Coastal reservoirs as a source of nitrous oxide: spatio-temporal patterns and assessment strategy

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ABSTRACT

Coastal reservoirs are widely regarded as a viable solution to the water scarcity problem faced by coastal cities with growing populations. As a result of the accumulation of anthropogenic wastes and the alteration of hydroecological processes, these reservoirs may also become the emission hotspots of nitrous oxide (N$_2$O). Hitherto, accurate global assessment of N$_2$O emission suffers from the scarcity and low spatio-temporal resolution of field data, especially from small coastal reservoirs with high spatial heterogeneity and multiple water sources. In this study, we measured the surface water N$_2$O concentrations and emissions at a high spatial resolution across three seasons in a subtropical coastal reservoir in southeastern China, which was hydrochemically highly heterogeneous because of the combined influence of river runoff, aquacultural discharge, industrial discharge and municipal sewage. Both N$_2$O concentration and emission exhibited strong spatio-temporal variations, which were correlated with nitrogen loading from the river and wastewater discharge. The mean N$_2$O concentration and mission were found to be significantly higher in the summer than in spring and autumn. The results of redundancy analysis showed that NH$_4^+$-N explained the greatest variance in N$_2$O emission, which implied that nitrification was the main microbial pathway for N$_2$O production in spite of the potentially increasing importance of denitrification of NO$_3^-$-N in the summer. The mean N$_2$O emission across the whole reservoir was 107 µg m$^{-2}$ h$^{-1}$, which was more than an order of magnitude higher than that from global lakes and reservoirs. Based on our results of
Monte Carlo simulations, a minimum of 15 sampling points per km² would be needed to produce representative and reliable N₂O estimates in such a spatially heterogeneous aquatic system. Overall, coastal reservoirs could play an increasingly important role in future climate change via their N₂O emission to the atmosphere as water demand and anthropogenic pressure continue to rise.

**Keywords:** Greenhouse gases; Water management; nitrous oxide; Spatial heterogeneity; Wastewater discharge; Coastal reservoir
1. Introduction

Over 30% of the world’s population currently live in water-scarce regions (United Nations, 2018). In many of these regions, water scarcity is caused not by water shortage but rather insufficient water storage, leading to the construction of reservoirs as one of the common solutions to help divert and retain surface runoff (Sitharam et al., 2020). Coastal reservoirs are particularly appealing because of their low cost, low ecological impacts on upstream areas, and close proximity to the coastal populations with growing water demand (Liu et al., 2013). Coastal reservoirs are considered a key water management solution in densely populated coastal cities around the world by providing portable water for irrigation, preventing flooding downstream, and treating the discharged wastewater (Sitharam et al., 2020).

By retaining surface runoff and lengthening the water residence time, coastal reservoirs may accumulate pollutants and alter various hydroecological processes, leading to the release of greenhouse gases (e.g., nitrous oxide or N₂O) into the atmosphere. This is of particular concern for reservoirs in densely populated areas with high loadings of nutrients and wastes. In general, high nutrient loadings will increase the microbial-mediated process of N₂O production (Davidson et al., 2015; Wang et al., 2017; Xiao et al., 2019a), subsequently triggering more N₂O emission from reservoirs to the atmosphere (Wang et al., 2017; He et al., 2017). In addition, high nutrient loads in the reservoirs can drive large oxygen depletion, further promoting denitrification as the primary pathway of N₂O production (Borges et al., 2015). Dammed rivers and
reservoirs are thus potentially N₂O emission hotspots.

N₂O is a highly potent greenhouse gas with a global warming potential almost 300 times higher than that of carbon dioxide (CO₂) over a 100-year period (IPCC, 2013), and is also one of the major ozone-depleting substances (Ravishankara et al., 2009; Shaaban et al., 2018). The average atmospheric N₂O concentration reached 331 ppbv in 2018, which was approximately 23% above the pre-industrial level (World Meteorological Organization, 2019) with a growth rate of 0.7–0.8 ppb yr⁻¹ over the last three decades (Davidson, 2009; Saikawa et al., 2014; Xiao, et al., 2019a). Despite the climatic importance of N₂O, accurate assessment of N₂O emissions are limited by the low availability of field data and the use of emission factor (EF) that is associated with the background N stock only. While it may be appropriate to apply EF for agriculture-based emission with easily quantifiable N input from fertilizers, applying specific EF in aquatic systems may yield large errors in the estimation of N₂O emission (Maavara et al., 2019), because of the highly variable N dynamics in both quantity and quality (Ivens et al., 2011). Furthermore, the assessment of N₂O emission is often done at low spatial resolutions (e.g., 1°×1° grids), which is too crude for small coastal reservoirs with high hydrographical heterogeneity and multiple water sources.

To address these limitations and explore the potential importance of coastal reservoirs as a net N₂O source, we measured in this study the surface water N₂O concentrations at a high spatial resolution in a subtropical coastal reservoir in southeast China, from which we calculated N₂O emissions using the wind-based thin boundary
layer model. We also examined the influence of environmental factors on the spatial variations of N$_2$O emissions and determined the optimal sampling strategy for assess N$_2$O emissions from spatially heterogeneous water bodies.

2. Materials and methods

2.1. Study area

The Wenwusha Reservoir (25°49′36″–25°54′00″ N, 119°35′12″–119°38′11″ E) is a coastal reservoir located at the mouth of the Nangyangdong River in Fujian Province in southeast China (Fig. 1). It has a surface area of 5.2 km$^2$, a mean depth of 9.0 m, a total volume of $3.20\times10^8$ m$^3$ and a catchment area of 275 km$^2$ (Yang et al., 2020a). Influenced by a humid subtropical monsoonal climate, this reservoir has an annual mean temperature of 19.3 °C and an annual precipitation of 1390 mm, with 75% of the precipitation occurring during the wet season (May - September).

The reservoir is divided into two basins, namely the north basin (NB) and the south basin (SB) (Fig. 1) (Yang et al., 2020a). NB (1.9 km$^2$; $1.40\times10^8$ m$^3$) is located in an urbanized area and is heavily impacted by aquacultural, industrial and municipal waste discharges while also receiving input from the Nanyangdong River. On the other hand, the watershed of SB (3.3 km$^2$; $1.69\times10^8$ m$^3$) is dominated by natural wetlands and forests. This study covered a total of 10 transects in NB and 11 transects in SB, with 3-10 sampling sites selected along each transect (Fig. 1). A total of 103 sampling
sites (56 in NB and 47 in SB) were included, which could be categorized into five different sections according to the types of discharge: industrial effluents (Section-I; \( n = 4 \)), town sewage (Section-T; \( n = 6 \)), river inputs (Section-R; \( n = 7 \)), aquacultural waste (Section-A; \( n = 22 \)) and non-wastewater discharge (Section-N; \( n = 64 \)).

2.2. Dissolved \( N_2O \) concentrations

Three sampling surveys covering the entire reservoir were conducted in November 2018, March 2019, and June 2019, respectively. In each survey, sample collection was completed within two consecutive days. We collected water samples at 20 cm depth using a 100-mL syringe equipped with three-way stopcock, and quickly transferred them into 55-mL glass serum bottles. We added 0.2 mL of saturated HgCl\(_2\) into each bottle to inhibit microbial activities (Taipale and Sonninen 2009; Zhang et al., 2013), and then immediately sealed the bottle with an open-topped screw cap equipped with a halobutyl rubber septum to exclude air bubbles (Borges et al., 2018; Xiao et al., 2019a; Yang et al., 2020b). All the water samples were stored in an ice box and analyzed within 48 hours of collection.

Dissolved \( N_2O \) concentrations were measured following the headspace equilibration method (Yu et al., 2013; Yu et al., 2017). In the laboratory, \( N_2 \) gas (>99.9% purity) was injected into each sample bottle to displace a 25-mL headspace. The bottles were then shaken vigorously for 10 minutes in an oscillator to gas achieve an
equilibrium in N\textsubscript{2}O between the headspace air and the liquid phase. After waiting for 30 minutes, 5 mL of headspace air sample was collected with a syringe and analyzed for N\textsubscript{2}O concentration using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with an electron capture detector. The detection limit was 0.02 ppm and the relative standard deviations of the measurements were \( \leq 5.0\% \) (Yang et al., 2020b). Dissolved N\textsubscript{2}O concentration was then calculated based on the temperature, Henry's law constant (salinity-dependent) and the measured head space N\textsubscript{2}O concentration according to Weiss and Price (1980).

2.3. N\textsubscript{2}O emission

N\textsubscript{2}O emission \( (F_{W-A}, \, \mu\text{mol m}^{-2} \text{ h}^{-1}) \) was calculated using the thin boundary layer model (Equation 1) that has been widely applied in the lentic ecosystem (e.g., Cole and Caraco, 1998; Musenze et al., 2014 Xiao et al., 2019b):

\[
F_{W-A} = k \times (C_{obs} - C_{eq}) \tag{Eq1}
\]

where \( k \) is the gas transfer velocity (m h\(^{-1}\)); \( C_{obs} \) is the measured N\textsubscript{2}O concentration (nmol L\(^{-1}\)) in the surface water; \( C_{eq} \) is the dissolved N\textsubscript{2}O concentration (nmol L\(^{-1}\)) in equilibrium with the atmospheric concentration at the prevailing \textit{in situ} conditions, as a function of atmospheric pressure, water temperature, and the ambient atmospheric N\textsubscript{2}O.

Because of negligible surface water flow at the reservoir, the N\textsubscript{2}O transfer velocity is primarily controlled by wind speed (Xiao et al., 2019b). In this study, we calculated \( k \) value using a wind-dependent equation (Eq 2) for small shallow lakes (Cole and Caraco,
1998):

\[ k = \left( \frac{Sc}{660} \right)^{-n}(2.07 + 0.215 \times U_{10}^{1/2}) \]  \quad \text{(Eq 2)}

where \( Sc \) is the temperature-dependent Schmidt number for N\(_2\)O; \( n \) is a constant that varies between 0.66 (for wind speeds \( \leq 3 \text{ m s}^{-1} \)) and 0.50 (wind speeds \( >3 \text{ m s}^{-1} \)); \( U_{10} \) is the frictionless wind speed (m s\(^{-1}\)) at 10 m height. \( Sc \) and \( U_{10} \) were calculated using the following equations (Crusius and Wanninkhof, 2003; Wanninkhof, 1992):

\[ Sc = 2055.6 - 137.11T + 4.3173T^2 - 0.054350T^3 \]  \quad \text{(Eq 3)}

\[ U_{10} = U_z \left[ 1 + \left( \frac{C_{d10}}{K} \right)^{1/2} \ln \left( \frac{10}{z} \right) \right] \]  \quad \text{(Eq 4)}

where \( U_z \) is the wind speed (m s\(^{-1}\)) at a height of \( z \) above the water surface (2.0 m in this study); \( C_{d10} \) is the drag coefficient at 10 m above the water surface (0.0013 m s\(^{-1}\)); and \( K \) is the von Karman constant (0.41).

2.4. Environmental variables

Water samples were collected at a depth of 20 cm with a 5 L organic glass hydrophore and transferred into 150 mL polyethylene bottles. About 0.5 mL of saturated HgCl\(_2\) solution was added to inhibit microbial activities (Zhang et al., 2013). The water samples were transported in an ice box back to the laboratory for analysis. In the laboratory, the water sample was filtered through a 0.45-\(\mu\)m cellulose acetate filter (Biotrans\textsuperscript{TM}) and then analyzed for the concentrations of total dissolved nitrogen (TDN), ammonium-nitrogen (NH\(_4\)\(^+-\)N), and nitrate-nitrogen (NO\(_3\)\(^-\)-N) using a flow injection
The detection limit and relative standard deviations were 3.0 μg L\(^{-1}\) and ≤2.0% for TDN, 0.6 μg L\(^{-1}\) and ≤3.0% for NH\(_4^+\)-N and 0.6 μg L\(^{-1}\) and ≤2.0% for NO\(_3^-\)-N.

Concurrent measurements of water temperature (\(T_w\)) and pH (IQ150 pH/mV/Temperature meter, IQ Scientific Instruments, USA), salinity (Eutech Instruments-Salt6 salinity meter, USA), dissolved oxygen (DO; 550A YSI sonde, USA) and electrical conductivity (EC; 2265FS electrical conductivity meter, Spectrum Technologies, USA) were also made at a water depth of 20 cm (Yang et al., 2020a). The relative standard deviations of the measurements were between ≤1.0% and ≤2.0%. Air temperature (\(T_A\)), wind speed at 2 m height (\(W_s\)) and atmospheric pressure (\(P_{atm}\)) were measured by a portable weather meter (Kestrel-3500, USA).

2.5. Statistical analysis

Results were presented as mean ± 1 standard error. Significant differences in hydrographical properties, dissolved N\(_2\)O concentration and N\(_2\)O emission among reservoir basins and discharge sections were tested by analysis of variance (ANOVA) using the statistical software SPSS 17.0 (SPSS Inc., USA). Correlations between hydrographical parameters and N\(_2\)O concentration or emissions were investigated by Pearson correlation analysis. The extent to which environmental variables affected the spatiotemporal variations in N\(_2\)O emissions was tested by Redundancy Analysis (RDA) using the software CANOCO 5.0 (Microcomputer Power, Ithaca, USA), with \(T_w\), pH,
DO, salinity, NO$_3^-$-N and NH$_4^+$-N, and TDN being inputted as environmental variables.

Monte Carlo analysis was used to determine the optimal sample size for assessing the mean N$_2$O emission at the whole-reservoir scale. The measured fluxes were resampled from the 103 sites with varying sample sizes ($n = 10, 20, 30…, 100$) without replacement for 10,000 times. The overall mean and standard deviation of N$_2$O emission were subsequently calculated for each sample size. Plots were created using OriginPro 7.5 (OriginLab Corp. USA). To illustrate the spatial variations in hydrographical parameters and N$_2$O concentration (or flux) in the reservoir, the Kriging method in ArcGIS 10.2 (ESRI Inc., Redlands, CA, USA) was employed for spatial interpolation.

3. Results

3.1. Hydrographical parameters

There were large differences in hydrographical parameters between NB and SB across the three campaigns. NB had lower DO, EC, and salinity (Fig. S1) and higher NO$_3^-$-N, NH$_4^+$-N and TDN concentrations as compared to SB (Fig. 2). $T_w$ and pH were comparable between the two basins (Fig. S2). Water chemistry also varied spatially depending on the discharge types. In Section-N without sewage discharge, the NO$_3^-$-N, NH$_4^+$-N, and TDN concentrations were significantly lower ($p<0.05$) while DO and salinity were generally higher than in other sections.
The hydrographical parameters also showed strong temporal variation across the whole reservoir \((p<0.001)\). Average water temperature was \(23.6\ ^\circ\text{C}\) during the study period, while the seasonal pattern was clear decreasing in the order: summer (\(29.3\ ^\circ\text{C}\)) > spring (\(23.1\ ^\circ\text{C}\)) > autumn (\(18.3\ ^\circ\text{C}\)) (Fig. S2). The seasonal mean concentrations of \(\text{NO}_3^-\)-N, \(\text{NH}_4^+\)-N and TDN ranged from 0.95 to 1.47, 0.28 to 0.57, and 1.43 to 1.82 mg L\(^{-1}\), respectively. The seasonal mean DO concentration, pH and salinity ranged from 4.60 to 8.87 mg L\(^{-1}\), 6.88 to 10.70, and 0.41 to 1.49‰, respectively. We observed generally higher \(\text{NO}_3^-\)-N, TDN, pH and salinity in autumn (Fig. 2 and Fig. S1), but higher DO and lower \(\text{NH}_4^+\)-N in spring (Fig. 2 and Fig. S1). The seasonal mean water depth ranged from 6.0 to 8.6 m, with a maximum value occurring in summer.

3.2. Spatial variation in \(\text{N}_2\text{O}\) concentration

Surface water \(\text{N}_2\text{O}\) concentrations in the reservoir showed high spatial heterogeneity, varying by 9 to >30 folds between the two basins (Fig. 3a-3c). The mean \(\text{N}_2\text{O}\) concentrations were significantly higher in NB than in SB (Table 1), while the overall mean \(\text{N}_2\text{O}\) concentrations in November, March and June were 46.4±2.0, 58.9±5.9, and 43.9±2.7 nmol L\(^{-1}\), respectively.

\(\text{N}_2\text{O}\) concentration also varied spatially depending on the discharge types, with significantly lower values in Section-N (non-wastewater discharge) and generally higher values in Section-T (town sewage) (Table 1). Overall, \(\text{N}_2\text{O}\) concentration showed significant differences between the sections with and without wastewater
discharge (Fig. 4a-4c).

3.3. Spatial variation in N₂O emission

From November to June, N₂O emission varied spatially by from >100 to >300 folds between the two basins (Fig. 3d-3f). N₂O emissions were generally high in NB, ranging from 6.9 to 711.5 μg m⁻² h⁻¹ with a mean of 153.1±8.6 μg m⁻² h⁻¹. In contrast, N₂O emissions were significantly lower in SB (Table 1), with occasional occurrence of negative values (i.e. net uptake of N₂O). The reservoir-wide mean N₂O emissions were 110.4±9.3, 78.5±6.3, and 153.1±8.6 μg m⁻² h⁻¹ in November, March, and June, respectively.

There were clear spatial differences in N₂O emission as a function of discharge types, with lower emissions in Section-N without wastewater discharge but higher emissions in Section-I (industrial effluent) and Section-R (river runoff) across the three seasons (Table 1). Overall, there were significant differences in mean N₂O emission between the sections with and without wastewater discharge (Fig. 4d-4f).

3.4. Effects of environmental variables on N₂O concentration and emission

Results of Pearson correlation analysis showed that the reservoir-wide N₂O concentration and emission were correlated negatively with DO, EC, and salinity (Table S2) and correlated positively with NO₃⁻-N, NH₄⁺-N and TDN concentrations across all surveys (Table S2, Fig. S3 and S4). Over the whole study period, the N₂O concentration and flux in the reservoir were positively correlated with air temperature but negatively
correlated with wind speed ($p<0.05$ or $< 0.01$; Table S2). Atmospheric pressure had no significant correlation with either N$_2$O concentration or flux ($p>0.05$; Table S2).

Based on the result of RDA analysis, EC, TDN and NO$_3^-$-N were the environmental variables that explained the most spatial variations in N$_2$O concentration and emission in November, March, and June, respectively (Fig. 5). Combining all the data together, NH$_4^+$-N had the largest explanatory power (50.4%) for the spatio-temporal variations in N$_2$O concentration and emission, followed by NO$_3^-$-N (23.5%) and salinity (20.2%) (Fig. 5).

3.5. Determining the optimal sample size

Based on the results of Monte Carlo analysis, the uncertainty of mean N$_2$O emission estimates across the reservoir decreased markedly with increasing number of sampling sites. For each sample size, we created the boxplots of N$_2$O resampling means after 10,000 times of resampling (Fig. 6a). The Highest Probability Density ranges converged with increasing sample size, indicating improved accuracy of N$_2$O emission. In addition, the bias of the resampling N$_2$O estimations declined and the standard deviations of resampling means decreased exponentially with sample size (Fig. 6b). Notably, an increase in sample size from 10 to 100 decreased the relative standard deviation of mean N$_2$O emission by nearly 20-fold, from 21% to 1.2%.

The results of Monte Carlo analysis could also be used to determine the optimal sample size for assessing the reservoir N$_2$O emission. To achieve an error of under 5%,
at least 80 samples over the whole reservoir (5.2 km$^2$) or ca. 15 samples per km$^2$ would be needed to resolve the high spatial heterogeneity of N$_2$O emissions. With a sample size of only 10, we obtained a high relative standard deviation of estimated average N$_2$O emission of 22.6% after 10,000 times of resampling. Despite this large deviation, the median of the resampled means was 107 μmol m$^{-2}$ h$^{-1}$ (Fig. 6a), which was close to the overall mean of all 103 samples combined. Therefore, the high spatial resolution measurements across multiple transects effectively reduced the estimation bias of N$_2$O emission in our study.

4. Discussion

4.1. N$_2$O production in reservoir systems

N$_2$O production is driven by microbial transformation of N substrates (Herrman et al., 2008; Liu et al., 2011; Phanwilai et al., 2020; Xiao et al., 2019b). Previous studies have reported significant relationships between sewage input, N substrates and N$_2$O emission in lakes (e.g., Xiao et al., 2019a), coastal river network (Yu et al., 2013) and inland reservoirs (e.g., He et al., 2017; Liu et al., 2011, 2017). In this study, while several environmental variables such as air temperature, wind speed, atmospheric pressure and solar radiation were quite homogeneous across the reservoir, the availability of N substrates as reflected by the concentrations of NH$_4^+$-N, NO$_3^-$-N and TDN showed high spatial variations (Fig. 2). Both the concentrations of N substrate and
N$_2$O emission rates were much higher in the north basin than in the south basin (Table 1). Located in an urbanized region, our reservoir was heavily impacted by anthropogenic activities, particularly the discharge of domestic and industrial wastewaters and the nutrient-rich effluents from aquaculture ponds. The highest average NO$_3^-$-N and NH$_4^+$-N concentrations were found in the section with river input, indicating the pronounced influence of the upstream area as a N source. In contrast, the N-section without wastewater discharge had better water quality as reflected by the lower NO$_3^-$-N and NH$_4^+$-N concentrations and higher DO level (Table S1). Taken together, our results showed that the high spatial heterogeneity of N$_2$O emission was closely linked to the input of localized N substrate into the reservoir.

N$_2$O can be produced via denitrification of NO$_3^-$ under oxygen-poor conditions, or nitrification of NH$_4^+$-N under aerobic conditions. The DO levels obtained in this reservoir were generally high enough (ca. $\geq$ 25% sat.) to favor nitrification process (Fig. S1), which was further supported by the results of RDA analysis in which NH$_4^+$-N explained the largest overall variance in N$_2$O emission (ca. 50%) during the study period (Fig. 5d). However, it should be noted that DO was only measured in the surface water in this study, but could drop to lower levels in deeper water, especially during the summer, where denitrification might dominate. This hypothesis was supported by the results of RDA analysis in which NO$_3^-$-N explained $>73\%$ of the variance in N$_2$O emission in June (Fig. 5c) when the average DO level was slightly lower (Table S1) and was the second most important factor in accounting for the overall variance (ca. 24%).
Interestingly, salinity also explained about 20% of the variance in N$_2$O emission (Fig. 5d). Because of the proximity of the south basin to the sea, salt intrusion and deposition has created spatial differences in salinity across the reservoir, which could adversely impact the abundance and activity of nitrifiers that are of freshwater in origin (Francis et al., 2003; Monteiro et al., 2017) and subsequently decrease N$_2$O production (Sun et al., 2013; Wang et al., 2018; Welti et al., 2017).

4.2. Spatial and temporal variations in N$_2$O emission

Large spatial variations in N$_2$O emission have been observed in freshwater lakes and reservoirs (Cheng et al., 2019; Musenze et al., 2014; Zhao et al., 2013). Based on field measurements with a high spatial resolution, we successfully characterized the spatial heterogeneity of N$_2$O emission from the Wenwusha Reservoir, which exhibited coefficients of variation ranging from 81% to 110% across the three seasons. Land use change in the catchment can disturb various biogeochemical processes on land and in adjacent waters (Hosen et al., 2014; Williams et al., 2016), with the consequence of changing the direction and magnitude of N$_2$O fluxes among different areas (He et al., 2017; Wang et al., 2017; Zhou et al., 2017). In our study, the concentrations of nitrogen substrate (NO$_3^-$-N, NH$_4^+$-N, and TDN) were substantially lower in sites without wastewater discharge than in those in close proximity with aquaculture ponds, municipal and agricultural lands with wastewater discharge (Table S1). The N$_2$O concentration and flux increased with nitrogen concentration across the whole reservoir. Most notably, the sites under the influence of wastewater discharge covered only 30%
of the reservoir area but contributed to approximately 60% of the total N\textsubscript{2}O emission from the whole reservoir. Our results demonstrated that the spatial variation in N\textsubscript{2}O flux in the subtropical Wenwusha reservoirs was largely affected by various anthropogenic activities (e.g., urbanization and land use) in the catchment area.

N\textsubscript{2}O emission also exhibited clear seasonal variation with significantly higher values in the summer (Table 1), similar to the observations made in other inland waters (Musenze et al., 2014; Xiao et al., 2019b; Zhu et al., 2013). Temperature is considered a dominant factor driving the temporal variation in N\textsubscript{2}O emission by regulating microbial activity (Beaulieu et al. 2010; Harrison and Matson, 2003; Hinshaw and Dahlgren 2013). The higher N\textsubscript{2}O emission observed in the summer coincided with the higher water temperature (Fig. S2c), which could have stimulated N\textsubscript{2}O production. Interestingly, we observed that the average N\textsubscript{2}O emission in spring was substantially lower than that in autumn (Fig. 3 and Table 1), which were opposite to the trend with water temperature (Fig. S2a and S2b). There were several heavy rain events that occurred in spring. Previous studies have shown that heavy precipitation could transport a greater amount of nutrients and greenhouse gases from the surrounding watershed into the lentic ecosystems (Dinsmore et al., 2013; Sinha et al., 2017; Xiao et al., 2021), thereby increasing the saturation level of dissolved greenhouse gases and stimulating microbial greenhouse gas production (Stanley et al., 2016; Yu et al., 2017). However, we found no significant increase in dissolved N\textsubscript{2}O concentrations and fluxes in association with the heavy spring precipitation, which was probably a result of the
dilution effects of precipitation on the biogeochemical reactions in the reservoir (Outram and Hiscock, 2012; He et al. 2017).

Furthermore, the drainage of aquaculture ponds after harvest in autumn as a common management practice could introduce N-rich effluent into the reservoir and stimulate N\textsubscript{2}O production. This was supported by the elevated concentrations of NO\textsubscript{3}\textsuperscript{-}-N, NH\textsubscript{4}\textsuperscript{+}-N, and TDN found near the aquaculture ponds in November (Fig. 2) and the strong positive correlations detected between N\textsubscript{2}O emission and the concentration of N substrates (Fig. S4).

### 4.3. Subtropical coastal reservoir as a net N\textsubscript{2}O source

The average N\textsubscript{2}O fluxes across the water-air interface from the Wenwusha Reservoir was 107.0±15.6 µg m\textsuperscript{-2} h\textsuperscript{-1}, which was about 3.9 and 1.9 times higher than that observed in the marsh (Wang et al., 2018) and aquaculture ponds (Yang et al., 2020b), respectively, in the same subtropical coastal region. This magnitude of N\textsubscript{2}O emission was also substantially higher than the average rate reported in the lakes (12.2 µg m\textsuperscript{-2} h\textsuperscript{-1}) and reservoirs (42.3 µg m\textsuperscript{-2} h\textsuperscript{-1}) of China (Li et al., 2018). When compared with the N\textsubscript{2}O emission rates globally (Table S3), the mean emission from our reservoir was lower than only a handful of tropical or subtropical reservoirs, but was over an order of magnitude greater than the global average of 7.2 µg m\textsuperscript{-2} h\textsuperscript{-1} for lakes and reservoirs spanning across the tropical to polar regions (Hu et al., 2016). Our results, along with similar findings by others (Chen et al., 2014), suggested that subtropical
coastal reservoirs were strong N$_2$O sources and could play an increasingly important climatic role as countries seek to construct more coastal reservoirs (Yang and Kelly, 2015).

4.4. Implications for N$_2$O assessment and reservoir management

Over the past two decades, researchers have increasingly recognized the role of reservoirs as a potential source of greenhouse gases including N$_2$O (Deemer et al., 2016; Descloux et al., 2017; Maavara et al., 2019). However, reliable assessment of N$_2$O emission from global reservoirs remains a challenge due to the scarcity and low resolution of field data. Results from this study highlighted the high spatial and temporal heterogeneities of N$_2$O concentration and emission in a subtropical coastal reservoir, with significant implications to representative sampling for flux estimation. Our results of Monte Carlo analysis suggested that reliance of field data from only one or a few sampling points, as quite commonly done in the literature (Table S3), could yield large errors. Urbanized, coastal reservoirs such as the one studied here are often influenced by runoff and discharge from multiple sources. Our Monte Carlo simulation results (section 3.5) suggested that a minimum of 15 sampling points per km$^2$ would be needed to produce a reliable and representative estimate of spatially-averaged N$_2$O emission in the spatially heterogeneous coastal reservoir system.

Our data showed a strong correlation between N$_2$O emission and wastewater discharges, affirming the important role of wastewater input in promoting N$_2$O
emission. This problem of greenhouse gas release can be exacerbated by the race to modernization and urbanization, particularly in developing countries where wastewater treatment remains inadequate (Hosen et al., 2014; Williams et al., 2016; Yang et al., 2013). Strengthening environmental regulation, particularly for wastewater treatment and discharge, will be key to mitigate water pollution and greenhouse gas emission in reservoir management (Yang, 2014; Yang et al., 2015). To the best of our knowledge, this study was the first attempt to characterize N\textsubscript{2}O concentration and emission from a coastal reservoir at a very high spatial resolution. Our data would be valuable for global biogeochemical modelling and prediction of N\textsubscript{2}O emission from costal reservoirs that are under intense human disturbance.

Several aspects of our study could be improved further in future studies. Firstly, several studies in inland waters have reported diurnal variations with higher N\textsubscript{2}O emissions at night (Baulch et al., 2012; Wu et al., 2018; Yang et al., 2011). Our measurements in the Wenwusha Reservoir were limited to daytime and therefore the emission might have been underestimated. Diurnal differences in N\textsubscript{2}O emission from the reservoir should be compared to improve the reliability of flux estimates. Secondly, in a thermally stratified water column, nitrogen transformation can differ between the epilimnion and the hypolimnion (Beaulieu et al., 2015; Liang et al., 2019; Salk et al., 2016), which was not included in our study. Future studies might use novel methods such as isotopic tracing to examine the relative importance of different nitrogen biogeochemical processes throughout the water column (Sebilo et al., 2006). While we
aimed to resolve the spatial heterogeneity with high spatial resolution sampling, our data also showed considerable seasonal variations in N₂O concentration and emission (Fig. 4). However, resolving temporal variation at a higher frequency (monthly to daily) could be challenging logistically without the help of in situ automated sampling systems. Lastly, the microbial abundance and functional groups (e.g. nitrifier, denitrifier, and ammonifier) should be further characterized to shed light on the dominant microbial pathways of N₂O production in the reservoir.

5. Conclusions

We characterized the variation of N₂O concentration and emission in a coastal, urbanized reservoir in southeastern China with a high spatial resolution in this study. We found that N₂O concentration and emission were strongly influenced by localized runoff and discharges, resulting in very high spatial heterogeneity in addition to the seasonal variations. Results of Monte Carlo simulations showed that a minimum of 15 sampling points per km² would be required to produce a reliable estimate of the whole-reservoir N₂O emission. Based on the analysis of water chemistry data, nitrification appeared to be the main pathway for N₂O production, although denitrification might become increasingly important in the summer months with reducing DO level. Total N₂O emission from our subtropical coastal reservoir was estimated to be 4.8 Mg yr⁻¹, which was considerably higher than the global average for
lakes and reservoirs. Continued construction of coastal reservoirs for water management may exacerbate future climate change by increasing N₂O emission.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgements**

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**References**


### Table 1. Summary of ANOVAs of the effects of sampling location, sampling time and their interactions on surface water N$_2$O concentration and emission in the Wenwusha Reservoir.

<table>
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<th>Month</th>
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<th>Discharge type **</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SB</td>
<td>NB</td>
</tr>
<tr>
<td>Nov 2018</td>
<td>30.1±2.5</td>
<td>60.1±1.8</td>
</tr>
<tr>
<td>May 2019</td>
<td>19.7±1.9</td>
<td>64.3±2.3</td>
</tr>
<tr>
<td>Jun 2019</td>
<td>29.1±5.2</td>
<td>84.0±8.6</td>
</tr>
<tr>
<td>Average</td>
<td>26.3</td>
<td>69.5</td>
</tr>
<tr>
<td>Nov 2018</td>
<td>53.5±6.5</td>
<td>158.2±13.2</td>
</tr>
<tr>
<td>May 2019</td>
<td>30.3±5.8</td>
<td>118.9±6.7</td>
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<td>Jun 2019</td>
<td>72.5±15.0</td>
<td>182.3±20.4</td>
</tr>
<tr>
<td>Average</td>
<td>52.1</td>
<td>153.1</td>
</tr>
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</table>

*SB and NB represent the south basin (n = 22 sampling sites) and north basin (n = 64 sampling sites), respectively, and the values are significantly different between basins at the p<0.05 level for all surveys. **Different sections impacted by different discharge types: Section-I (industrial effluents; n = 4), Section-T (municipal sewage; n = 6), Section-R (river input; n = 7), Section-A (aquacultural effluent; n = 22) and Section-N (non-discharge; n = 64). Across each row, values that share at least one letter are not significantly different from each other (p≥0.05) within the month. Average values are calculated across the three surveys for each column.
Fig. 1. Sampling sites within the Wenwusha Reservoir in Fujian Province, southeastern China. A total of 21 transects were sampled, with 11 transects in the south basin (SB) (1–11), and 10 in the north basin (NB) (12–21). Each circle represents a sampling site influenced by different discharge type: A: aquacultural effluent ($n = 4$); I: industrial effluent ($n = 6$); R: river input ($n = 7$); T: municipal sewage ($n = 22$); N: non-discharge ($n = 64$).
Fig. 2. Spatial distributions of surface-water NO$_3^-$-N (a-c), NH$_4^+$-N (d-f) and TDN (g-j) in the Wenwusha Reservoir.
Fig. 3. Spatial distributions of surface-water N$_2$O concentration (a-c) and fluxes (d-f) in the Wenwusha Reservoir.
Fig. 4. Boxplots of dissolved N\textsubscript{2}O concentrations and N\textsubscript{2}O fluxes for sampling sites with or without discharge. Each box shows the quartiles and median, while the square and whiskers represent the mean and values within 1.5 times of the interquartile range, respectively. Sites with discharge (n = 64) and without discharge (n = 39) are significantly different from each other (p<0.05) in all cases.
Fig. 5. The redundancy analysis (RDA) biplots of the N\textsubscript{2}O concentration / emission and hydrographical parameters in the Wenwusha Reservoir. The loadings of ancillary hydrographical parameters (arrows) and the scores of observations in the survey of November 2018 (a), March 2019 (b) and June 2019 (c) and combined data from all surveys (d). $W_T$, $EC$, $DO$, $CON$ and $FCole98$ represent water temperature, conductivity, dissolved oxygen, dissolved N\textsubscript{2}O concentration and N\textsubscript{2}O emission, respectively. The pie charts show the percentages of variance explained by the different parameters.
Fig. 6. (a) The box and whisker plots and (b) standard deviation of estimated N$_2$O fluxes from the Wenwusha Reservoir. Monte Carlo analysis was used to evaluate the effect of sample size on the emission estimate. Without replacements, the N$_2$O emission measurements were resampled from all sites ($n = 10, 20, 30…, 100$). The resampling process was repeated 10,000 times. For each sample size, the overall mean and standard deviation of N$_2$O emission were calculated. The N$_2$O emission are a function of the number of sampling sites selected from a total of 103 sites based on 1000 simulations. In panel $a$, the margins of the boxes are the upper and lower quartiles, and the line in the box represents the median. The whiskers are the extreme values that fall within 1.5 times the interquartile range and the circles represent extreme values that fall outside 1.5 times the interquartile range. The red line are the overall average N$_2$O flux based on the results from all 103 sites. The grey areas represent 70, 95 and 99% of the Highest Probability Density ranges.
Supporting Information

Coastal reservoirs as a source of the greenhouse gas nitrous oxide: spatial-temporal patterns and assessment strategy

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Supporting Information Summary

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Page S3: Fig. S2. Spatial distributions of surface-water WT (a–c), and pH (d–f) in the Wenwusha Reservoir.

Page S4: Fig. S3. Relationships between surface-water N2O concentration and NO3-N, NH4+-N and TDN concentrations in the Wenwusha Reservoir. Parameter bounds on the regression coefficients are at 95% confidence limits.

Page S5: Fig. S4. Relationships between N2O fluxes and surface-water NO3-N, NH4+-N and TDN concentrations in the Wenwusha Reservoir. Parameter bounds on the regression coefficients are at 95% confidence limits

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Fig. S2. Spatial distributions of surface-water $T_w$ (a-c), and pH (d-f) in the Wenwusha Reservoir.
Fig. S3. Relationships between surface-water \( \text{N}_2\text{O} \) concentration and \( \text{NO}_3^-\text{-N}, \text{NH}_4^+\text{-N} \) and TDN concentrations in the Wenwusha Reservoir. Parameter bounds on the regression coefficients are at 95% confidence limits.
Fig. S4. Relationships between N\textsubscript{2}O fluxes and surface-water NO\textsubscript{3}-N, NH\textsubscript{4}+-N and TDN concentrations in the Wenwusha Reservoir. Parameter bounds on the regression coefficients are at 95% confidence limits.
Table S1. Hydrographical parameters in the different areas based on discharge types in the Wenwusha Reservoir.

<table>
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<th>Month</th>
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<th>DO (mg L&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Salinity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt;-N (mg L&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NH&lt;sub&gt;4&lt;/sub&gt;+-N (mg L&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TDN (mg L&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tr>
<td>Nov-2018</td>
<td>Section-I</td>
<td>4.50±0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.08±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.96±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.87±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Section-T</td>
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<td>1.59±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>0.72±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.27±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
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<td><strong>1.43</strong></td>
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*Different sections impacted by different discharge types: Section-I (industrial effluents; n = 4), Section-T (municipal sewage; n = 6), Section-R (river input; n = 7), Section-A (aquacultural effluent; n = 22) and Section-N (no discharge; n = 64). DO, and TDN represent dissolved oxygen and total dissolved nitrogen, respectively. Different letters across each row indicate significant differences at the p<0.05 level. Averages across sections are calculated for each month. *Data are taken from Yang et al. (2020).*
Table S2. Pearson correlation coefficients between \( \text{N}_2\text{O} \) concentration / emission and different environmental variables in the Wenwusha Reservoir.

<table>
<thead>
<tr>
<th>Environmental variables</th>
<th>( \text{N}_2\text{O} ) concentration</th>
<th>( \text{N}_2\text{O} ) emission</th>
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<td>Meteorologic variables</td>
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<td>Air temperature</td>
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<td>NS</td>
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<tr>
<td>Wind speed</td>
<td>NS</td>
<td>-0.607**</td>
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<td>Atmospheric pressure</td>
<td>NS</td>
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<td>Hydrographical parameters</td>
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<td>Water temperature</td>
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<td>pH</td>
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<td>Dissolved oxygen (DO)</td>
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<td>NH(4^+)-N</td>
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<tr>
<td>TDN</td>
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<td>0.873**</td>
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The symbols * and ** denote significant correlations at \( p < 0.05 \) and \( p < 0.01 \), respectively. NS means non-significant relationship.
Table S3. Comparison of the N$_2$O fluxes (µg m$^{-2}$ h$^{-1}$) in reservoirs and lakes across different countries and climate zones.

<table>
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<tr>
<th>Ecosystem Type</th>
<th>Location</th>
<th>Climate</th>
<th>N$_2$O emission</th>
<th>Reference</th>
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<td>Reservoirs</td>
<td>Samuel Reservoir and Tucurui Reservoir, Brazil</td>
<td>Tropical</td>
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<td>Lima et al., 2002</td>
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<td>Petit Saut Reservoir and Fortuna Reservoir</td>
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<td>-247.5 to 401.6  (167.2)</td>
<td>Guérin et al., 2008</td>
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<td>Serra de Mesa Reservoir, Brazil</td>
<td>Tropical</td>
<td>5.7</td>
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<td>Manso Reservoir, Brazil</td>
<td>Tropical</td>
<td>5.7</td>
<td>Sikar et al., 2005</td>
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<td></td>
<td>Three Gorges Reservoir, China</td>
<td>Subtropical</td>
<td>-0.64 to 5.17 (---)</td>
<td>Yu et al., 2018</td>
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<td>Chongqing, Southwest China</td>
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<td>3.67 to 1221.0 (240.17)</td>
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<td>4.4 to 58.1 (19.8)</td>
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<td>0.4 to 26.8 (8.4)</td>
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<td>0.6 to 1.1 (---)</td>
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<td>-0.2 to 3.5 (0.4)</td>
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<td>Wenwusha Reservoir, China</td>
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<td>-10.8 to 711.5 (107.0)</td>
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<td>24.2 to 25.1 (24.8)</td>
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<td>4.9 to 142.1 (31.5)</td>
<td>Descloux et al., 2017</td>
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<td>-3.5 to 11.4 (---)</td>
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<td>-0.9 to 10.6 (---)</td>
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<td>31.2 to 78.8 (47.1)</td>
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<td>Lakes on the Tibetan Plateau, China</td>
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<td>0.1 to 38.3 (11.9)</td>
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Numbers in brackets are averages. “---” indicates no data.
References


