N\textsubscript{2}O emission and its influencing factors in subtropical streams, China

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Abstract

Background: Rivers and streams are one of the primary sources of nitrous oxide (N\textsubscript{2}O) which is an important greenhouse gas with great global warming potential. Yet, over the past century, human activities have dramatically increased reactive nitrogen loadings into and consequently led to increased N\textsubscript{2}O emission from the river ecosystems. Here, we carried out a study in two subtropical rivers, i.e., Jinshui River and Qi River with slight and intense human disturbance in their respective catchments in China. The study intended to explore spatial variability and seasonality in N\textsubscript{2}O emissions, and the relative importance of physicochemical variables, nitrification and denitrification potentials, and functional genes abundance influencing N\textsubscript{2}O emissions.

Results: N\textsubscript{2}O concentration, N\textsubscript{2}O saturation, and N\textsubscript{2}O flux of Jinshui River peaked in high flow season. N\textsubscript{2}O concentration, N\textsubscript{2}O saturations, and N\textsubscript{2}O flux in Qi River and downstream of Jinshui River were significantly higher than that in other areas in normal and low flow seasons. N\textsubscript{2}O concentration was positively correlated with water temperature, water NO\textsubscript{3}\textsuperscript{-}, and DOC, negatively correlated with water NH\textsubscript{4}\textsuperscript{+} and DOC/NO\textsubscript{3}\textsuperscript{-} (the ratio of dissolved organic carbon to NO\textsubscript{3}\textsuperscript{-} in water), and positively correlated with potential nitrification rate in high flow season, but not correlated with functional genes abundance. Both rivers had lower N\textsubscript{2}O saturation and flux than many freshwater systems, and their EFr-5 (N\textsubscript{2}O emission factor for river) was lower than the recommended values of IPCC.

Conclusions: While the two rivers were moderate sources of N\textsubscript{2}O and N\textsubscript{2}O emissions in river systems were normally elevated in the summer, areas with intense human disturbance had higher N\textsubscript{2}O concentration, N\textsubscript{2}O saturations, and N\textsubscript{2}O flux than those with slight human disturbance. Physicochemical variables were good indicators of N\textsubscript{2}O emissions in the river ecosystems.

Keywords: River, N\textsubscript{2}O concentration, N\textsubscript{2}O saturation, N\textsubscript{2}O flux, N\textsubscript{2}O emission factor

Introduction

Rivers are one of the primary sources of nitrous oxide (N\textsubscript{2}O) which is an important contributor to global climate change (Stocker et al. 2013; Hu et al. 2016; Marzadri et al. 2017). But in recent decades, increasing human activities, such as land use change (e.g., deforestation, urbanization, etc.) have released large quantities of pollutants, leading to increasing nitrogen loadings which affect nitrogen cycling in and increase N\textsubscript{2}O emission from the river ecosystems (Kim et al. 2014; Hou et al. 2015; Liu et al. 2015; Chen et al. 2019; Zheng et al. 2019). The influence of N\textsubscript{2}O emissions in the river system on the atmospheric N\textsubscript{2}O balance is becoming much more important (Seitzinger and Kroeze 1998).

N\textsubscript{2}O is a byproduct of different microorganisms’ transformations in the nitrogen cycling, including nitrification, denitrification, and dissimilatory nitrate reduction to ammonium (DNRA) (Cole and Caraco 2001). Nitrification and denitrification appear to be the dominant sources of N\textsubscript{2}O in most natural systems (Firestone and Davidson 1989; Wang et al. 2009). The microorganisms driving nitrification process contain AOA-amoA and
AOB-amoA genes (Kowalchuk et al. 2000). nirS, nirK, and nosZ are the key genes in the denitrification process (Braker et al. 2000; Wyman et al. 2013). N₂O emission in the environment is generally associated with these gene encoding enzymes and bacteria in the nitrogen cycling (Nie et al. 2015; Nie et al. 2016; Ma et al. 2017; Cocco et al. 2018; Black et al. 2019). These genes are often used to study the relationship between N₂O emission and microorganisms (Cocco et al. 2018).

Aquatic N₂O production is complex and sensitive to environmental variables (Wang et al. 2009), which arises from the complexity of the nitrogen cycling, the difficulty of decoupling hydrologic and biogeochemical processes, and increasing human disturbance in the river ecosystems (Liu et al. 2015). Increasing human disturbance, such as conversion of natural land use (e.g., forests and wetlands) to human land use (e.g., cropland and urban areas), releases large quantities of pollutants, including nitrogen, and has widespread effects on biodiversity and ecological function of rivers (Müller et al. 1998; Liu et al. 2015). The increase of inorganic nitrogen concentration can promote nitrification and denitrification, leading to increase in N₂O production (Weathers 1984; Herbert 1999; McMahon and Dennehy 1999; Naqvi et al. 2000; Cole and Caraco 2001). Other environmental factors, such as temperature, DO, C/NO₃⁻, can affect the nitrogen cycle processes and subsequently N₂O release (Kelso et al. 1997; Liikanen and Martikainen 2003; Baulch et al. 2012; Rosamond et al. 2012; Deng et al. 2015; Quick et al. 2019). Land use could also indirectly affect sediment denitrification and N₂O emission in headwater streams by influencing the river water quality or sediment characteristics (Inwood et al. 2007). But the relative contributions of different environmental factors and biogeochemical processes to N₂O emissions are widely debated (Bollmann and Conrad 1998; Soued et al. 2015; Gardner et al. 2016; Voigt et al. 2017), and few studies have addressed the indirect effects of catchment human disturbance on river N₂O emission. IPCC proposed a method to estimate N₂O emission flux from rivers by using emission factor (EF5-r). The recommended value of EF5-r was revised to 0.0025 in 2006 (IPCC 2006). However, due to the difference of N₂O generation mechanism in different geographical regions, the universal applicability of EF5-r is widely disputed (Wang et al. 2012).

Here, we investigated environmental factors, dissolved N₂O concentration, N₂O saturation, N₂O flux, and N₂O emission factor in different hydrological regimes (i.e., high, normal, and low flow seasons) in two subtropical rivers with different human disturbance intensities in their respective catchments in China. Our objects are to (1) assess spatial variability and seasonality in dissolved N₂O concentration, N₂O saturation, N₂O flux, and N₂O emission factor and (2) assess relationships between physicochemical factors, functional genes abundance, nitrification rates, denitrification rates, and N₂O concentration.

Materials and methods
Study area
Our study areas were located in Jinshui River and Qi River in China (Fig. 1). Jinshui River is a mountainous river, a secondary tributary of the Yangtze River and a primary tributary of the Han River. The catchment area of Jinshui River is 731 km². Mean annual temperature is 11.8°C. Annual precipitation ranges from 950 to 1200 mm (Zhang et al. 2010; Wang et al. 2015). Rainfall is highly variable, with July to October being high flow season, November and April to June is normal flow season, and December to March is low flow season (Wang et al. 2015). Elevation ranges from 363 to 2884 m in the catchment (Fig. 1).

Qi River is a plain river, a tertiary tributary of the Yangtze River and a secondary tributary of Han River. The basin area of Qi River is 1501 km². The average annual temperature is about 15.1 °C, and the annual precipitation ranges from 860 to 935 mm. June to August is high flow season, March to May and September to November is normal flow season, and December to February is low flow season (Xiong 2018). Elevation ranges from 161 to 2018 m in the catchment (Fig. 1).

The highest temperatures occur in high flow season, followed by normal and low flow seasons in the Jinshui River and Qi River. For Jinshui River, the catchment can be divided into three zones representing varying human disturbance intensities (i.e., slightly, moderately and intensively disturbed areas) from upstream to downstream based upon population density, area of cropland, and disturbance history (Zhang et al. 2010, 2013; Wang et al. 2015). Upstream of Jinshui River is in the Foping National Nature Reserve of the Qinling Mountains, which is mostly uninhabited with extensive forest cover (Zhang et al. 2010, 2013). Cultivated lands and small towns are primarily located along the downstream and midstream of Jinshui River catchment. There are no industries in Jinshui River catchment. However, there are cultivation of edible fungi, fruit trees and traditional Chinese medicine herbs, power stations, and pharmaceutical factories in Qi River catchment (Zhao et al. 2020). In general, human disturbance in Qi River catchment has been more intensive than that in Jinshui River catchment, and the area of small towns and cultivated lands are larger in Qi River catchment than those in Jinshui River (Table S1, land use attribute table of Jinshui River and Qi River catchments; Zhao et al. 2020).
Field sampling
We sampled sediment, overlying water, and air samples at nine locations from upstream to downstream in Jinshui River (Zhang et al. 2013; Wang et al. 2015) (Fig. 1). Sites J1, J2, and J3 were located downstream, sites J4, J5, and J6 were located midstream, and sites J7, J8, and J9 were located in the upstream. Similarly, we sampled samples at nine locations from upstream to downstream in Qi River (Fig. 1). Sites Q1, Q2, and Q3 were located downstream, sites Q4, Q5, and Q6 were located midstream, and sites Q7, Q8, and Q9 were located upstream.

According to the hydrological regime, samples were collected in high flow season (August 2018), normal flow season (November 2018), and low flow season (March 2019) in both rivers. We sampled sediment using sterile sampling bags, and each sediment sample was mixed with three parallel samples. Overlying water (0–10 cm) was collected with polyethylene plastic bottles, filtered the samples through filter membranes (0.45 μm), and stored them at 4 °C for the determination of physicochemical factors, nitrification and denitrification rates. Overlying water (0–10 cm) was collected with 60 mL serum bottle (Thermo Fisher) for the determination of dissolved N2O concentration, and to prevent microbial activity, these samples were poisoned with 500 μL of a saturated aqueous mercury chloride (HgCl₂) solution.

The surface sediment samples (0–5 cm) were collected with sterilized shovel and stored the sediment in sterile TWIRL® EPR-3050 sample bags (Labplas, Quebec). Sediment samples for molecular analyses were stored in liquid nitrogen immediately, and samples for nitrogen transformation rate and physicochemical factors detections were stored in 4 °C. We sampled air samples with 12 mL gas-tight vials (Labco Exetainers) for air N₂O detections above 0.5 m the water surface. Wind speed at 2 m above water surface was measured by hand-held anemometer (Kestrel 2500, USA).
Measurement of water and sediment physicochemical variables

Water temperature was measured with a YSI Professional ProPlus probe in the field. NO$_3^-$ and NH$_4^+$ concentrations of water (w-NO$_3^-$ and w-NH$_4^+$) and sediments (s-NO$_3^-$ and s-NH$_4^+$) were measured with an automatic continuous flow analyzer (AMS westco, Smartchem 200, Italy) in the lab. NO$_3^-$ and NH$_4^+$ concentrations of sediments were extracted from fresh soil with KCl (2.0 mol/L) in the lab. Dissolved organic carbon (DOC) concentration of water and sediment organic carbon (SOC) were measured with a TOC analyzer (Elementar, Vario TOC, Germany) in the lab.

Detection of dissolved N$_2$O concentration, N$_2$O saturation, N$_2$O flux, and N$_2$O emission factor

Static headspace gas chromatography was used to determine dissolved N$_2$O concentration in water samples (Walter et al. 2005). During the water sample pretreatment, a needle was inserted into the rubber stopper of the serum bottle under the condition of sealing the serum bottle in the lab. NO$_3^-$ automatic continuous flow analyzer (AMS westco, Smartchem 200, Italy) in the lab. N$_2$O concentration to the equilibrium N$_2$O concentration in water was calculated according to the headspace N$_2$O concentration (C$_{eq}$) (Eq. 2). The equilibrium N$_2$O concentration was calculated by Henry formula (Yang et al. 2013; Liu et al. 2011b; Eq. 3).

$$ C_{eq} = \beta \times C_A $$

where $C_{eq}$ is the equilibrium concentration of N$_2$O in water at the given water temperature (nmol/L), and $C_A$ is the atmospheric N$_2$O concentration of the sampling sites (nmol/L).

The flux of N$_2$O was calculated as follows (Wanninkhof 1992, 2014; Cole and Caraco 2001; Crusius and Wanninkhof 2003; Eq. 4):

$$ F = k \times \Delta N_2O $$

where $\Delta N_2O$ is the N$_2$O net increase and is the difference between the dissolved N$_2$O concentration to the equilibrium N$_2$O concentration (Eq. 5). $k$ denotes gas exchange rate (Cole and Caraco 1998; Crusius and Wanninkhof 2003; Eqs. 6 and 7).

$$ \Delta N_2O = C_{N2O} \cdot C_{eq} $$

$$ k = (2.07 + 0.215U_{10}^{1.7}) \times \left( \frac{S_C}{600} \right)^{0.7} $$

for $U_{10} < 3.7m/s$ (6)

$$ k = (4.33U_{10} - 13.3) \times \left( \frac{S_C}{600} \right)^{-0.2} $$

for $U_{10} > 3.7m/s$ (7)

where $U_{10}$ denotes wind speed at 10 m above water surface (m/s), and $U_{10}$ was calculated by wind speed at 2 m above water surface ($U_2$) (Yang et al. 2015; Eq. 8; Table S2). $S_C$ denotes viscosity coefficient of N$_2$O (Wanninkhof 2014; Eq. 9).

$$ \frac{U_2}{U_{10}} = \frac{\lg 200}{\lg 1000} $$

$$ S_C = 2141.2 - 152.56 T + 5.8963 T^2 - 0.12411 T^3 + 0.0010655 T^4 $$

where $T$ denotes water temperature (°C). N$_2$O emission factor for river (EF5-r) is ratios of dissolved N$_2$O-N ($\mu$g N/L) to NO$_3^-$-N ($\mu$g N/L) (Eq. 10).

$$ EF5-r = \frac{[N_2O]}{[NO3]} \times 100\% $$

Statistical analysis

Linear mixed modeling was performed to examine spatial variability and seasonality in dissolved N$_2$O concentration in water, N$_2$O saturation in water, N$_2$O flux and N$_2$O emission factor using SPSS 20 (SPSS*, version 20; IBM*, Armonk, New York). Sampling sites were random effects, and sampling seasons and rivers were fixed effects. Linear mixed modeling was performed to examine the difference in N$_2$O concentration, N$_2$O saturation,
N$_2$O flux, and N$_2$O emission factor among sampling areas of each river using SPSS 20. Sampling sites were random effects, and sampling areas were fixed effects.

A series of stepwise multiple regression analyses with backward selection ($P < 0.05$) were then applied to identify the determinants of N$_2$O concentration using SPSS 20 (the independent variables were water and sediment physicochemical variables). Correlations between dissolved N$_2$O concentration, nitrification genes abundance (Fig. S1, AOA-amoA, AOB-amoA), denitrification genes abundance (Fig. S2, nirK; Fig. S3, nirS; Fig. S4, nosZ), denitrification rates (Fig. S5), and nitrification rates (Fig. S6) were analyzed with the Pearson correlation analyses using Origin 9.0.

The Structural Equation Modeling (SEM) was performed to further elucidate the direct and indirect effects of key explanatory variables on N$_2$O concentration. First, a conceptual path model was developed according to existing literature and basic ecological principles (Liu et al. 2015; Feng et al. 2018). Second, promising explanatory variables were selected to include in path analysis mainly based on the results of Pearson correlation (Table S3) and stepwise multiple regression analyses. The abundance of nitrification genes (AOB-amoA and AOA-amoA) and three denitrification genes (nirK, nirS, and nosZ) were found to be highly positively correlated with each other (Table S4). Afterwards, a principal component analysis (PCA) was conducted to reduce the number of variables using SPSS 20.0 (SPSS, Chicago, IL, USA). The principal component 1 (PC1) extracted from two nitrification gene explained 54.71% of the total variance and was thus considered as the representative of the overall variation in nitrification genes. The principal component 1 (PC1) extracted from three denitrification genes explained 70% of the total variance and was thus considered as the representative of the overall variation in denitrification genes (Feng et al. 2018). amoA and denitrification genes were introduced as new variables into the SEM. Third, path coefficients, $R^2$, direct and indirect effects, and model fit parameters were calculated by AMOS 20.0. The low $\chi^2$ (chi-squared test), $P$ value $> 0.05$, a comparative fit index (CFI) value $> 0.95$, Tucker-Lewis index (TLI) value $> 0.90$, and root square error of approximation (RMSEA) $< 0.05$ indicated that the final path model had an acceptable fit with the data (Schmelleh-Engel et al. 2003; Fan et al. 2016). Statistical analyses were conducted at a 0.05 significant level.

**Results**

**Physicochemical variables**

For Jinshui River, water temperature, s-NO$_3^-$, w-NH$_4^+$, s-NH$_4^+$, DOC, and SOC in high flow season were higher than those in other seasons (Tables 1 and 2). DOC/NO$_3^-$ (the ratio of dissolved organic carbon to NO$_3^-$ in

| Table 1 Water physicochemical variables of Jinshui River and Qi River |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Sampling season** | **River** | **Sampling area** | **Temp (°C)** | **w-NO$_3^-$ (mg L$^{-1}$)** | **w-NH$_4^+$ (mg L$^{-1}$)** | **DOC (mg L$^{-1}$)** | **DOC/NO$_3^-$** |
| High flow season | Jinshui River | Downstream | 28.72 ± 0.51$^a$ | 0.94 ± 0.30 | 0.78 ± 0.19 | 7.67 ± 1.06$^b$ | 9.10 ± 4.49 |
| | | Midstream | 25.23 ± 4.71$^a$ | 1.03 ± 0.23 | 0.71 ± 0.11 | 8.63 ± 0.59$^b$ | 10.45 ± 2.21 |
| | | Upstream | 16.04 ± 0.53$^a$ | 1.15 ± 0.07 | 0.56 ± 0.03 | 10.61 ± 2.17$^b$ | 11.32 ± 1.57 |
| | Qi River | Downstream | 32.07 ± 0.97$^a$ | 1.44 ± 0.33 | 0.67 ± 0.19 | 7.12 ± 1.58 | 8.69 ± 2.55 |
| | | Midstream | 29.78 ± 1.05$^{ab}$ | 1.09 ± 0.12 | 0.49 ± 0.05 | 7.45 ± 0.60 | 8.57 ± 1.18 |
| | | Upstream | 23.92 ± 5.58$^a$ | 1.68 ± 0.52 | 0.67 ± 0.42 | 8.84 ± 0.76 | 7.66 ± 1.57 |
| Normal flow season | Jinshui River | Downstream | 8.73 ± 0.32$^a$ | 1.23 ± 1.12 | 0.04 ± 0.00 | 7.49 ± 2.28$^a$ | 10.08 ± 7.30 |
| | | Midstream | 7.58 ± 0.78$^a$ | 0.60 ± 0.11 | 0.04 ± 0.01 | 5.02 ± 0.20$^{ab}$ | 12.83 ± 1.91 |
| | | Upstream | 4.77 ± 0.42$^c$ | 0.91 ± 0.08 | 0.04 ± 0.00 | 4.60 ± 0.48$^b$ | 11.88 ± 0.54 |
| | Qi River | Downstream | 12.13 ± 1.37$^a$ | 0.86 ± 0.38 | 0.04 ± 0.01 | 14.04 ± 4.34$^a$ | 8.55 ± 16.95 |
| | | Midstream | 10.73 ± 0.50$^{ab}$ | 1.28 ± 0.32 | 0.04 ± 0.01 | 9.84 ± 1.39$^{ab}$ | 6.86 ± 2.77 |
| | | Upstream | 8.47 ± 1.99$^b$ | 1.29 ± 0.72 | 0.04 ± 0.01 | 6.57 ± 0.55$^b$ | 5.69 ± 2.38 |
| Low flow season | Jinshui River | Downstream | 10.43 ± 0.64$^a$ | 0.50 ± 0.01$^c$ | 0.38 ± 0.02 | 12.35 ± 0.54$^b$ | 24.63 ± 0.84$^a$ |
| | | Midstream | 7.66 ± 1.23$^b$ | 0.59 ± 0.02$^b$ | 0.32 ± 0.03 | 8.08 ± 0.62$^b$ | 21.47 ± 1.61$^b$ |
| | | Upstream | 4.34 ± 0.08$^a$ | 0.91 ± 0.04$^a$ | 0.47 ± 0.14 | 6.44 ± 0.07$^a$ | 17.52 ± 0.29$^a$ |
| | Qi River | Downstream | 9.87 ± 0.38 | 0.56 ± 0.20$^b$ | 0.39 ± 0.09 | 26.28 ± 4.15$^a$ | 13.76 ± 46.82 |
| | | Midstream | 9.79 ± 2.18 | 1.13 ± 0.18$^a$ | 0.39 ± 0.02 | 20.81 ± 2.41$^{ab}$ | 10.92 ± 1.51 |
| | | Upstream | 8.87 ± 1.88 | 0.84 ± 0.23$^a$ | 0.42 ± 0.01 | 15.77 ± 1.17$^b$ | 8.94 ± 5.35 |

Values are presented as mean ± SD (n = 3); different letters indicate significant differences among sampling areas by linear mixed modeling ($P < 0.05$).

Abbreviations: temperature is denoted by Temp, the NO$_3^-$ and NH$_4^+$ concentration of water is denoted by w-NO$_3^-$ and w-NH$_4^+$. The dissolved organic carbon concentration of water is denoted by DOC. The ratio of dissolved organic carbon to NO$_3^-$ in water is denoted by DOC/NO$_3^-$.
water) and SOC/NO$_3^-$ (the ratio of sediment organic carbon to sediment NO$_3^-$) in low flow season were higher than those in other seasons ($P < 0.05$). There was no significant seasonal difference in w-NO$_3^-$ ($P > 0.05$). Temperature in downstream was the highest in all seasons ($P < 0.05$). DOC in upstream was the highest in high flow season ($P < 0.05$). DOC in downstream was the highest in normal flow and low flow seasons ($P < 0.05$). w-NO$_3^-$ in downstream was the lowest in low flow season ($P < 0.05$). s-NO$_3^-$ and SOC in midstream were the lowest in high flow season ($P < 0.05$). And DOC/NO$_3^-$ in downstream was the highest in low flow season ($P < 0.05$).

For Qi River, water temperature, w-NO$_3^-$, s-NO$_3^-$, w-NH$_4^+$, s-NH$_4^+$ and SOC in high flow season were higher than those in other seasons ($P < 0.05$). DOC and DOC/NO$_3^-$ in low flow season were higher than those in other seasons ($P < 0.05$). There was no significant seasonal difference in SOC/NO$_3^-$ ($P > 0.05$). Temperature in downstream was highest in high and normal flow seasons ($P < 0.05$). w-NO$_3^-$ in downstream was the lowest in high flow season ($P < 0.05$). s-NO$_3^-$ in upstream was the lowest in high flow season ($P < 0.05$). DOC in downstream was the lowest in normal and low flow seasons ($P < 0.05$). And SOC/NO$_3^-$ in upstream was the lowest in low flow season ($P < 0.05$).

Comparatively, water temperature in Qi River was higher than that in Jinshui River in normal flow season ($P < 0.05$). w-NO$_3^-$ concentration in Qi River was higher than that in Jinshui River in high flow season ($P < 0.05$). DOC in Qi River was higher than that in Jinshui River in normal and low flow seasons ($P < 0.05$).

### Dissolved N$_2$O concentration

N$_2$O concentrations in Qi River were higher, 1.51 and 1.37 times of that in Jinshui River in normal and low flow seasons (Fig. 2, $P < 0.01$ and $P < 0.05$), respectively. For Jinshui River, there was no significant seasonal difference in N$_2$O concentration ($P > 0.05$). N$_2$O concentration in normal flow season in downstream was higher ($P < 0.05$), and 1.12 and 1.12 times of that in midstream and upstream, respectively. There was no difference in N$_2$O concentration between sampling sites in high and low flow seasons ($P > 0.05$). For Qi River, there was no significant seasonal difference in N$_2$O concentration ($P > 0.05$). N$_2$O concentration of normal flow season in midstream was higher, 1.23 times of that in downstream ($P < 0.05$).

### N$_2$O saturation

N$_2$O saturation in Qi River was higher, 1.69 times of that in Jinshui River in normal flow season (Fig. 3, $P < 0.01$ and $P < 0.05$). For Jinshui River, N$_2$O saturation

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### Table 2 Sediment physicochemical variables of Jinshui River and Qi River

| Sampling season | River       | Sampling area | s-NO$_3^-$ (mg kg$^{-1}$) | s-NH$_4^+$ (mg kg$^{-1}$) | SOC (g kg$^{-1}$) | SOC/NO$_3^-$ |
|-----------------|-------------|--------------|--------------------------|--------------------------|-----------------|--------------|
| High flow season| Jinshui River | Downstream   | 6.04 ± 2.12$^a$          | 5.74 ± 1.95              | 7.42 ± 1.87$^a$ | 1.30 ± 0.39  |
|                 |             | Midstream    | 5.97 ± 4.47$^b$          | 4.83 ± 0.98              | 3.14 ± 0.48$^b$ | 2.09 ± 1.66  |
|                 |             | Upstream     | 6.70 ± 2.34$^a$          | 5.99 ± 1.39              | 10.06 ± 0.80$^a$| 1.80 ± 0.49  |
|                 | Qi River    | Downstream   | 6.79 ± 0.42$^{ab}$       | 6.17 ± 1.24$^b$          | 5.55 ± 2.39     | 1.35 ± 0.39  |
|                 |             | Midstream    | 7.98 ± 0.74$^d$          | 8.61 ± 1.05$^d$          | 7.93 ± 6.14     | 0.65 ± 0.68  |
|                 |             | Upstream     | 5.33 ± 1.74$^b$          | 5.52 ± 0.55$^b$          | 4.38 ± 0.72     | 1.03 ± 0.49  |
| Normal flow season | Jinshui River | Downstream   | 1.34 ± 0.09              | 0.80 ± 0.47              | 3.47 ± 2.05     | 2.62 ± 1.61  |
|                 |             | Midstream    | 0.82 ± 0.25              | 0.89 ± 0.55              | 5.74 ± 4.53     | 4.85 ± 4.71  |
|                 |             | Upstream     | 1.22 ± 0.07              | 0.60 ± 0.10              | 9.48 ± 3.88     | 5.09 ± 2.83  |
|                 | Qi River    | Downstream   | 0.98 ± 0.22              | 1.19 ± 0.46              | 2.58 ± 3.43     | 7.01 ± 3.11  |
|                 |             | Midstream    | 0.83 ± 0.32              | 1.13 ± 1.02              | 1.79 ± 1.13     | 5.32 ± 2.34  |
|                 |             | Upstream     | 1.06 ± 0.73              | 1.26 ± 0.79              | 5.50 ± 3.53     | 7.50 ± 6.14  |
| Low flow season | Jinshui River | Downstream   | 0.60 ± 0.16              | 3.43 ± 0.97              | 2.64 ± 1.11     | 4.59 ± 2.29  |
|                 |             | Midstream    | 1.00 ± 0.07              | 2.19 ± 1.14              | 5.21 ± 3.46     | 3.32 ± 3.25  |
|                 |             | Upstream     | 1.03 ± 0.37              | 2.44 ± 1.92              | 2.72 ± 0.40     | 5.36 ± 0.75  |
|                 | Qi River    | Downstream   | 1.81 ± 1.43              | 2.89 ± 0.67              | 2.87 ± 1.75     | 5.16 ± 1.21$^a$|
|                 |             | Midstream    | 0.94 ± 0.25              | 2.10 ± 0.72              | 1.47 ± 0.64     | 5.06 ± 0.20$^b$|
|                 |             | Upstream     | 0.58 ± 0.32              | 1.73 ± 0.59              | 3.26 ± 1.00     | 2.74 ± 4.39$^b$|

Values are presented as mean ± SD ($n = 3$); different letters indicate significant differences among sampling areas by linear mixed modeling ($P < 0.05$).

Abbreviations: the NO$_3^-$ and NH$_4^+$ concentration of sediment are denoted by s-NO$_3^-$ and s-NH$_4^+$, Sediment organic carbon concentration is denoted by SOC. The ratio of sediment organic carbon to NO$_3^-$ in sediment is denoted by SOC/NO$_3^-$.
in high flow season was higher \((P < 0.01)\), 1.61 and 1.45 times of that in normal and low flow seasons, respectively. The \(\text{N}_2\text{O}\) saturation in downstream was the highest for all three seasons \((P < 0.05)\). For Qi River, there was no significant seasonal difference in \(\text{N}_2\text{O}\) saturation in Qi River \((P > 0.05)\). \(\text{N}_2\text{O}\) saturation of normal flow season in midstream was higher, 1.24 times of that in upstream \((P < 0.05)\).

\textbf{N}_2\text{O} \text{ flux}

\(\text{N}_2\text{O}\) flux of Qi River was 13.28 and 4.79 times of that in Jinshui River in normal and low flow seasons,
respectively (Fig. 4, $P < 0.01$ and $P < 0.05$). For Jinshui River, the N$_2$O flux in high flow season was higher ($P < 0.01$), 7.95 and 1.77 times of that in normal and low flow seasons, respectively. N$_2$O flux of downstream was higher than midstream and upstream in normal and low flow seasons ($P < 0.05$). N$_2$O flux in few downstream sampling sites was less than zero. For Qi River, there was no significant seasonal difference in N$_2$O flux ($P > 0.05$). The N$_2$O flux in upstream was higher, 1.63 times of that in downstream in high flow season ($P < 0.05$).

**N$_2$O emission factor**

N$_2$O emission factor in Jinshui River varied from 0.042–0.054%, 0.047–0.072%, and 0.047–0.089%, and in Qi River varied from 0.034–0.049%, 0.057–0.083%, and 0.054–0.113% in high, normal, and low flow seasons, respectively (Table 3). There was no significant difference in N$_2$O emission factor between the two rivers ($P > 0.05$). For Jinshui River, N$_2$O emission factor in low flow season was higher than that in high flow season ($P < 0.05$). N$_2$O emission factor in upstream was lower than that in downstream and midstream in low flow season ($P < 0.05$). For Qi River, N$_2$O emission factor in low flow season was higher than that in high and normal flow season ($P < 0.05$). There was no significant difference among sampling sites in all seasons ($P > 0.05$).

**Relationship between N$_2$O concentration, physicochemical variables, nitrification rate, denitrification rate, and functional genes abundance**

Stepwise multiple regression revealed that w-NO$_3^-$, DOC, w-NH$_4^+$, and DOC/NO$_3^-$ explained a relatively large portion of the variances in N$_2$O concentration at annual level for both rivers (Table 4, $P < 0.05$). w-NO$_3^-$ explained a relatively large portion of the variances in N$_2$O concentration in high flow season (Table 4, $P < 0.05$). Water temperature explained a relatively large portion of the variances in N$_2$O concentration in normal flow season (Table 4, $P < 0.01$). For Jinshui River, DOC/NO$_3^-$ explained a relatively large portion of the variances in N$_2$O concentration in normal flow season (Table 4, $P < 0.05$). For Qi River, w-NH$_4^+$ explained a relatively large portion of the variances in N$_2$O concentration at annual level (Table 4, $P < 0.05$).

Pearson’s correlation analyses showed that there was no significant correlation between functional genes abundance and N$_2$O concentration (Table S5, $P > 0.05$). There was significantly positive correlation between N$_2$O concentration and nitrification rate in normal flow season (Table S5, $r = 0.52$, $P < 0.05$).

SEM result ($R^2 = 0.431$, $P = 0.977$, $\chi^2 = 0.467$, CFI = 1.00, TLI = 1.151, RMSEA = 0.000) showed water temperature, w-NO$_3^-$, w-NH$_4^+$, DOC, and DOC/NO$_3^-$ could affect N$_2$O concentration both directly and indirectly (Fig. 5, Table S6, $P < 0.05$).
Seasonal and spatial variabilities of N\textsubscript{2}O emission

N\textsubscript{2}O emission (N\textsubscript{2}O concentration, N\textsubscript{2}O saturation, and N\textsubscript{2}O flux) showed significant seasonality in present study. Similar to other studies (Hasegawa et al. 2000; Harrison and Matson 2003; Garnier et al. 2009; Beaulieu et al. 2010; Rosamond et al. 2012; Burgos et al. 2015), N\textsubscript{2}O concentration, N\textsubscript{2}O saturation and flux of Jinshui River were peak in high flow season (summer) (Figs. 2 and 4). The higher NO\textsubscript{3}\textsuperscript{−} and organic carbon in high flow season were important factors and tended to enhance microbial processes including those producing N\textsubscript{2}O, such as nitrification and denitrification (Starry et al. 2005; Wang et al. 2018; Liu et al. 2019). Temperature is the key driver of the temporal dynamics of N\textsubscript{2}O emission (Wang et al. 2018), and higher temperature in high flow season affects the decomposition rate of organic matter through its effect on microbial activity and consequently regulates N\textsubscript{2}O production rate in the present study (Wang et al. 2018). This finding confirms that N\textsubscript{2}O emissions in subtropical river systems are normally elevated in the summer (Musenze et al. 2014, 2015; Allen et al. 2011).

Significant spatial differences in N\textsubscript{2}O emission were also observed in present study, N\textsubscript{2}O concentration, N\textsubscript{2}O saturation, and N\textsubscript{2}O flux were higher in areas with intensive human disturbance (Figs. 2 and 4). As previously noted, significant variability in water physicochemical variables was observed in the sampling areas, and these variables could be considered here as possible factors influencing the spatial differences in N\textsubscript{2}O emission. The effects of human disturbance on river N\textsubscript{2}O emission were more likely driven through changes of water physicochemical variables (Liu et al. 2015). Water characteristics were significantly affected by human disturbance (Sponseller et al. 2001; Huang et al. 2012). Disturbance gradient followed an elevational gradient in the present study, and the elevational gradient also had driven higher temperatures in the lower-elevation intensely disturbed areas. Higher temperature enhanced the N\textsubscript{2}O production processes (Rosamond et al. 2012; Burgos et al. 2015). The increase of agricultural

Table 3  N\textsubscript{2}O emission factor (EF5-r, dissolved N\textsubscript{2}O-N:NO\textsubscript{3}\textsuperscript{−}-N) of Jinshui River and Qi River

| River     | Sampling area | High flow season (%) | Normal flow season (%) | Low flow season (%) |
|-----------|---------------|----------------------|------------------------|--------------------|
| Jinshui   | Downstream    | 0.054 ± 0.013        | 0.062 ± 0.023          | 0.089 ± 0.007\textsuperscript{a} |
|           | Midstream     | 0.049 ± 0.002        | 0.072 ± 0.008          | 0.076 ± 0.005\textsuperscript{a} |
|           | Upstream      | 0.042 ± 0.001        | 0.047 ± 0.003          | 0.047 ± 0.004\textsuperscript{b} |
| Qi River  | Downstream    | 0.039 ± 0.026        | 0.083 ± 0.026          | 0.113 ± 0.028      |
|           | Midstream     | 0.046 ± 0.011        | 0.061 ± 0.011          | 0.054 ± 0.002      |
|           | Upstream      | 0.034 ± 0.012        | 0.057 ± 0.012          | 0.075 ± 0.016\textsuperscript{b} |

Values are presented as mean ± SD (n = 3); different letters indicate significant differences among sampling areas by linear mixed modeling (P < 0.05)

Table 4 Results of stepwise multiple regression analyses to predict N\textsubscript{2}O concentration

| Sampling season | Independent variables | Coefficient | Adjusted $R^2$ | P value |
|-----------------|-----------------------|-------------|----------------|---------|
| Two Rivers Whole year | w-NO\textsubscript{3}\textsuperscript{−} | 0.28        | 0.08           | 0.04    |
|                  | DOC                   | 0.73        | 0.21           | 0.00    |
|                  | w-NH\textsubscript{4}\textsuperscript{+} | -0.28       | 0.27           | 0.02    |
|                  | DOC/NO\textsubscript{3}\textsuperscript{−} | -0.46       | 0.33           | 0.03    |
|                  | High flow season | w-NO\textsubscript{3}\textsuperscript{−} | 0.59        | 0.301    | 0.01    |
|                  | Normal flow season | Temp        | 0.65         | 0.39     | 0.00    |
|                  | Low flow season     | -           | -             | -        | -       |
| Jinshui River Whole year | DOC/NO\textsubscript{3}\textsuperscript{−} | -0.53       | 0.25           | 0.01    |
|                  | High flow season    | -           | -             | -        | -       |
|                  | Normal flow season  | -           | -             | -        | -       |
|                  | Low flow season     | -           | -             | -        | -       |
| Qi River Whole year | w-NH\textsubscript{4}\textsuperscript{+} | -0.44       | 0.16           | 0.02    |
|                  | High flow season    | -           | -             | -        | -       |
|                  | Normal flow season  | -           | -             | -        | -       |
|                  | Low flow season     | -           | -             | -        | -       |

Correlation coefficients with P values less than 0.05 were shown. “-” represent P > 0.05
and urban land use could lead to the decline of river water quality including increased reactive nitrogen and degrading organics in the terrestrial biosphere (Sponseller et al. 2001; Huang et al. 2012; Kim et al. 2014; Hou et al. 2015), leading to higher denitrification rate (Jung et al. 2014; Harrison et al. 2011; Morse et al. 2012). Therefore, the Qi River and downstream of Jinshui River with higher water temperature, DOC and NO$_3^-$, had promoted the occurrence of nitrification and denitrification which enhanced N$_2$O concentration.

**Influencing factors of dissolved N$_2$O concentration**

N$_2$O concentration has been shown to be associated with many physicochemical variables. Higher temperature can increase microbial enzyme activity in denitrification and nitrification processes (Chen et al. 2011; Zheng et al. 2016), which was demonstrated by the positive correlation between N$_2$O concentration and water temperature in present study (Table 4). On the other hand, expression and activity of key enzymes in denitrification and nitrification processes are strongly dependent on the carbon substrate (Philippot et al. 2013; Sigleo 2019). Higher organic carbon content leads to proliferation of heterotrophic bacteria, large consumption of dissolved oxygen (Wang et al. 2015), and the anaerobic environment is more suitable for denitrification (Chapin et al. 2011; Hou et al. 2013; Ma et al. 2014). Also, many nitrification microorganisms can use organic carbon as carbon source (Hallam et al. 2006), and these may explain the positive correlation between N$_2$O concentration and DOC (Table 4).

Previous studies found that DOC/NO$_3^-$ was significantly negatively correlated with nitrification (Schade et al. 2016; Zhao et al. 2020) and reported higher sediment denitrification rates under optimal DOC/NO$_3^-$ range (0.35–3.5) (Hansen et al. 2016). DOC/NO$_3^-$ was beyond this range in present study, and higher DOC/NO$_3^-$ might have inhibited N$_2$O production from denitrification and nitrification. Denitrification is positively correlated with NO$_3^-$ concentration (Jung et al. 2014; Liu et al. 2019), so higher NO$_3^-$ concentration may promote N$_2$O production. In the present study, N$_2$O concentration was positively correlated with w-NO$_3^-$ concentrations (Table 4), but the relationship was not always significant (Fig. 5; Reay et al. 2003). These results indicated uncertainty of the correlation between NO$_3^-$ and N$_2$O emission, which suggests complexity of N$_2$O production in rivers (Liu et al. 2011a). Also, heterotrophic microorganisms consume NH$_4^+$ with rapid propagation, providing an anaerobic environment for denitrification; therefore, N$_2$O concentration from denitrification might increase as w-NH$_4^+$ decreased (Liu et al. 2015).

The correlation between functional genes abundance and N$_2$O concentration was weak, but N$_2$O concentration was positively correlated with potential nitrification rate in high flow season (Table S5). Several studies have shown that nitrification rate can be greater than denitrification rate in rivers (Holmes et al. 1996; Webster et al. 1997; Goedeking et al. 2003; Naus et al. 2005; Reay et al. 2003).
N$_2$O emission factor, N$_2$O saturations, and N$_2$O flux

IPCC recommended value of N$_2$O emission factor for river (EF5-r) was 0.0025 (IPCC 2006). Similar to other studies (Clough et al. 2006; Yang et al. 2015), our measured EF5-r values ranged from 0.00034 to 0.00113 in the present study (Table 3). According to the IPCC definition, the amount of N$_2$O released estimated by IPCC release coefficient may be overestimated because dissolved N$_2$O concentration in river includes part of N$_2$O dissolved in water to reach equilibrium, which is not a source of atmospheric N$_2$O (Wang et al. 2012). A discrete measurement of EF5-r is extremely difficult, and its values were different in different rivers (Table 3). New measurement and estimation techniques are needed to minimize errors of N$_2$O flux by applying single model (Clough et al. 2006).

Interestingly, seasonal difference of N$_2$O saturation and N$_2$O flux was significant in Jinshui River (Figs. 3 and 4), but not in Qi River. Spatial variations of N$_2$O saturation and N$_2$O flux of Qi River were inconsistent (Figs. 3 and 4). This may be due to larger direct discharge of sewage in Qi River, and N$_2$O in the water body far exceeds the amount of N$_2$O formed in the process of nitrogen migration and transformation, which makes the seasonal difference of N$_2$O emission smaller. Our study showed N$_2$O saturations and flux in Jinshui River and Qi River were similar to most freshwater systems in China (Yan et al. 2004; Zhao et al. 2009; Wang et al. 2012; Xu et al. 2016) and lower than those in other countries (García-Ruiz et al. 1999; McMahon and Dennehy 1999; Dong et al. 2004; Rosamond et al. 2011). N$_2$O saturations of most samples in Jinshui River and Qi River were greater than 100% (Fig. 3), which indicated that both rivers were sources of atmospheric N$_2$O (Yang et al. 2013). Overall, the two rivers had high N$_2$O fluxes in most of their areas, and they were moderate sources of the atmospheric N$_2$O.

Conclusions

We investigated N$_2$O concentration, N$_2$O saturation, N$_2$O flux, and N$_2$O emission factor of two subtropical rivers, China. Our results revealed that: (1) N$_2$O concentration, N$_2$O saturation, and N$_2$O flux of Jinshui River peaked in high flow season, and areas with intensive human disturbance had higher N$_2$O concentration, N$_2$O saturation, and N$_2$O flux in normal and low flow seasons. (2) Our present study rivers had lower N$_2$O saturation and flux than many freshwater systems, and they were moderate sources of N$_2$O. (3) Physicochemical variables including temperature, NO$_3$-, NH$_4$+, DOC, SOC, DOC/NO$_3$- and SOC/NO$_3$- were good indicators of N$_2$O emissions in the river ecosystems.

Abbreviations

N$_2$O: Nitrous oxide; EF5-r: N$_2$O emission factor for river; Temp: Temperature; w-NO$_3$-, NO$_3$- concentration of water; w-NH$_4$+, NH$_4$+ concentration of water; DOC: Dissolved organic carbon concentration of water; DOC/NO$_3$−: The ratio of dissolved organic carbon to NO$_3$− in water; s-NO$_3$-, NO$_3$− concentration of sediment; s-NH$_4$+, NH$_4$+ concentration of sediment; SOC: Sediment organic carbon concentration; SOC/NO$_3$−: The ratio of sediment organic carbon to NO$_3$− in sediment; SEM: The Structural Equation Modeling; PC1: The principal component 1; CFI: Comparative fit index; TLI: Tucker-Lewis index; RMSEA: Root square error of approximation.

Supplementary Information

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correlation analyses between N\textsubscript{2}O concentration, functional genes abundance, nitrification and denitrification rates \((n = 54)\). * represent \(P < 0.05\). Table S6. Total, direct, and indirect effects of explanatory variables on N\textsubscript{2}O concentration. Table S7. The primers and primer sequences of functional genes qPCR.

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Authors’ contributions

BJZ and QFZ designed the experiment. BJZ performed the experiment, processed the data, and performed the statistical analyses. The manuscript was drafted by BJZ and QFZ. The authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

Allen D, Dalal RC, Rennenberg H, Schmidt S (2011) Seasonal variation in nitrous oxide and methane emissions from subtropical estuary and coastal mangrove sediments, Australia. Plant Biol 13(1):126–133. https://doi.org/10.1111/j.1438-8677.2010.00331.x

Arango C, Tark J, (2008) Land use influences the spatiotemporal controls on nitrification and denitrification in headwater streams. J North Am Benthological Soc 27(1):90–107. https://doi.org/10.1899/07-024.1

Bauch HM, Dillon PJ, Maninger R, Venkiteswaran JJ, Wilson HF, Schiff SL (2012) Night and day: short-term variation in nitrogen chemistry and nitrous oxide emissions from streams. Freshw Biol 57(3):509–525. https://doi.org/10.1111/j.1365-2427.2011.02720.x

Bauza JF, Morell JM, Corredor JE (2002) Biogeochemistry of nitrous oxide. Appl Environ Microbiol 68(6):2006–2014. https://doi.org/10.1128/AEM.68.6.2006-2014.2000

Burgos M, Sierra A, Ortega T, Forja JM (2015) Anthropogenic effects on greenhouse gas (CH\textsubscript{4} and N\textsubscript{2}O) emissions in the Guadalete River Estuary (SW Spain). Sci Total Environ 503–504:179–189

Chapin FS, Matson PA, Vitousek PM (2011) Principles of terrestrial ecosystem ecology, 2nd edn. Springer, New York

Chen LM, Liu ST, Chen Q, Zhu GB, Wu X, Wang JW, Li XF, Hou LJ, Ni JR (2019) Anammox response to natural and anthropogenic impact over the Yangtze River. Sci Total Environ 665:171–180. https://doi.org/10.1016/j.scitotenv.2019.02.096

Chen NW, Wu JZ, Hong HS (2011) Preliminary results concerning summer-time denitrification in the Jiulong River Estuary. Environ Sci 32:3229–3234

Clough TJ, Bertram JE, Sherlock RR, Leonard RL, Nowicki BL (2006) Comparison of measured and EFs-rivered N\textsubscript{2}O fluxes from a spring-fed rive. Glob Chang Biol 12(3):477–488. https://doi.org/10.1111/j.1365-2486.2005.01092.x

Cocco E, Bertola C, Squarini A, Delle Vedove G, Berti A, Grignani C, Lazzaro B, Morari F (2018) How shallow water table conditions affect N\textsubscript{2}O emissions and associated microbial abundances under different nitrogen fertilisations. Agric Ecosyst Environ 261:1–11. https://doi.org/10.1016/j.agee.2018.03.018

Cole JJ, Caraco NF (1998) Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF\textsubscript{6}. Limnol Oceanogr 43(4):647–656. https://doi.org/10.4319/lo.1998.43.4.0647

Cole JJ, Caraco NF (2001) Emissions of nitrous oxide (N\textsubscript{2}O) from a tidal, freshwater river, the Hudson River, New York. Environ Sci Technol 35(6):991–996. https://doi.org/10.1021/es015848f

Crusius J, Wanninkhof R (2003) Gas transfer velocities measured at low wind speed over a lake. Limnol Oceanogr 48(3):1010–1017. https://doi.org/10.4349/lo.2003.48.3.1010

Deng FY, Hou LJ, Liu M, Zheng YL, Yin YG, Li XF, Lin XB, Chen F, Gao J, Jiang XF (2015) Dissimilatory nitrate reduction processes and associated contribution to nitrogen removal in sediments of the Yangtze Estuary. J Geophys Res Biogeosci 120(8):1521–1531. https://doi.org/10.1002/2015JG003007

Dong LF, Nedwell DB, Colbeck I, Finch J (2004) Nitrous oxide emission from some English and Welsh rivers and estuaries. Water Air Soil Pollut Focus 4:127–134. https://doi.org/10.1007/s11267-004-0022-4

Fan Y, Chen J, Shikey G, John R, Wu R, Park H, Shao C (2016) Applications of structural equation modeling (SEM) in ecological research: an updated review. Ecol Process 5:19. https://doi.org/10.1186/s13717-016-0063-3

Feng J, Xu X, Wu JJ, Zhang Q, Zhang DD, Li QX, Long CY, Chen Q, Chen JW, Cheng XL (2018) Inhibited enzyme activities in soil macroaggregates contribute to enhanced soil carbon sequestration under afforestation in central China. Sci Total Environ 640–641:653–661

Firestone MK, Davidson EA (1989) Microbial basis of NO and N\textsubscript{2}O production and consumption in soil. In: Andrew MO, Schimmel DS (eds) Exchange of trace gases between terrestrial ecosystems and the atmosphere. Wiley, New York, pp 7–21

Garcia-Ruiz R, Patterson SN, Whitton BA (1999) Nitrous oxide production in the river Swale-Duse, North-East England. Water Res 33(5):1231–1237. https://doi.org/10.1016/S0043-1354(98)00324-8

Gardner JR, Fisher TR, Jordan TE, Knee KL (2016) Balancing watershed nitrogen budgets: accounting for biogenic gases in streams. Biogeochemistry 127(2–3):231–253. https://doi.org/10.1007/s10533-015-0177-1

Garner J, Billen G, Vlaicu G, Martinez A, Silvestre M, Mournier E, Toche F (2009) Nitrous oxide (N\textsubscript{2}O) in the Seine river and basin: observations and budgets. Agric Ecosyst Environ 133(4):223–233. https://doi.org/10.1016/j.agee.2009.04.024

Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, Richardson PM, DeLong EF (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. PLoS Biol 4(4):e95. https://doi.org/10.1371/journal.pbio.0040095

Hansen AT, Dolph CL, Finlay JC (2016) Do wetlands enhance downstream denitrification in agricultural landscapes? Ecosphere 7(10):e01516. https://doi.org/10.1002/ecs2.1516

Harrison JA, Matson PA, Fendorf SE (2005) Effects of a diel oxygen cycle on nitrogen transformations and greenhouse gas emissions in a eutrophied subtropical stream. Aquat Sci 67(3):308–315

Braker G, Zhou JH, Wu LY, Devol AH, Tiedje JM (2000) Nitrite reductase genes \((nirK \text{ and } nirS)\) as functional markers to investigate diversity of denitrifying bacteria in Pacific Northwest marine sediment communities. Appl Environ Microbiol 66(5):2006–2014. https://doi.org/10.1128/AEM.66.5.2006-2014.2000

Bollmann A, Conrad R (1998) Influence of \(Q\) availability on NO and N\textsubscript{2}O release by nitrification and denitrification in soils. Glob Chang Biol 4(4):387–396. https://doi.org/10.1046/j.1365-2486.1998.00161.x
Schmelleleh-Engel K, Moosbrugger H, Müller H (2003) Evaluating the fit of structural equation models: tests of significance and descriptive goodness-of-fit measures. Method Psychol Res 8:23–74

Seitzinger SP, Kroeze C (1998) Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. Glob Biogeochem Cycles 12(1):93–113. https://doi.org/10.1029/97GC03657

Sigleo AC (2019) Denitrification rates across a temperate North Pacific Estuary, Yaquina Bay, Oregon. Estuar Coast 42(6):655–664

Smith CJ, Dong LF, Wilson J, Stott A, Osborn AM, Nedwell DB (2015) Seasonal variation in denitrification and dissimilatory nitrate reduction to ammonia process rates and corresponding key functional genes along an estuarine nitrate gradient. Front Microbiol 6:542

Soued C, del Giorgio PA, Manziger R (2015) Nitrous oxide sink and emissions in boreal aquatic networks in Québec. Nat Geosci 9:1–7

Sponseller RA, Benfield EF, Valett HM (2001) Relationships between land use, spatial scale and stream macroinvertebrate communities. Freshw Biol 46(10):1409–1424. https://doi.org/10.1046/j.1365-2427.2001.00758.x

Stryn OS, Valett HM, Schreiber ME (2005) Nitrification rates in a headwater stream: influences of seasonal variation in C and N supply. J North Am Benthol Soc 24(4):753–768. https://doi.org/10.1899/05-015.1

Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Midgley PM (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York

Vogt C, Marušchák ME, Lampecht RE, Jackowiak-Koczyszy M, Lindgren A, Mestapanov M, Granlund L, Christensen TR, Talhavainiene T, Markinkaia PJ, Biais C (2017) Increased nitrous oxide emissions from Arctic peatslands after permafrost thaw. Proc Natl Acad Sci 114(24):6238–6243. https://doi.org/10.1073/pnas.1702902114

Walter S, Peeken I, Lochte K, Webb A, Bange HW (2005) Nitrous oxide measurements during EFEX, the European iron fertilization experiment in the subpolar South Atlantic Ocean. Geophys Res Lett 32(23):L23613. https://doi.org/10.1029/2005GL024619

Wang J, Yuan J, Tan X, Li SY, Zhang QF (2020) Influence factors of potential nitrification rates and functional genes abundance in the Jinshui River and the Qihe River of the Han River basin. Environ Sci 41:5419–5427

Zhao J, Zhang GL, Wu Y, Zhang J (2009) Distribution and emission of nitrous oxide from the Changjiang River. Acta Sci Circumst 29:1995–2002

Zheng LZ, Cardenas MB, Wang LC (2016) Temperature effects on nitrogen cycling and nitrate removal-production efficiency in bed form-induced hyporheic zones. J Geophys Res Biogeosci 121(4):1086–1103. https://doi.org/10.1002/2015JG003162

Zheng YL, Hou LJ, Liu M, Yin GY (2019) Dynamics and environmental importance of anaerobic ammonium oxidation (anammox) bacteria in urban river networks. Environ Pollut 254(Pt A):112998. https://doi.org/10.1016/j.envpol.2019.112998

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