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# $N_2O$ 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 ($N_2O$) 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 $N_2O$ 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 $N_2O$ emissions, and the relative importance of physicochemical variables, nitrification and denitrification potentials, and functional genes abundance influencing $N_2O$ emissions.

**Results:** $N_2O$ concentration, $N_2O$ saturation, and $N_2O$ flux of Jinshui River peaked in high flow season. $N_2O$ concentration, $N_2O$ saturations, and $N_2O$ flux in Qi River and downstream of Jinshui River were significantly higher than that in other areas in normal and low flow seasons. $N_2O$ concentration was positively correlated with water temperature, water $NO_3^-$, and DOC, negatively correlated with water $NH_4^+$ and DOC/$NO_3^-$ (the ratio of dissolved organic carbon to $NO_3^-$ in water), and positively correlated with potential nitrification rate in high flow season, but not correlated with functional genes abundance. Both rivers had lower $N_2O$ saturation and flux than many freshwater systems, and their EFr-5 ($N_2O$ emission factor for river) was lower than the recommended values of IPCC.

**Conclusions:** While the two rivers were moderate sources of $N_2O$ and $N_2O$ emissions in river systems were normally elevated in the summer, areas with intense human disturbance had higher $N_2O$ concentration, $N_2O$ saturations, and $N_2O$ flux than those with slight human disturbance. Physicochemical variables were good indicators of $N_2O$ emissions in the river ecosystems.


**Keywords:** River, $N_2O$ concentration, $N_2O$ saturation, $N_2O$ flux, $N_2O$ emission factor

## Introduction

Rivers are one of the primary sources of nitrous oxide ($N_2O$) 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 $N_2O$ 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 $N_2O$ emissions in the river system on the atmospheric $N_2O$ balance is becoming much more important (Seitzinger and Kroeze 1998).

$N_2O$ 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 $N_2O$ in most natural systems (Firestone and Davidson 1989; Wang et al. 2009). The microorganisms driving nitrification process contain AOA-*amoA* and

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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). $N_2O$ 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 $N_2O$ emission and microorganisms (Cocco et al. 2018).

Aquatic $N_2O$ 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 $N_2O$ production (Weathers 1984; Herbert 1999; McMahon and Dennehy 1999; Naqvi et al. 2000; Cole and Caraco 2001). Other environmental factors, such as temperature, DO, $C/NO_3^-$, can affect the nitrogen cycle processes and subsequently $N_2O$ 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 $N_2O$ 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 $N_2O$ 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 $N_2O$ emission. IPCC proposed a method to estimate $N_2O$ 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 $N_2O$ 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 $N_2O$ concentration, $N_2O$ saturation, $N_2O$ flux, and $N_2O$ 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 $N_2O$ concentration, $N_2O$ saturation, $N_2O$ flux, and $N_2O$ emission factor and (2) assess relationships between physicochemical factors, functional genes abundance, nitrification rates, denitrification rates, and $N_2O$ 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 km$^2$. 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 km$^2$. 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).

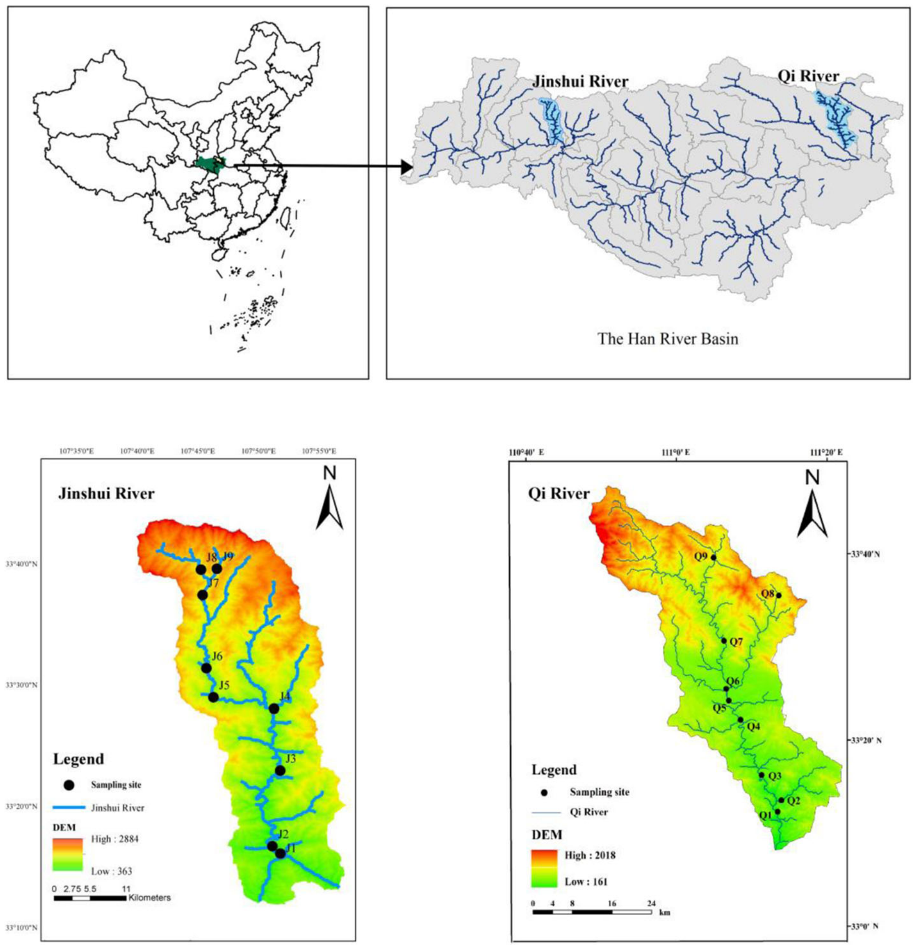


**Fig. 1** Sampling sites of the Jinshui River and Qi River. The source of DEM is the free data from https://earthexplorer.usgs.gov/. The map was made in ArcGIS 10.2

## 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 $N_2O$ concentration, and to prevent microbial activity, these samples were poisoned with 500 μL of a saturated aqueous mercury chloride ($HgCl_2$) solution.

The surface sediment samples (0–5 cm) were collected with sterilized shovel and stored the sediment in sterile TWIRL'EM® 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 $N_2O$ 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 $N_2O$ concentration, $N_2O$ saturation, $N_2O$ flux, and $N_2O$ emission factor

Static headspace gas chromatography was used to determine dissolved $N_2O$ 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. The 30 mL of the water sample was replaced with $N_2$ (purity > 99.999%). After the sample bottle was placed on the shaker for 4 h at room temperature to release the dissolved $N_2O$ from the water, 5 ml of gas was extracted from the top of the sample bottle with a syringe and injected into a vacuum tube. The headspace $N_2O$ sample was determined by gas chromatography (Agilent, 7890A, USA; column temperature: 60 °C; chromatographic column: hayesp Q 80-mesh packed column, 8FT; pre-column flow: 21 ml/min; separation column: constant pressure, 33.5 psi; detector ECD: 300 °C; tail blowing 5 ml/min). Finally, the dissolved $N_2O$ concentration in water was calculated according to the headspace $N_2O$ concentration (Johnson et al. 1990; Eq. 1).

$$C_{N_2O} = C_g \times \left(\frac{\beta R T_k}{22.4} + \frac{V_g}{V_1}\right) \quad (1)$$

where $C_{N2O}$ is the dissolved $N_2O$ concentration (nmol/L), $C_g$ is the headspace $N_2O$ concentration (nmol/L), β is the Bunsen coefficient (Liu et al. 2011b), 22.4 is the molar volume of $N_2O$, R is the gas constant 0.082, and $T_k$ is in K. $V_g$ is the volume of the gas phase, $V_l$ is the volume of the liquid phase.

The $N_2O$ saturation was the ratio of the dissolved $N_2O$ concentration to the equilibrium $N_2O$ concentration ($C_{eq}$) (Eq. 2). The equilibrium $N_2O$ concentration was calculated by Henry formula (Yang et al. 2013; Liu et al. 2011b; Eq. 3).

$$N_2O_{\text{saturation}=\frac{C_{N_2O}}{C_{eq}}\times 100\%} \quad (2)$$

$$C_{eq} = \beta \times C_A \quad (3)$$

where $C_{eq}$ is the equilibrium concentration of $N_2O$ in water at the given water temperature (nmol/L), and $C_A$ is the atmospheric $N_2O$ concentration of the sampling sites (nmol/L).

The flux of $N_2O$ was calculated as follows (Wanninkhof 1992, 2014; Cole and Caraco 2001; Crusius and Wanninkhof 2003; Eq. 4):

$$F = k \times \Delta N_2O \quad (4)$$

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

$$\Delta N_2O = C_{N_2O} - C_{eq} \quad (5)$$

$$k = \left(2.07 + 0.215 {U_{10}}^{1.7}\right) \times \left(\frac{S_C}{600}\right)^{-\frac{2}{3}} \text{for } U_{10} < 3.7 \text{m/s} \quad (6)$$

$$k = (4.33 U_{10} - 13.3) \times \left(\frac{S_C}{600}\right)^{-\frac{2}{3}} \text{for } U_{10} > 3.7 \text{m/s} \quad (7)$$

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). Sc denotes viscosity coefficient of $N_2O$ (Wanninkhof 2014; Eq. 9).

$$\frac{U_2}{U_{10}} = \frac{\lg 200}{\lg 1000} \quad (8)$$

$$S_C = 2141.2 - 152.56T + 5.8963T^2 - 0.12411T^3 + 0.0010655T^4 \quad (9)$$

where T denotes water temperature (°C).

$N_2O$ emission factor for river (EF5-r) is ratios of dissolved $N_2O$-N (μg N/L) to $NO_3^-$-N (μg N/L) (Eq. 10).

$$\text{EF5-r} = \frac{\lfloor N_2O \rfloor}{[NO_3]} \times 100\% \quad (10)$$

### Statistical analysis

Linear mixed modeling was performed to examine spatial variability and seasonality in dissolved $N_2O$ concentration in water, $N_2O$ saturation in water, $N_2O$ flux and $N_2O$ 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 $N_2O$ concentration, $N_2O$ saturation,

$N_2O$ flux, and $N_2O$ 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 $N_2O$ concentration using SPSS 20 (the independent variables were water and sediment physicochemical variables). Correlations between dissolved $N_2O$ 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 $N_2O$ 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 (Schermelleh-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$^a$ | 10.45 ± 2.21 |
| | | Upstream | 16.04 ± 0.53$^b$ | 1.15 ± 0.07 | 0.56 ± 0.03 | 10.61 ± 2.17$^a$ | 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$^b$ | 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$^b$ | 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$^a$ | 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$^c$ | 0.91 ± 0.04$^a$ | 0.47 ± 0.14 | 6.44 ± 0.07$^c$ | 17.52 ± 0.29$^c$ |
| | Qi River | Downstream | 9.87 ± 0.38 | 0.56 ± 0.26$^b$ | 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/$NO_3^-$

**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$^b$ | 1.30 ± 0.39 |
| | | Midstream | 5.97 ± 4.47$^b$ | 4.83 ± 0.98 | 3.14 ± 0.48$^c$ | 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$^a$ | 8.61 ± 1.05$^a$ | 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 ± 0.27$^a$ |
| | | 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/$NO_3^-$

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 low 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 $N_2O$ concentration

$N_2O$ 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 $N_2O$ concentration ($P$ > 0.05). $N_2O$ 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 $N_2O$ concentration between sampling sites in high and low flow seasons ($P$ > 0.05). For Qi River, there was no significant seasonal difference in $N_2O$ concentration ($P$ > 0.05). $N_2O$ concentration of normal flow season in midstream was higher, 1.23 times of that in downstream ($P$ < 0.05).

### $N_2O$ saturation

$N_2O$ 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, $N_2O$ saturation

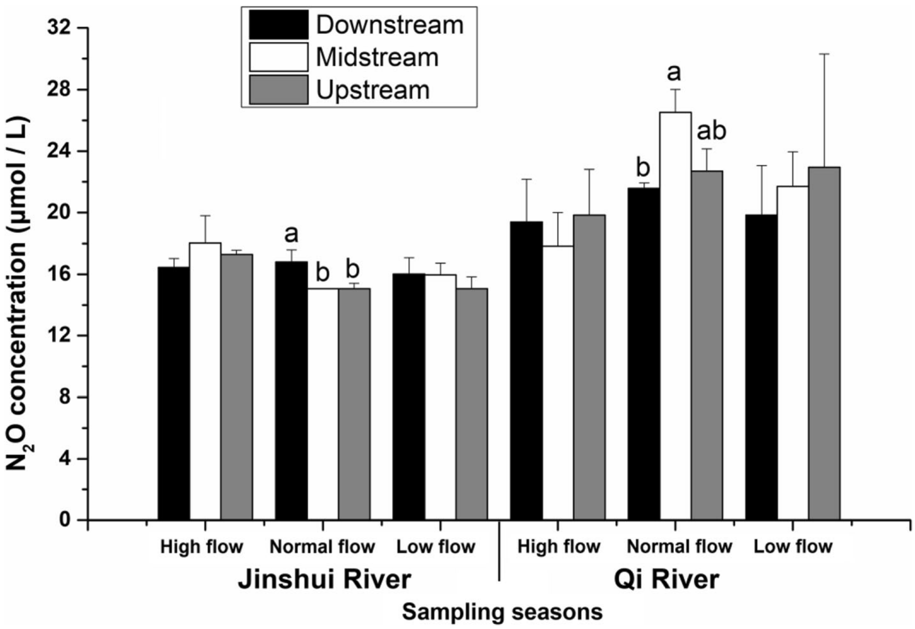


**Fig. 2** $N_2O$ concentration in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples. Different letters indicate significant differences among sampling areas by linear mixed modeling ($P$ < 0.05)

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 $N_2O$ saturation in downstream was the highest for all three seasons ($P$ < 0.05). For Qi River, there was no significant seasonal difference in $N_2O$ saturation in Qi River ($P$ > 0.05). $N_2O$ saturation of normal flow season in midstream was higher, 1.24 times of that in upstream ($P$ < 0.05).

### $N_2O$ flux

$N_2O$ flux of Qi River was 13.28 and 4.79 times of that in Jinshui River in normal and low flow seasons,

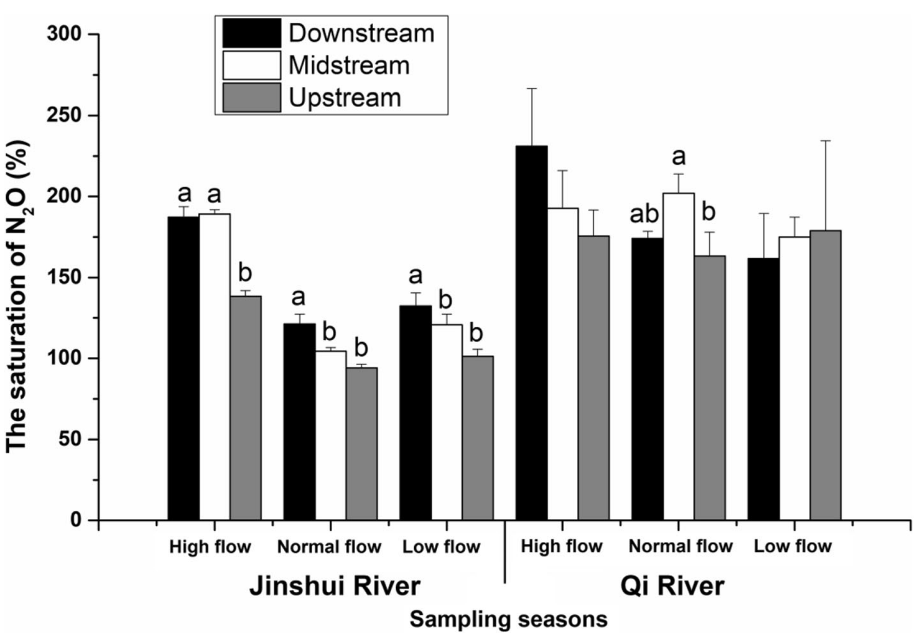


**Fig. 3** The saturation of $N_2O$ in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples. Different letters indicate significant differences among sampling areas by linear mixed modeling ($P$ < 0.05)

respectively (Fig. 4, $P < 0.01$ and $P < 0.05$). For Jinshui River, the $N_2O$ 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. $N_2O$ flux of downstream was higher than midstream and upstream in normal and low flow seasons ($P < 0.05$). $N_2O$ flux in few downstream sampling sites was less than zero. For Qi River, there was no significant seasonal difference in $N_2O$ flux ($P > 0.05$). The $N_2O$ flux in upstream was higher, 1.63 times of that in downstream in high flow season ($P < 0.05$).

### $N_2O$ emission factor

$N_2O$ 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 $N_2O$ emission factor between the two rivers ($P > 0.05$). For Jinshui River, $N_2O$ emission factor in low flow season was higher than that in high flow season ($P < 0.05$). $N_2O$ emission factor in upstream was lower than that in downstream and midstream in low flow season ($P < 0.05$). For Qi River, $N_2O$ 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 $N_2O$ 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 $N_2O$ concentration at annual level for both rivers (Table 4, $P < 0.05$). w-$NO_3^-$ explained a relatively large portion of the variances in $N_2O$ concentration in high flow season (Table 4, $P < 0.05$). Water temperature explained a relatively large portion of the variances in $N_2O$ 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 $N_2O$ concentration at annual level (Table 4, $P < 0.05$). For Qi River, w-$NH_4^+$ explained a relatively large portion of the variances in $N_2O$ 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 $N_2O$ concentration (Table S5, $P > 0.05$). There was significantly positive correlation between $N_2O$ 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 $N_2O$ concentration both directly and indirectly (Fig. 5, Table S6, $P < 0.05$).

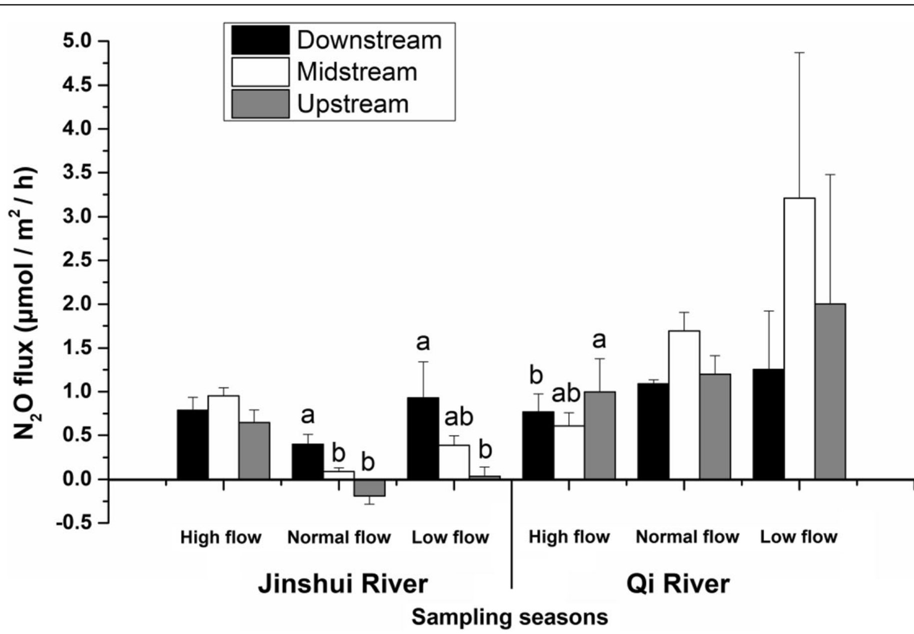


**Fig. 4** $N_2O$ flux in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples. Different letters indicate significant differences among sampling areas by linear mixed modeling ($P < 0.05$)

**Table 3** $N_2O$ emission factor (EF5-r, dissolved $N_2O$-N:$NO_3^-$-N) of Jinshui River and Qi River

| River | Sampling area | High flow season (%) | Normal flow season (%) | Low flow season (%) |
|---|---|---|---|---|
| Jinshui River | Downstream | 0.054 ± 0.013 | 0.062 ± 0.023 | 0.089 ± 0.007[a] |
| | Midstream | 0.049 ± 0.002 | 0.072 ± 0.008 | 0.076 ± 0.005[a] |
| | Upstream | 0.042 ± 0.001 | 0.047 ± 0.003 | 0.047 ± 0.004[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% |

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

## Discussion

### Seasonal and spatial variabilities of $N_2O$ emission

$N_2O$ emission ($N_2O$ concentration, $N_2O$ saturation, and $N_2O$ 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), $N_2O$ concentration, $N_2O$ saturation and flux of Jinshui River were peak in high flow season (summer) (Figs. 2 and 4). The higher $NO_3^-$ and organic carbon in high flow season were important factors and tended to enhance microbial processes including those producing $N_2O$, 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 $N_2O$ 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 $N_2O$ production rate in the present study (Wang et al. 2018). This finding confirms that $N_2O$ emissions in subtropical river systems are normally elevated in the summer (Musenze et al. 2014, 2015; Allen et al. 2011).

Significant spatial differences in $N_2O$ emission were also observed in present study, $N_2O$ concentration, $N_2O$ saturation, and $N_2O$ flux were higher in areas with intensive human disturbance (Figs. 2, 3, 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 $N_2O$ emission. The effects of human disturbance on river $N_2O$ 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 $N_2O$ production processes (Rosamond et al. 2012; Burgos et al. 2015). The increase of agricultural

**Table 4** Results of stepwise multiple regression analyses to predict $N_2O$ concentration

| | Sampling season | Independent variables | Coefficient | Adjusted $R^2$ | $P$ value |
|---|---|---|---|---|---|
| Two Rivers | Whole year | w-$NO_3^-$ | 0.28 | 0.08 | 0.04 |
| | | DOC | 0.73 | 0.21 | 0.00 |
| | | w-$NH_4^+$ | − 0.28 | 0.27 | 0.02 |
| | | DOC/$NO_3^-$ | − 0.46 | 0.33 | 0.03 |
| | High flow season | w-$NO_3^-$ | 0.59 | 0.301 | 0.01 |
| | Normal flow season | Temp | 0.65 | 0.39 | 0.00 |
| | Low flow season | - | - | - | - |
| Jinshui River | Whole year | DOC/$NO_3^-$ | − 0.53 | 0.25 | 0.01 |
| | High flow season | - | - | - | - |
| | Normal flow season | - | - | - | - |
| | Low flow season | - | - | - | - |
| Qi River | Whole year | w-$NH_4^+$ | − 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

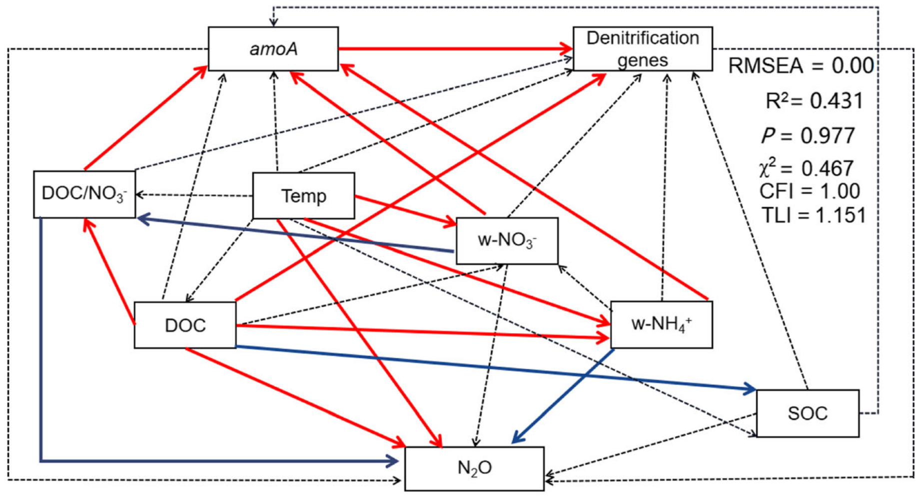


**Fig. 5** SEM estimating the direct and indirect effects of physicochemical variables and functional genes on $N_2O$ concentration. The *amoA* in this figure is the principal component 1 (PC1) extracted from two nitrification genes. The denitrification genes in this figure are the PC1 extracted from three denitrification genes. Direct effect: the direct influence of explanatory variables on $N_2O$ concentration, and the value is the path coefficient from cause variable to result variable. Indirect effect: the influence of explanatory variables on $N_2O$ concentration through one or more mediating variables. The value is the product of the path coefficients starting from the explanatory variables and ending at the $N_2O$ concentration through all intermediary variables. Red solid lines demonstrate significantly positive effects ($P< 0.05$), blue solid lines demonstrate significantly negative effects ($P< 0.05$), and black dashed lines indicate insignificant effects. Single-headed arrows refer to unidirectional causal relationships

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 $N_2O$ concentration.

#### Influencing factors of dissolved $N_2O$ concentration

$N_2O$ 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 $N_2O$ 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, larges 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 $N_2O$ 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 $N_2O$ 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 $N_2O$ production. In the present study, $N_2O$ 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 $N_2O$ emission, which suggests complexity of $N_2O$ production in rivers (Liu et al. 2011a). Also, heterotrophic microorganisms consume $NH_4^+$ with rapid propagation, providing an anaerobic environment for denitrification; therefore, $N_2O$ concentration from denitrification might increase as w-$NH_4^+$ decreased (Liu et al. 2015).

The correlation between functional genes abundance and $N_2O$ concentration was weak, but $N_2O$ 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.

2003; Arango and Tank 2008). Nitrification produces twice as much $N_2O$ per unit N converted as compared to denitrification (Mosier et al. 1998). Therefore, our results did not exclude the possibility of $N_2O$ production through denitrification but identifies nitrification as the major $N_2O$ source in sediments (Koike and Terauchi 1996; Bauza et al. 2002). Direct measurement of functional genes abundance could not be an indicator of their activities, and more research on substantial post-transcriptional, protein assembly, and/or environmental factors to determine what ultimately controls activity is much needed (Ikeda et al. 2009; Liu et al. 2010; Smith et al. 2015).

Overall, the influencing factors of $N_2O$ concentration varied in different seasons and rivers in the present study (Table 4; Harrison et al. 2005; Liu et al. 2015; Yang and Lei 2018). There were more influencing factors at the annual level than the monthly level, due to large variation of physicochemical variables at the annual level. Spatially, the dominant control factor was DOC/$NO_3^-$ for Jinshui River, and the dominant control factor for Qi River was $NH_4^+$ (Table 4), implying difference in controlling factors on river $N_2O$ concentration with different human activity intensities in the uplands.

### $N_2O$ emission factor, $N_2O$ saturations, and $N_2O$ flux

IPCC recommended value of $N_2O$ 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 $N_2O$ released estimated by IPCC release coefficient may be overestimated because dissolved $N_2O$ concentration in river includes part of $N_2O$ dissolved in water to reach equilibrium, which is not a source of atmospheric $N_2O$ (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 $N_2O$ flux by applying single model (Clough et al. 2006).

Interestingly, seasonal difference of $N_2O$ saturation and $N_2O$ flux was significant in Jinshui River (Figs. 3 and 4), but not in Qi River. Spatial variations of $N_2O$ saturation and $N_2O$ flux of Qi River were inconsistent (Figs. 3 and 4). This may be due to larger direct discharge of sewage in Qi River, and $N_2O$ in the water body far exceeds the amount of $N_2O$ formed in the process of nitrogen migration and transformation, which makes the seasonal difference of $N_2O$ emission smaller. Our study showed $N_2O$ 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). $N_2O$ 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 $N_2O$ (Yang et al. 2013). Overall, the two rivers had high $N_2O$ fluxes in most of their areas, and they were moderate sources of the atmospheric $N_2O$.

## Conclusions

We investigated $N_2O$ concentration, $N_2O$ saturation, $N_2O$ flux, and $N_2O$ emission factor of two subtropical rivers, China. Our results revealed that: (1) $N_2O$ concentration, $N_2O$ saturation, and $N_2O$ flux of Jinshui River peaked in high flow season, and areas with intensive human disturbance had higher $N_2O$ concentration, $N_2O$ saturation, and $N_2O$ flux in normal and low flow seasons. (2) Our present study rivers had lower $N_2O$ saturation and flux than many freshwater systems, and they were moderate sources of $N_2O$. (3) Physicochemical variables including temperature, $NO_3^-$, $NH_4^+$, DOC, SOC, DOC/$NO_3^-$ and SOC/$NO_3^-$ were good indicators of $N_2O$ emissions in the river ecosystems.

#### Abbreviations

$N_2O$: Nitrous oxide; EFr-5: $N_2O$ 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

The online version contains supplementary material available at https://doi.org/10.1186/s13717-021-00307-3.

**Additional file 1: Fig. S1.** *amoA* genes abundance in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples (Zhao et al. 2020). The genes primers were shown in Table S7. **Fig. S2.** *nirK* gene abundance in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples (The data have not yet been published). The measured method was the same as *amoA* genes. The gene primer was shown in Table S7. **Fig. S3.** *nirS* genes abundance in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples (The data have not yet been published). The measured method was the same as *amoA* genes. The gene primer was shown in Table S7. **Fig. S4.** *nosZ* genes abundance in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples (The data have not yet been published). The measured method was the same as *amoA* genes. The gene primer was shown in Table S7. **Fig. S5.** Potential nitrification rates in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples (Zhao et al. 2020). **Fig. S6.** Potential nitrification rates in the Jinshui River and Qi River. Vertical bar denote SE of triplicate samples (The data have not yet been published). **Table S1.** The land use attribute table of the Jinshui River and Qi River. **Table S2.** Wind speed (m/s) at 2 m above water surface of Jinshui River and Qi River. **Table S3.** Pearson's correlation analyses between $N_2O$ concentration and physicochemical variables. * represent $P < 0.05$, **represent $P < 0.01$. **Table S4.** Pearson's correlation analyses between nitrogen transformation genes abundance ($n$ = 54). **Table S5.** Pearson's correlation analyses between $N_2O$ concentration, functional genes abundance, nitrification and denitrfication rates ($n$ = 54). * represent $P <$ 0.05. **Table S6.** Total, direct, and indirect effects of explanatory variables on $N_2O$ 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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**Availability of data and materials**
Not applicable.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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