Variable contribution of wastewater treatment plant effluents to nitrous oxide emission

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Abstract
Nitrous oxide (N\textsubscript{2}O), 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\textsubscript{2}O into the atmosphere during the nitrogen removal process. However, the impact of WWTPs on N\textsubscript{2}O emissions in downstream aquatic systems remains poorly constrained. By measuring N\textsubscript{2}O 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\textsubscript{2}O concentrations and fluxes: N\textsubscript{2}O 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\textsubscript{2}O emissions downstream of WWTPs. N\textsubscript{2}O 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\textsubscript{2}O from WWTPs into the aquatic system or a higher N\textsubscript{2}O 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\textsubscript{2}O emissions downstream of the WWTPs would better constrain the global N\textsubscript{2}O emissions. Efficient N\textsubscript{2}O 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$_2$O in downstream aquatic environments is less constrained. We found spatially and temporally variable but overall higher N$_2$O concentrations and fluxes in waters downstream of WWTPs, pointing to the need for efficient N$_2$O 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$_2$O – is produced (Quick et al., 2019).

Aquatic systems are large but highly variable sources of N$_2$O to the atmosphere (Wang et al., 2023). For example, on a global basis, 0.04 - 0.291 Tg N yr$^{-1}$ and 0.15 - 0.91 Tg N yr$^{-1}$ of N$_2$O are estimated to outgas from rivers and estuaries, respectively (Murray et al., 2015; Maavara et al., 2019; Yao et al., 2019). The high end of the estimates in these inland and coastal waters approaches the scale of the global marine N$_2$O emissions (2.5 - 4.3 Tg N yr$^{-1}$ in Tian et al., 2020). The large uncertainty in the estimate of aquatic N$_2$O emission is partly due to high spatial and temporal variabilities of N$_2$O flux within/ across rivers and estuaries and the lack of observations to capture such variability. Therefore, sampling and measurements of N$_2$O concentration at high spatial and temporal resolutions would be desirable to constrain aquatic N$_2$O emission.

The major factors that appear to correlate with N$_2$O 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. Meanwhile, waste and wastewater emit ~0.3 Tg N yr$^{-1}$ of N$_2$O (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$_2$O emission from WWTPs accounts for ~5.2% of total N$_2$O emission in 2021 in US (EPA, 2023). N$_2$O emission from different WWTPs is highly variable, and is normally calculated as a function of DIN loading into the WWTPs, using an N$_2$O emission factor (Kampschreur et al., 2009). N$_2$O emission factors range from 0.16% to 4.5% (N$_2$O emitted/DIN loading) (De Haas and Andrews, 2022; Eggleston et al., 2006). In addition to direct emission from the WWTPs, N$_2$O 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$_2$O concentration is less studied and the downstream N$_2$O emission remains poorly constrained. Here we specifically compared the N$_2$O concentration upstream and downstream of the WWTPs in order to assess the
impact of WWTPs on N$_2$O 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 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$_2$O production and concentration downstream (McElroy et al., 1978). Thanks to higher standards mandated by governmental agencies (nitrogen concentration 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$_2$O 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$_2$O. Therefore, we collaborated with DEQ of Virginia to measure the spatial and temporal variation of N$_2$O concentration in the Potomac River Estuary.
Materials and Methods

Sample collection for N\textsubscript{2}O 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) for the analysis of nitrogen concentration, and both concentration and nitrogen isotopes of N\textsubscript{2}O from April 2022 to May 2023 (Figure 1). The eleven stations are characterized into 3 groups: embayments downstream of WWTPs, embayments not associated with WWTPs, and the central channel of the Potomac River. 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 nitrogen nutrients and N\textsubscript{2}O, and to compare nitrogen nutrients and N\textsubscript{2}O 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). Water samples for N\textsubscript{2}O concentration were collected via a submersible pump into 60 mL serum bottles after overflowing three times the bottle’s volume. After creating a 3 mL air headspace, the serum bottles were immediately sealed with butyl stoppers and aluminum crimps and preserved with 0.5 mL of 10M NaOH solution to stop biological activities. NaOH has been shown to be an effective and less environmentally hazardous preservative for N\textsubscript{2}O 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.

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\textsubscript{2}O concentration. 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). Mooney WWTP discharges ~54.9 million liters of water and 147 kg N per day into the Neabsco Creek while Noman Cole WWTP discharges ~140.8 million liters of water and 370 kg N per day into Pohick Creek. Because Aquia WWTP discharges much less water and N into the Aquia Creek (~21.2 million liters per day and 35 kg N per day), its impact was not specially 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\textsubscript{2}O sampling, temperature, salinity, and dissolved O\textsubscript{2} concentration 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\textsubscript{2}O and nutrient concentrations**

N\textsubscript{2}O in the serum bottles was stripped by helium carrier gas into a Delta V Plus mass spectrometer (Thermo) for the analyses of N\textsubscript{2}O concentration and isotope ratio (m/z = 44, 45, 46) (Tang et al., 2022). The total amount of N\textsubscript{2}O in the serum bottles was determined using a standard curve of N\textsubscript{2}O peak area with N\textsubscript{2}O standards containing a known amount of N\textsubscript{2}O 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 accounting for the amount of N₂O in 3 mL air headspace. 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 δ¹⁵N of N₂O samples.

After analyzing N₂O concentration, samples were neutralized to pH ~7 by adding 10% hydrochloric acid. NO₂⁻ + NO₃⁻ (NOₓ⁻) concentration in these samples was measured using the vanadium (III) reduction method by converting NOₓ⁻ to NO, which was then quantified by chemiluminescence analyzer (Braman and Hendrix, 1989). The detection limit of NOₓ⁻ concentration is 0.15 µM. NH₄⁺ and NO₂⁻ 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 NO₃⁻ alone, mostly accounting for less than 10% of the DIN concentration. Therefore, we only present NOₓ⁻ data in this study.

**N₂O flux calculation**

Surface N₂O flux is calculated using the following equation: $Flux = k \times (N₂O_{measured} - N₂O_{equilibrium})$. The equilibrium N₂O concentration ($N₂O_{equilibrium}$) was calculated based on the solubility of N₂O (Weiss and Price, 1980) and the gas transfer velocity ($k$) was estimated based on the empirical relationship between physical conditions and gas fluxes: $k = 0.251 \times U^2 \times \left(\frac{Sc}{660}\right)^{-0.5}$ (Wanninkhof, 2014). $U$ is the wind speed at the 10 m height obtained from NCEP reanalysis (Kalnay et al., 1996) and Sc is the Schmidt number. We acknowledge large variations in estimating $k$ values in the riverine and estuarine aquatic systems by using different empirical models (Rosentreter et al., 2021; Raymond and Cole, 2001; Borges et al., 2004). For instance, the effect of water velocity and water depth on gas transfer velocity was not considered in the parameterization above. Therefore, we focus on comparing the N₂O fluxes among different stations and their driving factors instead of their absolute magnitude.
Results and discussion

Spatial and temporal variations of N₂O concentrations in the Potomac River Estuary

Along the roughly 50 km sampling transect in the Potomac River Estuary, NOₓ concentration decreased from 98 to <1 μM from upstream to downstream (Figure 2a). NOₓ concentration also showed a clear seasonal pattern: higher in winter and spring while lower in summer and fall. The spatial and temporal patterns are 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 (Figure 2b). Since temperature decreased from ~31°C in summer to 4°C in winter (Supplementary Figure 1a), 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 (Figure 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 flux ranged from 0.4 to 14.5 μmol N₂O m⁻² d⁻¹ (Figure 2d). N₂O concentration (median: 18.2 nM) and flux (median: 2.4 μmol N₂O m⁻² d⁻¹) in the Potomac River Estuary were substantially higher than in the mainstem of the Chesapeake Bay (2.6-20.9 nM N₂O with a median value at 10.6 nM and -0.3-4.3 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 of the Chesapeake Bay (i.e., Potomac River) are intense sources of N₂O to the atmosphere.
Figure 2. Spatial and temporal variations of NO\textsubscript{x} concentration (a), N\textsubscript{2}O concentration (b), N\textsubscript{2}O saturation (c), N\textsubscript{2}O flux (d) and $\delta^{15}$N of N\textsubscript{2}O (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\textsubscript{2}O concentration, N\textsubscript{2}O saturation, N\textsubscript{2}O flux and $\delta^{15}$N values compared to other stations ($p<0.01$, $t$-test) but not significantly different NO\textsubscript{x} concentration.
Stations close to each other had similar NOx- 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 varied within locations according to the station category: N2O concentrations were substantially higher at stations downstream of WWTPs (except downstream of the Aquia WWTP, which discharges a much smaller amount of treated water and N into the river) (p<0.01, t-test). N2O 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 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 ~1.8-4.2 km downstream of the WWTPs. Particularly, the highest N2O concentration of up to 40 nM was found at two stations downstream of the Noman Cole and Mooney WWTPs on August 23, 2022. High-resolution spatial and temporal sampling allowed us to capture these N2O hotspots. Previous studies have shown the impact of WWTPs on downstream N2O concentration. For example, the highest N2O concentration ~675 nM in the Potomac River was measured near the discharge of the Blue Plains WWTP in 1977 (McElroy et al., 1978). Highest N2O emissions in the Ohio River near Cincinnati were attributed to direct input of N2O from WWTPs effluent (Beaulieu et al., 2010).

In addition, higher nitrogen isotopic signature (δ15N) of N2O also suggests the distinct sources or cycling processes of N2O associated with WWTPs in the Potomac River Estuary (median δ15N of N2O at 13‰ vs 6‰ for stations with or without the influence by WWTPs, Figure 2e). In comparison, the average δ15N of N2O in the tropospheric air is around 6.55‰ (Snider et al., 2015). δ15N of N2O produced in WWTPs depends on the treatment stages and aeration conditions (Toyoda et al., 2011; Tumendelger et al., 2014). For example, the average δ15N values were reported to be -24.5‰ and 0‰ respectively for N2O produced from nitrification during oxic treatment versus N2O produced from anaerobic denitrification in a California WWTP (Townsend-Small et al., 2011). Our observed δ15N of N2O downstream of WWTPs was higher than the values found in these urban WWTPs. One of the reasons for the increased δ15N of N2O may be partial N2O reduction via denitrification in the WWTPs or in downstream creeks; this denitrification effect has been seen in the marine oxygen minimum zones (Kelly et al., 2021). Denitrification as the cause of the elevated δ15N is partly supported by the higher δ15N of N2O when NOx- was
reduced to less than 40 μM, suggesting the occurrence of N₂O reduction when the concentration of other denitrification substrates became low (Supplementary Figure 2). The influence of denitrification on unique isotopic signatures of N₂O produced from WWTPs has also been observed in Tama River in Japan (Toyoda et al., 2009).

Environmental controls on N₂O concentrations

N₂O concentrations showed a strong positive correlation with total N (r=0.62, p<0.01) and NO₃⁻ concentrations (r=0.51, p<0.01) (Figure 3a). Correlation analyses done separately for stations with or without WWTPs had similar patterns (Supplementary Figure 3). A better correlation between the N₂O concentration and total N may indicate the contribution of other N sources besides NO₃⁻ to N₂O production. N₂O could be produced from nitrification in the process of oxidizing NH₄⁺ to NO₃⁻ 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 N₂O production from denitrification associated with anaerobic microsites in particles or in sediment (Beaulieu et al., 2011; Wan et al., 2023). Future investigations with ¹⁵N 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 (Zheng et al., 2022; Rosamond et al., 2012; Wang et al., 2015), we did not find a strong dependence of N₂O on oxygen concentrations in the Potomac River Estuary (Figure 3a), probably because of the overall oxygenated conditions (Supplementary Figure 1c).
Figure 3. (a) Correlation coefficients among different environmental factors and N$_2$O concentration. (b) Relationship between N$_2$O and NO$_x$-concentration. P: total phosphorus concentration; [Chla]: chlorophyll a concentration.

The significant positive relationship between N$_2$O and NO$_x$-concentration existed for samples collected at stations from all three different categories (Figure 3b). N$_2$O 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$_2$O concentration vs NO$_x$-concentration at stations downstream of WWTPs may be related to the direct input of N$_2$O from WWTPs into the downstream waters or different N$_2$O production pathways and production yields that deserve further investigations. The DIN concentration has been found to be a good predictor of N$_2$O concentration and emission in many other rivers and estuaries (Zheng et al., 2022; Reading et al., 2020; Murray et al., 2015). However, the correlation varied spatially, which may be affected by the variable N$_2$O 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$_2$O input (e.g., input from WWTPs) could also affect the relationship between N$_2$O and DIN concentration (Dong et al., 2023). Compared to DIN (~28 to 71 μM) and N$_2$O 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$_2$O concentrations have slightly decreased. Thus, an additional benefit of nutrient regulation is the reduction of greenhouse gas - N$_2$O - emission, beyond improving water quality.
Since N$_2$O concentrations had the strongest correlation with total N concentrations (reflecting the N$_2$O production potential) and temperature (affecting N$_2$O solubility), we developed a predictive model of N$_2$O concentration based on total N and temperature. Predictions were performed separately for stations with WTTPs ($N_2O$ concentration = 0.115 $\times$ total N – 0.241 $\times$ temperature + 17.185), ($r$=0.78; $p<0.01$) and without WTTPs ($N_2O$ concentration = 0.049 $\times$ total N – 0.298 $\times$ temperature + 18.888), ($r$=0.81, $p<0.01$). The observed N$_2$O variability was generally captured by these simple linear models (Supplementary Figure 4) 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 estimated the N$_2$O concentration at an embayment station in the Occoquan River using total N concentration and temperature that were measured since 2008 by the DEQ of Virginia monitoring program. N$_2$O concentrations showed a clear seasonality: higher in winter (up to 24.8 nM) and lower in summer (down to 11.7 nM) (Supplementary Figure 5). N$_2$O concentrations decreased slightly (-0.1 nM/year, not statistically significant) possibly due to the nutrient reduction (total N concentration decreasing at non-statistically significant rate of 0.5 μM/year) in the Occoquan River over the last 14 years. Continuation of environmental monitoring in the Potomac River (e.g., N nutrients and temperature), which is much easier than sampling and measuring N$_2$O gas, could be used to indirectly estimate the changes in N$_2$O concentration 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$_2$O concentrations and emissions**

To further evaluate how WWTPs affect the N$_2$O distribution in the Potomac River, we measured N$_2$O 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$_2$O concentrations measured in two creeks that do not have WWTPs. Interestingly, the N$_2$O concentration at the station upstream of Mooney WWTP in Neabsco Creek was higher than the N$_2$O 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$_2$O concentration Potomac water (12.1 nM at the outflow of Neabsco Creek into the Potomac River Estuary). In contrast, we found substantially higher N$_2$O 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$_2$O concentration may suggest the direct addition of N$_2$O from WWTP effluent to the downstream environment. Furthermore, $\delta^{15}$N of N$_2$O 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$_2$O production by WWTPs found in the Potomac River Estuary. Overall, the influence of WWTP effluents on downstream distribution of N$_2$O is variable, and could be affected by the physical movement of water.

Figure 4. (a) Color-coded N$_2$O 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$_2$O concentration 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$_2$O flux and $\delta^{15}$N of N$_2$O 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$_2$O flux and $\delta^{15}$N of N$_2$O 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\textsubscript{2}O emissions globally. Here we compiled data from previous studies with direct N\textsubscript{2}O measurements in aquatic systems associated with WWTPs (not included in Dong et al., 2023) to assess the global impact of WWTPs on aquatic N\textsubscript{2}O 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\textsubscript{2}O concentrations and fluxes observed in the aquatic system (Table 1 and Supplementary Figure 6). For example, up to 12,411.4% saturation of N\textsubscript{2}O was measured in the effluent of WWTPs in the Tama River in Japan (Toyoda et al., 2009). In addition, N\textsubscript{2}O flux up to 40,800 µmol N\textsubscript{2}O-N m\textsuperscript{-2} d\textsuperscript{-1} was found downstream of the Regina WWTP in the Wascana Creek in Canada (Dylla, 2019). The downstream N\textsubscript{2}O flux was >300 times higher than the N\textsubscript{2}O flux upstream of the Regina WWTP.

In comparison, the maximum N\textsubscript{2}O saturation and flux previously reported in a global riverine N\textsubscript{2}O dataset were around 2,500% and 12,754 N\textsubscript{2}O-N m\textsuperscript{-2} d\textsuperscript{-1} (Hu et al., 2016). Across the sites listed in Table 1, N\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O emission factors in the WWTPs, the ratio of river discharge vs WWTP effluent, the distance from the WWTPs where measurements were conducted and the direction of water flow. Overall, failing to account for the N\textsubscript{2}O emission downstream of the WWTPs and its variability would substantially bias estimates of aquatic N\textsubscript{2}O emissions. This uncertainty is increased by the fact that only a few observations are available (all in the northern hemisphere) (Supplementary Figure 6) compared to >58 000 WWTPs present globally (Ehalt Macedo et al., 2022). It is also important to restrict the N\textsubscript{2}O emission via efficient N\textsubscript{2}O reduction in the WWTPs considering the projected increase in future wastewater production (Qadir et al., 2020).
<table>
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<th>River/location</th>
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<th>N$_2$O upstream or in tributaries without WWTP</th>
<th>N$_2$O in WWTP effluent</th>
<th>N$_2$O downstream or in tributaries with WWTP</th>
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<td>219.3% saturation</td>
<td>3326.2% saturation</td>
<td>1029.6% saturation</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Grand River/ Ontario, Canada</td>
<td>e.g., 4-12 µmol N$_2$O-N m$^{-2}$ d$^{-1}$</td>
<td>9.113 µmol N$_2$O-N m$^{-2}$ d$^{-1}$</td>
<td>374</td>
<td></td>
<td>9.4</td>
<td>Rosamond et al., 2012</td>
</tr>
<tr>
<td>Wascana Creek/ Saskatchewan, Canada</td>
<td>32.5 to 109 µmol N$_2$O-N m$^{-2}$ d$^{-1}$</td>
<td>227 to 72800 µmol N$_2$O-N m$^{-2}$ d$^{-1}$</td>
<td>398 to 40800 µmol N$_2$O-N m$^{-2}$ d$^{-1}$</td>
<td>374</td>
<td>Dylla, 2019</td>
<td></td>
</tr>
<tr>
<td>Han River/ Seoul, Korea</td>
<td>JNW WWTP</td>
<td>39.7 nM</td>
<td>602.1 nM</td>
<td>441.6 nM</td>
<td>11.1</td>
<td>Chun et al., 2020</td>
</tr>
<tr>
<td>A-river</td>
<td>A-WWPT</td>
<td>61 nM</td>
<td>493 nM</td>
<td>180 nM</td>
<td>3</td>
<td>Masuda et al., 2021</td>
</tr>
<tr>
<td>B-river</td>
<td>B-WWPT</td>
<td>95 nM</td>
<td>246</td>
<td>286</td>
<td>3</td>
<td>Masuda et al., 2018</td>
</tr>
<tr>
<td>C-river/Miyagi, Japan</td>
<td>C-WWTP</td>
<td>100 nM</td>
<td>319</td>
<td>145</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td>Potomac River Estuary/Virginia, USA</td>
<td>Noman Cole Mooney Aquia</td>
<td>10.8-29.7 nM</td>
<td>11.87-39.5 nM</td>
<td></td>
<td>1.6</td>
<td>This study</td>
</tr>
<tr>
<td>Neabsco Creek/Virginia, USA</td>
<td>Mooney</td>
<td>20.1 nM</td>
<td>15.0 nM</td>
<td></td>
<td>0.75</td>
<td>This study</td>
</tr>
<tr>
<td>Pohick Creek/Virginia, USA</td>
<td>Noman Cole</td>
<td>16.7 nM</td>
<td>30.8 nM</td>
<td></td>
<td>1.84</td>
<td>This study</td>
</tr>
</tbody>
</table>

### Conclusion

Taking advantage of the routine water monitoring program by the DEQ of Virginia, we detected strong spatial and temporal variabilities of N$_2$O 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$_2$O emissions associated with WWTPs effluents. Higher N$_2$O concentrations downstream of WWTPs compared to regions with similar nitrogen nutrient concentrations suggested the direct discharge of dissolved N$_2$O from WWTPs and/or intense N$_2$O production. A survey of globally available data shows N$_2$O concentrations or emissions are consistently elevated in waters downstream from WWTPs. Future $^{15}$N tracer incubations would...
help to explain the high N\textsubscript{2}O concentration downstream of WWTPs by disentangling the N\textsubscript{2}O production pathways. In addition, concurrent measurements of the N flux and N\textsubscript{2}O concentration downstream of WWTPs will help to constrain overall N\textsubscript{2}O 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\textsubscript{2}O and other greenhouse gases along with many regularly monitored environmental factors like temperature and nutrients. Such efforts may identify previously overlooked sources of N\textsubscript{2}O emission and help to better estimate the N\textsubscript{2}O emission 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\textsubscript{2}O 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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