will help to constrain overall N_2O emission factors associated with WWTPs. Our work could encourage potential collaborations between scientific community and governmental agencies/the public to better observe the environmental pollution or quality, e.g., increasing the frequency and resolution of observations for N_2O and other greenhouse gases along with many regularly monitored environmental factors like temperature and nutrients. Such efforts

may identify previously overlooked sources of N_2O emission and help to better estimate N_2O emissions from aquatic systems.

Data availability

Data presented in this study has been deposited in Zenodo repository: https://doi.org/10.5281/zenodo.10775250.

Author contribution

W.T. conceived the study. J.T., T.J., and W.T. collected N_2O samples from the Potomac River Estuary. W.T. analyzed samples and interpreted data with other coauthors. W.T. wrote the first draft of the manuscript with input from B.B.W. All coauthors contributed to the result discussion and manuscript writing.

Competing interests

The authors declare that they have no conflict of interest.

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