Dissimilatory Nitrate/Nitrite Reduction Processes in River Sediments Across Climatic Gradient: Influences of Biogeochemical Controls and Climatic Temperature Regime

Xiaofei Li1,2,3, Jordi Sardans4,5, Lijun Hou1, Dengzhou Gao2, Min Liu2, and Josep Peñuelas4,5

1Key Laboratory for Humid Subtropical Eco-geographical Processes of the Ministry of Education, Fujian Normal University, Fuzhou, China, 2Key Laboratory of Geographic Information Science of the Ministry of Education, School of Geographic Sciences, East China Normal University, Shanghai, China, 3State Key Laboratory of Estuarine and Costal Research, East China Normal University, Shanghai, China, 4CSIC, Global Ecology CREAF-CEAB-CSIC-UAB, Barcelona, Spain, 5CREAF, Barcelona, Spain

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Abstract Dissimilatory nitrate/nitrite reduction processes play an important role in controlling nitrogen loading in river environments. However, the relative importance of climatic temperature regime and biogeochemical controls to dissimilatory nitrate/nitrite reduction processes remains unclear. We used nitrogen isotope tracer approach to investigate geographical variabilities of denitrification, anaerobic ammonium oxidation (anammox), and dissimilatory nitrate reduction to ammonium (DNRA) in river sediments from temperate to tropical climates of China. Denitrification, anammox, and DNRA varied greatly across the climatic gradient, with potential rates of 1.47–25.7, 0.54–3.4, and 0.15–7.17 nmol N g⁻¹ h⁻¹, respectively. Mean measured rates throughout the sampling sites were 9.73 nmol N g⁻¹ h⁻¹ for denitrification, 1.29 nmol N g⁻¹ h⁻¹ for anammox, and 1.61 nmol N g⁻¹ h⁻¹ for DNRA. Denitrification and DNRA rates increased significantly from temperate to tropical climates, while no significantly spatial difference was observed for anammox rates along the climatic gradient. Mean annual temperature, total organic carbon, dissolved organic carbon, pH, NH₄⁺, NO₃⁻, C/N, Fe²⁺, and functional genes were the crucial factors affecting denitrification, anammox, and DNRA. High dissolved organic carbon and NO₃⁻ availability determined nitrogen removal capacity in river sediments. Mean annual temperature was the most important factor explaining the geographical variabilities of denitrification and DNRA, while the critical predictor of anammox variance was sediment pH along the climatic gradient. Our results highlight that biogeochemical controls and climatic temperature regime are important coregulators affecting the geographical variabilities of dissimilatory nitrate/nitrite reduction processes in river sediments at the continental-scale variation.

1. Introduction

Rivers are important environments for nitrogen transformation (Lansdown et al., 2016; Stelzer & Scott, 2018), which contributes greatly to global biogeochemical cycling (Gomez-Velez et al., 2015; Lansdown et al., 2016; Vilmin et al., 2008). Undoubtedly, nitrogen from soil leaching and agricultural and industrial discharge is ultimately transported into rivers, and thus leads to enhanced nitrogen loading (Stelzer & Scott, 2018; Strokal et al., 2016; Zhao et al., 2015). Recently, most of rivers around the world, particularly in the developing countries, have suffered from high nitrogen loading which poses a great threat to water quality and ecological functions (Gruber & Galloway, 2008; Strokal et al., 2016; Tomasek et al., 2017; Vilmin et al., 2008; Zhao et al., 2015). River nitrogen pollution is also expected to cause microbial community alterations (Dodds, 2006; Reisinger et al., 2016; Tatariw et al., 2013) and further influence nitrogen transformation processes (Gruber & Galloway, 2008; Kim et al., 2016). Denitrification, anaerobic ammonium oxidation (anammox), and dissimilatory nitrate reduction to ammonium (DNRA) are the important dissimilatory nitrate/nitrite reduction processes controlling nitrogen removal and retention (Stelzer et al., 2011; Stelzer et al., 2014; Reisinger et al., 2016; Xiong et al., 2017). Therefore, it is of ecological and environmental significance to elucidate the crucial factors controlling denitrification, anammox, and DNRA in river environments.
Denitrification is an important process of nitrogen removal through reducing nitrate/nitrite to N₂O and N₂ (Kreiling et al., 2011; Stelzer & Bartsch, 2012). In river systems, denitrification generally accounts for the majority of the nitrate loss (Kreiling et al., 2011). Although rivers are the hot spots of denitrification activity, the capability of denitrification varies largely across different river environments, mainly depending on nitrate and organic carbon availability (Kreiling et al., 2011; Reisinger et al., 2016; Stelzer et al., 2014; Tomasek et al., 2017). In organic carbon-enriched rivers, denitrification is mainly affected by nitrate/nitrite content (Reisinger et al., 2016; Tomasek et al., 2017; Xiong et al., 2017). Denitrification is also limited by organic carbon types including dissolved organic carbon and labile organic carbon regardless of total organic carbon abundance in rivers (Perryman et al., 2011; Reisinger et al., 2016; Stelzer et al., 2014; Tomasek et al., 2017). Temperature is also an important factor affecting denitrification rates in river sediments (Xiong et al., 2017) because high temperature can provide a higher respiration energy yield of organic matter consumption for denitrify bacteria (Reisinger et al., 2016; Strohm et al., 2007). Temperature is thus assumed to strongly mediate denitrification rates along a climate gradient. However, covariant environmental factors (e.g., nitrate/nitrite and dissolved organic carbon) affected by temperature can have complicate influences on denitrification in the rivers.

Anammox is an autotrophic process of nitrogen removal through using nitrite as the electron acceptor to oxidize ammonium (Thamdrup & Dalsgaard, 2002). River sediments, the anoxic and low reducing environment, are an important environment for anammox (Teixeira et al., 2016; Zhu et al., 2013, 2015). Recently, many studies have documented that anammox plays an important role in nitrate removal and is affected by some environmental factors (Lansdown et al., 2016; Zhang et al., 2017; Zhu et al., 2013). NO₂⁻ is observed to be a key factor affecting anammox rates in river sediments (Teixeira et al., 2016). Although NO₂⁻ is mobile and limited in rivers, anaerobic NO₂⁻ reduction or ammonium oxidation appears to be a potential source of NO₂⁻ to anammox (Greaver et al., 2016; Teixeira et al., 2016). It has been reported that pH may have a significant influence on anammox rates because anammox bacteria is sensitive to pH changes (Zhu et al., 2015). Generally, neutral and weak alkaline conditions are the most favorable for anammox activity (Tomaszewski et al., 2017). The pH has been shown to decrease greatly from temperate to tropical climates due to intensive water leaching and microbial respiration in warmer climates (Wang et al., 2016). Therefore, pH is a potential factor driving the variations of anammox rates across the different river environments.

Unlike denitrification and anammox, DNRA is a heterotrophic process converting nitrate/nitrite into more bioavailable ammonium, which retains nitrogen for recycling within aquatic environments (Kim et al., 2016; Lin et al., 2017; Nogaro & Burgin, 2014; Shelley et al., 2017). It has been reported that DNRA can proceed using organic carbon for fermentative pathway or sulfide and Fe²⁺ for chemomautrophic metabolisms (Giblin et al., 2013). Organic carbon quantity has been shown to influence DNRA rates (Kim et al., 2016) because high organic carbon loading can create a strongly reducing condition and thus favor DNRA activity (Hardison et al., 2015; Nizzoli et al., 2010). In addition, more formation of Fe²⁺ driven by the strongly reducing conditions can also fuel DNRA process (Robertson et al., 2016). It has been reported that DNRA rates are closely correlated with organic carbon availability in aquatic sediments (Hardison et al., 2015). The available portions of organic carbon including dissolved and labile organic carbon are affected by water content, and vary largely across different temperature conditions (Perryman et al., 2011; Tomasek et al., 2017). Dissolved and labile organic carbon may thus have important influence on DNRA in river environments. DNRA is a temperature preference pathway of nitrate/nitrite reduction and occurs strongly in tropical aquatic environments (Dong et al., 2011; Giblin et al., 2013). Therefore, DNRA rates are likely to exhibit a latitude gradient because climatic temperature increases from high to low latitudes.

Denitrification, anammox, and DNRA can occur synchronously in river environments, but their extent and contribution are highly variable due to their different preferences of substrates availability and environmental conditions (Kim et al., 2016; Teixeira et al., 2016; Tomasek et al., 2017; Xiong et al., 2017). Denitrification, anammox, and DNRA can compete for nitrate/nitrite under different substrates contents and environmental conditions (Hardison et al., 2015; Kessler et al., 2018), having a large influence on the fate of nitrogen in river environments. Therefore, many studies devote attention to reveal the balance between removal and retention of nitrogen, and explore the critical determinants of nitrogen removal efficiency in the high nitrogen-polluted rivers (Reisinger et al., 2016; Stelzer et al., 2014; Stelzer & Scott, 2018; Zhao et al., 2015). However, the environmental conditions and substrate availability in river environments vary largely...
across different climates, and it remains unclear which factors determine the variations of nitrogen removal and retention. This information can exactly evidence the resilience of nitrogen removal to excess nitrogen loading in river environments under human impacts and global change. More importantly, environmental variables in rivers are usually covariant and dependent on climatic regimes, which can have an interactive influence on nitrogen cycling. Therefore, we propose that both climatic temperature and sediment variables have crucial effects on denitrification, anammox, and DNRA in river environments along the climate gradient.

In this study, we used nitrogen isotope tracer approach to investigate spatial distributions of dissimilatory nitrate/nitrite reduction processes in river sediments along the climatic gradient from temperate to tropical climate zones in China. The main objectives of this study are (1) to measure potential rates of denitrification, anammox, and DNRA, and their relative contributions to total nitrate/nitrite reduction in river sediments from temperate to tropical climates; (2) to quantify the relative importance of climatic temperature regime and sediment properties to the variations of dissimilatory nitrate reduction processes; and (3) to estimate the environmental implications of nitrogen removal or conservation caused by dissimilatory nitrate/nitrite reduction processes. This work provides novel insights for understanding the influences of climatic temperature regime and biogeochemical controls on nitrogen cycling in river environments at a large geographical scale.

2. Material and Methods

2.1. Study Area and Sediment Collection

China is characterized by a high density of river networks, spanning from temperate to tropical climates (Strokal et al., 2016). There are more than 50,000 rivers, each with a catchment area of larger than 100 km². The total catchment area of Chinese rivers is equivalent to nearly 0.8% of China land area. However, most of Chinese rivers are heavily polluted by high nitrogen loading owing mainly to overuse of agricultural nitrogen fertilizer and discharge of household and industry wastewater (Strokal et al., 2016; Zhao et al., 2015). Nitrogen removal potential in the rivers affected by excess nitrogen loading has thus attracted an increasing concern (Zhao et al., 2015). In this study, 16 rivers were collected across the climatic gradient, including middle temperate climate zones (HLJ, JL, and LN), north subtropical climate zones (HB, AH, JS, and ZJ), middle subtropical climate zones (SC, YN, GZ, HN, JX, and FJ), and south subtropical and tropical climate zones (GX, GD, and HAN) in July 2017 (Figure 1). The air temperature of sampling sites was measured and varied slightly between 29.3 °C and 32.2 °C across these sites (Table S1). However, these sites experience a great variability of mean annual temperature, which varies from 4.7 °C to 23.9 °C (Table S1). During the field work, triplicate sediment cores (0–5 cm) were collected from each site, and 48 sediment cores were totally obtained along the climatic gradient. The sediment samples were stored at sterile plastic bags with ice packs and transferred to the laboratory within 12 hr. Upon return to the lab, each sediment core was homogenized thoroughly under helium and divided into two subsamples. One subsample was used for determination of dissimilatory nitrate/nitrite reduction rates and sediment pH, total organic carbon, total nitrogen, dissolved organic carbon, labile organic carbon, ammonium, nitrate, nitrite, ferrous iron, and ferric iron. The other subsample was stored at −80 °C for measurement of microbial abundances.

2.2. Measurements of Dissimilatory Nitrate/Nitrite Reduction Rates

Denitrification and anammox rates were measured through sediment-slurry experiments combined with nitrogen isotope-tracing technique (Deng et al., 2015; Hou et al., 2013). In brief, slurry was prepared with fresh sediment and helium-purged deionized water at a ratio of 1:7 (sediment/water). The slurry was stirred homogeneously by magnetic stirrer for 25 min and transferred into helium-purged 12-mL vials (Labco Exetainers, UK) in an anaerobic glove box filled with high-purity helium. Subsequently, these vials were in dark preincubated at near in situ temperature for 36–48 hr to eliminate residual oxygen, nitrate, and nitrite. During the preincubation, the contents of nitrate and nitrite in the slurries were measured. Preincubation was stopped for subsequent experiments until nitrate and nitrite were not detectable. After the preincubation, these vials were divided into three treatments: (1) 15NH4+ (100 μmol 15N/L), (2) 15NH4+ + 14NO3− (100 μmol 15N/L), and (3) 15NO3− (100 μmol 15N/L; Hou et al., 2013). Treatment 1 was used to further verify if nitrate and nitrite were completely eliminated by the preincubation, treatment 2 was used to assess the occurrence of anammox, and treatment 3 was used to measure the potential rates
of denitrification and anammox (Hou et al., 2013). The vials in these treatments were spiked with 0.2 mL ZnCl₂ (50%) to stop microbial activity after 0-, 2-, 4-, 6-, and 8-hr time series incubation, respectively. Produced ²⁹N₂ and ³⁰N₂ gases within the incubation were determined with a membrane inlet mass spectrometer (MIMS; An et al., 2001). The potential rates of both anammox and denitrification were calculated using the methods developed by Thamdrup and Dalsgaard (2002) and Trimmer et al. (2003). Specifically, the respective contributions of denitrification and anammox to total ²⁹N₂ production were quantified by equation (1):

\[ P_{29} = A_{29} + D_{29} \] (1)

where \( P_{29} \) (nmol N g⁻¹ hr⁻¹) represents the total ²⁹N₂ production rate, \( A_{29} \) (nmol N g⁻¹ hr⁻¹) represents ²⁹N₂ production from anammox in the incubation slurries, respectively. \( D_{29} \) was estimated by equation (2), assuming random isotope pairing of ¹⁵N and ¹⁴N from ¹⁴NO₃⁻ or ¹⁵NO₃⁻ (Risgaard-Petersen et al., 2003):

\[ D_{29} = P_{30} \times 2 \times (1 - F_N) \times F_N^{-1} \] (2)

where \( P_{30} \) (nmol N g⁻¹ hr⁻¹) represents the total ³⁰N₂ production rate and \( F_N \) (%) denotes the fraction of ¹⁵N in the ¹⁵NO₃⁻ treatment, which was obtained according to the injected ¹⁵NO₃⁻ and measured residual NO₃⁻ concentrations in the slurries. Potential rates of denitrification and anammox were finally estimated by equations (3) and (4):

\[ D_t = D_{29} + 2 \times D_{30} \] (3)

\[ A_{29} = P_{29} - D_{29} \] (4)

where \( D_t \) and \( A_{29} \) (nmol N g⁻¹ hr⁻¹) represent the rates of denitrification and anammox, respectively.

Potential rates of DNRA were measured using the OX/MIMS method (¹⁵NH₄⁺ oxidation combined with MIMS measurement; Yin et al., 2014). Briefly, sediment slurry was prepared and preincubated as the experiments for denitrification and anammox. After the preincubation, the vials were spiked with ¹⁵NO₃⁻ (100 μmol ¹⁵N/L in each vial) and stopped with 0.2 mL ZnCl₂ (50%) at the 0-, 2-, 4-, 6-, and 8-hr time series incubation, respectively. The ¹⁵NH₄⁺ produced from DNRA was oxidized with 0.2 mL hypobromite iodine...
solution, and was then determined with MIMS (An & Gardner, 2002; Yin et al., 2014). A standard curve was also constructed with known concentrations (0, 5, 10, and 20 μmol/L) of $^{15}$NH$_4^+$ (Yin et al., 2014). The concentrations of $^{15}$NH$_4^+$ produced from the sediment-slurry incubation were thus quantified based on the standard curve. Potential rates of DNRA were calculated from concentration changes of the process-specific $^{15}$N-labeled product ($^{15}$NH$_4^+$) during the time series incubations according to equation (5) (An & Gardner, 2002; Porubsky et al., 2008; Yin et al., 2014):

$$R_{DNRA} = S \times V \times W^{-1}$$

where $R_{DNRA}$ (nmol $^{15}$N g$^{-1}$ hr$^{-1}$) is the DNRA rate, $S$ (nmol $^{15}$N L$^{-1}$ hr$^{-1}$) is the slope of $^{15}$NH$_4^+$ contents versus incubation time, $V$ (L) is the vial volume, and $W$ (g) denotes the sediment dry weight converted from fresh weight.

### 2.3. Analyses of Sediment Geochemical Properties

Sediment pH was determined at a 1:2.5 ratio of sediment to water (w/v) using a Mettler-Toledo pH meter. Dissolved organic carbon (DOC) in sediment was extracted with deionized water and determined using a TOC-VCPH analyzer (Shimadzu, Japan). Labile oxidized organic carbon (LOC) in sediment was determined using a spectrophotometer colorimetry after oxidation with 333 mmol/L KMnO$_4$ (Vieira et al., 2007). Total organic carbon (TOC) and nitrogen (TN) in sediment were measured using an Elemental Analyzer (Elementar analyzer vario MaxCNOHS, Germany) after leached by 1 mol/L HCl (Hou et al., 2013). Sediment NH$_4^+$, NO$_3^-$, and NO$_2^-$ were determined using a continuous-flow nutrient autoanalyzer (SAN plus, Skalar Analytical B.V., Breda, Netherlands) after extraction with 2 mol/L KCl. Microbially oxidizable ferrous iron (Fe$^{2+}$) and reducible ferric iron (Fe$^{3+}$) in sediment were analyzed according to the method described by Lovley and Phillips (1987). All these sediment properties were measured in triplicate in this study.

### 2.4. DNA Extraction and qPCR Analysis

Total DNA in sediment was extracted using Power soil DNA Isolation Kits according to the accompanying instruction. Purity and concentrations of DNA were measured using a NanoDrop 2000 Spectrophotometer (Thermo, USA). Extracted DNA was then examined using 1.0% agarose gel electrophoresis and used for subsequent analysis. Nitrous oxide reductase encodes gene ($nosZ$) conducts the final step reduction of N$_2$O to N$_2$ in denitrification, which is widely used to reveal the denitrifying activity (Henry et al., 2006). Hydrazine oxidizing enzyme ($hzsB$) catalyzes the final step of anammox process, so the functional $hzsB$ gene is usually identified as a specific gene marker for quantifying and characterizing anammox activity (Bai et al., 2015). Because the pentaheme cytochrome C nitrite reductase ($nrfA$) is the major enzyme for conducting DNRA, $nrfA$ gene is frequently targeted as a functional gene to indicate DNRA activity (Smith et al., 2007). Thus, the copy numbers of $nosZ$, $hzsB$, and $nrfA$ genes were amplified with the primers $nosZ1F$ (5'–ATG TCG ATC ARC TGV KCR TTY TC-3') and $nosZ1R$ (5'–WCS YTG TTC MTC GAC AGC CAG-3'; Henry et al., 2006), $hzsB396F$ (5'–ARG GHT GGG GHA GYT GGA AG-3') and $hzsB742R$ (5'–GTA GCC ACR TCA TGV GTG TGT G-3'; Bai et al., 2015), and $nrfA2F$ (5'–CAC GAC AGC AAC ACT GCC G-3') and $nrfA2R$ (5'–CAG GCA CTT TCG AGC CC-3'; Smith et al., 2007), respectively. The qPCR analysis was performed using an ABI 7500 Sequence Detection System (Applied Biosystems, Canada) with the SYBR Green qPCR method. The thermal reaction cycling conditions for the $nosZ$, $hzsB$, and $nrfA$ genes can be found in Henry et al. (2006), Bai et al. (2015), and Smith et al. (2007), respectively. Standard curves were constructed from a series of tenfold dilutions of a known copy number of plasmid DNA comprising the functional $nosZ$, $hzsB$, and $nrfA$ genes. In all qPCR assays, three negative controls without DNA were conducted to confirm any possible contamination.

### 2.5. Statistical Analyses

Statistical analyses were performed using SPSS software for Windows 19.0 and Canoco software for Windows 4.5. Level of significance was chosen at $p < 0.05$. Comparison of dissimilatory nitrate/nitrite reduction rates among the climatic zones was analyzed using one-way analysis of variance (ANOVA) with Tukey's HSD test. Statistical significance was identified using a sequential Bonferroni adjustment to correct for multiple comparisons (Morrissey et al., 2014). Pearson’s correlation analyses were also used to evaluate the relationships among the functional gene abundance, dissimilatory nitrate/nitrite reduction rates, and sediment
Sediment Properties Across the Different Climatic Zones

| Location | pH    | TOC (%) | TN (%) | DOC (mg/g) | LOC (mg/g) | NH₄⁺ (μg/g) | NO₃⁻ (μg/g) | NO₂⁻ (μg/g) | Fe²⁺ (mg/g) | Fe³⁺ (mg/g) | C/N |
|----------|-------|---------|--------|------------|------------|-------------|-------------|-------------|------------|------------|-----|
| HLJ      | 7.7 ± 0.08 | 0.85 ± 0.06 | 0.12 ± 0.04 | 0.10 ± 0.01 | 0.16 ± 0.01 | 5.01 ± 0.99 | 2.01 ± 0.35 | 0.08 ± 0.01 | 2.17 ± 0.38 | 0.53 ± 0.20 | 7.2 ± 1.5 |
| JL       | 7.7 ± 0.02 | 1.68 ± 0.18 | 0.18 ± 0.02 | 0.15 ± 0.01 | 0.97 ± 0.21 | 3.67 ± 0.54 | 1.99 ± 0.04 | 0.07 ± 0.01 | 0.12 ± 0.11 | 1.81 ± 0.18 | 9.4 ± 0.5 |
| LN       | 8.2 ± 0.08 | 1.28 ± 0.20 | 0.22 ± 0.02 | 0.13 ± 0.005 | 0.33 ± 0.05 | 2.08 ± 0.07 | 1.83 ± 0.04 | 0.11 ± 0.001 | 0.27 ± 0.06 | 1.94 ± 0.21 | 5.8 ± 0.8 |
| JS       | 8.0 ± 0.06 | 1.51 ± 0.22 | 0.21 ± 0.03 | 0.19 ± 0.03 | 1.41 ± 0.40 | 6.81 ± 1.79 | 2.51 ± 0.07 | 0.09 ± 0.01 | 3.05 ± 0.21 | 0.93 ± 0.32 | 7.1 ± 0.4 |
| AH       | 7.8 ± 0.02 | 0.95 ± 0.07 | 0.08 ± 0.02 | 0.10 ± 0.01 | 0.11 ± 0.01 | 3.49 ± 1.10 | 2.09 ± 0.88 | 0.10 ± 0.03 | 1.50 ± 0.47 | 1.47 ± 0.46 | 12.3 ± 2.4 |
| ZJ       | 7.5 ± 0.05 | 1.81 ± 0.27 | 0.27 ± 0.03 | 0.15 ± 0.03 | 0.78 ± 0.15 | 4.54 ± 0.29 | 2.58 ± 0.05 | 0.14 ± 0.05 | 2.54 ± 0.23 | 1.04 ± 0.09 | 6.9 ± 1.6 |
| HB       | 6.5 ± 0.32 | 2.36 ± 0.44 | 0.25 ± 0.01 | 0.11 ± 0.01 | 1.23 ± 0.10 | 2.76 ± 0.22 | 1.70 ± 0.07 | 0.19 ± 0.03 | 0.45 ± 0.10 | 2.67 ± 0.54 | 9.3 ± 1.9 |
| SC       | 7.4 ± 0.20 | 1.45 ± 0.09 | 0.15 ± 0.02 | 0.17 ± 0.003 | 0.56 ± 0.09 | 2.57 ± 0.84 | 2.36 ± 0.06 | 0.15 ± 0.13 | 3.47 ± 0.44 | 0.78 ± 0.28 | 9.5 ± 0.9 |
| GZ       | 8.1 ± 0.02 | 1.42 ± 0.16 | 0.13 ± 0.03 | 0.13 ± 0.001 | 0.56 ± 0.12 | 2.99 ± 0.27 | 3.30 ± 0.18 | 0.09 ± 0.01 | 1.23 ± 0.19 | 0.40 ± 0.03 | 11.1 ± 1.1 |
| YN       | 7.9 ± 0.04 | 1.15 ± 0.10 | 0.14 ± 0.02 | 0.20 ± 0.02 | 0.32 ± 0.03 | 5.87 ± 0.12 | 2.55 ± 0.01 | 0.13 ± 0.01 | 1.14 ± 0.08 | 5.55 ± 1.35 | 8.2 ± 0.7 |
| LN       | 7.4 ± 0.03 | 1.25 ± 0.13 | 0.16 ± 0.03 | 0.28 ± 0.02 | 1.92 ± 0.08 | 11.6 ± 0.57 | 5.29 ± 0.24 | 0.24 ± 0.03 | 4.85 ± 0.13 | 0.65 ± 0.39 | 7.7 ± 0.9 |
| JX       | 6.9 ± 0.02 | 1.29 ± 0.14 | 0.14 ± 0.04 | 0.15 ± 0.03 | 0.86 ± 0.09 | 2.70 ± 0.88 | 3.84 ± 0.30 | 0.16 ± 0.02 | 1.10 ± 0.22 | 1.84 ± 0.09 | 9.7 ± 1.5 |
| FJ       | 6.4 ± 0.04 | 1.44 ± 0.26 | 0.20 ± 0.02 | 0.20 ± 0.02 | 1.91 ± 0.28 | 4.18 ± 1.60 | 2.93 ± 0.02 | 0.14 ± 0.01 | 0.86 ± 0.19 | 0.46 ± 0.15 | 7.3 ± 1.1 |
| GX       | 7.4 ± 0.08 | 2.31 ± 0.15 | 0.18 ± 0.02 | 0.21 ± 0.01 | 1.26 ± 0.15 | 12.6 ± 1.69 | 4.28 ± 0.08 | 0.17 ± 0.03 | 3.43 ± 0.59 | 0.34 ± 0.36 | 13.0 ± 1.7 |
| GD       | 7.8 ± 0.07 | 2.78 ± 0.14 | 0.18 ± 0.02 | 0.22 ± 0.02 | 0.23 ± 0.03 | 5.54 ± 0.12 | 3.03 ± 0.10 | 0.21 ± 0.02 | 3.03 ± 0.50 | 2.03 ± 0.08 | 15.8 ± 1.2 |
| HAN      | 7.1 ± 0.05 | 1.59 ± 0.15 | 0.18 ± 0.02 | 0.15 ± 0.01 | 0.87 ± 0.08 | 4.00 ± 0.65 | 2.35 ± 0.14 | 0.11 ± 0.03 | 3.40 ± 0.52 | 0.60 ± 0.24 | 9.0 ± 0.7 |

Values were the mean ± SD of triplicate.

3. Results

3.1. Sediment Characteristics

Sediment characteristics are provided in Table 1. Sediment pH showed a significantly spatial variation (one-way ANOVA, F = 79.3, df = 15, p < 0.001), ranging from 6.4 (FJ) to 8.2 (LN). TOC and TN varied from 0.85 to 2.78% and 0.08 to 0.27%, resulting in a C/N ratio of 5.8–15.8. DOC varied between 0.10 and 0.28 mg/g, and LOC was in the range of 0.11–1.92 mg/g. Sediment NH₄⁺ was the dominant form of inorganic nitrogen, which ranged from 2.70 to 12.6 μg/g and accounted for 39.8–73.8% of total inorganic nitrogen. Sediment NO₃⁻ and NO₂⁻ varied from 1.20 to 2.64 and 0.07 to 0.24 μg/g, respectively. Sediment NO₃⁻ and NO₂⁻ occupied for 25.2–57.8% and 1.0–4.1% of total inorganic nitrogen, respectively. Sediment Fe²⁺ and Fe³⁺ contents were in the range of 0.12–4.85 and 0.34–5.55 mg/g, respectively. TOC, DOC, NO₃⁻, NO₂⁻, Fe²⁺, and C/N varied significantly across the climate gradient (one-way ANOVA, adjusted p < 0.011), which all showed an increase from temperate to tropical climates. DOC, LOC, and Fe²⁺ were significantly related to dissolved inorganic nitrogen (two-tailed, p < 0.04; Table S2). Mean annual temperature showed a negative influence on pH, and positive effects on TOC, DOC, NO₃⁻, NO₂⁻, and Fe²⁺ (two-tailed, p < 0.01; Table S2).
3.2. Dissimilatory Nitrate/Nitrite Reduction Rates

There was no significant $^{29}\text{N}_2$ and $^{30}\text{N}_2$ production in the incubations spiked with only $^{15}\text{NH}_4^+$, suggesting that residual nitrate and nitrite was almost eliminated during the preincubation (Figure 2). For the treatment of $^{15}\text{NH}_4^+ + ^{14}\text{NO}_3^-$, only $^{29}\text{N}_2$ accumulation was detected, indicating that anammox occurred in the incubations (Figure 2). Significant accumulation of both $^{29}\text{N}_2$ and $^{30}\text{N}_2$ was observed within the incubations spiked with $^{15}\text{NO}_3^-$ (Figure 2), indicating the co-occurrences of denitrification and anammox in the study area. Potential rates of denitrification and anammox were thus estimated from the treatment spiked with only $^{15}\text{NO}_3^-$. In this study, the estimated rates of denitrification and anammox ranged from 1.47 to 25.7 and from 0.35 to 3.40 nmol N g$^{-1}$ hr$^{-1}$, respectively, while potential rates of DNRA varied between 0.15 and 7.17 nmol N g$^{-1}$ hr$^{-1}$ across the sediment samples (Figure 3). Figure 4 shows that denitrification and DNRA rates differed significantly along the climate gradient (one-way ANOVA, $F = 34.17$, df = 3, $p = 0.000$ for denitrification; $F = 64.92$, df = 3, $p = 0.000$ for DNRA), while no significant difference in anammox rates was observed along the climate gradient (one-way ANOVA, $F = 1.431$, df = 3, $p = 0.247$). Denitrification rates increased greatly from middle temperate to tropical climate zones (Figure 4a). Meanwhile, DNRA rates were also significantly higher in south subtropical and tropical climate zones than in middle temperate climate zones (Figure 4c). In addition, denitrification rates showed a significant correlation with DNRA rates throughout the sampling sites ($r^2 = 0.76$, $p < 0.01$), implying a close interaction between denitrification and DNRA (Figure S1).

Based on the dissimilatory nitrate/nitrite reduction rates, 60.3−89.9% of total nitrate reduction was caused by denitrification (Figure 3), while the remaining was attributed to anammox (2.1−33.4%) and DNRA (3.3−21.9%).

3.3. Abundances of nosZ, hzsB, and nrfA Genes

Copy numbers of nosZ, hzsB, and nrfA genes are shown in Figure 5. Abundance of nosZ gene varied between $8.89 \times 10^4$ and $9.12 \times 10^6$ gene copies/g. The highest abundance of nosZ gene was observed at site HN, which was 100-fold of the lowest value measured at site LN. nosZ gene abundance exhibited an increase from high to low latitudes (Figure 5a). hzsB gene abundance in the sediments was in the range of $0.27$−$6.3 \times 10^4$ gene copies/g, with no significantly spatial variation along the latitude gradient (Figure 5b). nrfA gene abundance ranged from $2.39 \times 10^5$ to $3.07 \times 10^6$ copies/g. Relatively high abundance of nrfA gene was recorded at the sites AH, GX, GD, and HAN (Figure 5c). Similar to nosZ gene, nrfA gene...
abundance across all the sampling sites (except site AH) followed a latitude gradient change, generally increasing from middle to tropical climate zones (Figure 5c).

3.4. Effects of Climatic and Sediment Variables on Dissimilatory Nitrate/Nitrite Reduction Rates

Mean annual temperature showed a significant influence on denitrification and DNRA, while sediment pH strongly affected anammox via partial correlation analyses controlling for sediment TOC, DOC, NO$_3^-$, and NO$_2^-$ (Table 2). Only DOC, NH$_4^+$, NO$_3^-$, and Fe$^{2+}$ remained strongly correlated with denitrification rates from the partial correlation analysis controlling for mean annual temperature (Table 3). The pH, NH$_4^+$, and Fe$^{2+}$ showed negative correlation with anammox rates ($r_l > 0.37$, $p < 0.01$). DNRA rates were significantly and positively correlated with TOC, DOC, NH$_4^+$, NO$_3^-$, Fe$^{2+}$, C/N, and nrfA gene abundance ($r > 0.44$, $p < 0.04$). However, only NH$_4^+$, Fe$^{2+}$, C/N, and nrfA remained positively covaried with DNRA rates, following a partial correlation analysis to account for mean annual temperature ($r > 0.34$, $p < 0.03$).

The generalized linear model analyses controlling spatial autocorrelation were conducted to examine the effects of sediment variables on dissimilatory nitrate/nitrite reduction processes (Table 4). The results further confirmed that both denitrification and DNRA rates were significantly and positively affected by TOC, DOC, NH$_4^+$, NO$_3^-$, Fe$^{2+}$, C/N, nosZ, and nrfA gene abundances, whereas pH, NH$_4^+$, and Fe$^{2+}$ had significant and negative influence on anammox rates. Mean annual temperature had positive influence on the three processes, but showed a stronger influence on both denitrification and DNRA than on anammox.

3.5. Redundancy Analysis and Principal Component Analysis

Mean annual temperature and sediment variables explained 84%, 72.3%, and 79.8% of denitrification, anammox, and DNRA variances, respectively, throughout the rivers (Figure 6). Mean annual temperature ($F = 53.72$, $p = 0.002$, 499 permutations) and Fe$^{2+}$ ($F = 34.17$, $p = 0.002$, 499 permutations) were crucial factors affecting denitrification, accounting for 54% and 20% of total denitrification variation, respectively. The pH ($F = 16.08$, $p = 0.002$, 499 permutations), TN ($F = 10.63$, $p = 0.008$, 499 permutations), and NH$_4^+$ ($F = 11.56$, $p = 0.004$, 499 permutations) were significant factors affecting anammox, explaining 26%, 14%, and 13% of total variation, respectively. DNRA rates were strongly influenced by mean annual temperature ($F = 30.88$, $p = 0.002$, 499 permutations), NH$_4^+$ ($F = 18.67$, $p = 0.002$, 499 permutations), LOC ($F = 14.09$, $p = 0.002$, 499 permutations), explaining 40%, 18%, and 10% of DNRA variance throughout the rivers. Overall, climatic temperature explained mostly 54% and 40% of total denitrification and DNRA variances, respectively; however, it explained only 0.4% of anammox variation which was explained mostly 71.9% by sediment parameters.

Principal component analysis suggested that denitrification showed a close correlation with DNRA, and anammox was independent of denitrification and DNRA (Figure 7). In addition, we observed that Fe$^{2+}$...
was positively linked with denitrification and DNRA but not with anammox. Anammox was related to NO$_2^-$, while denitrification and DNRA were positively correlated with NO$_3^-$ and NH$_4^+$. The denitrification, anammox, and DNRA were consistently and positively related with the concentrations of other detected variables in the correlation analyses (Table 4).

4. Discussion

4.1. Features of Dissimilatory Nitrate/Nitrite Reduction at a Continental-Scale Variation

Terrestrial rivers are assumed to be important environments of dissimilatory nitrate/nitrite reduction processes controlling nitrogen loading (Lansdown et al., 2016; Stelzer & Scott, 2018). Although dissimilatory nitrate/nitrite reduction processes in river environments have attracted considerable attention at both regional and local scales (Reisinger et al., 2016; Stelzer et al., 2014; Stelzer & Bartsch, 2012; Strokal et al., 2016; Tomasek et al., 2017; Xiong et al., 2017; Zhao et al., 2015), their geographical patterns at a continental scale remain unclear. In addition, limited information on the effects of climate variations on dissimilatory nitrate/nitrite reduction processes in rivers is currently available. In this study, we measured the denitrification, anammox, and DNRA rates in river sediments from temperate to tropical climate zones to reveal the spatial patterns along the climate gradient. The spatial variation in anammox rates appeared to be largely independent of denitrification and DNRA throughout the rivers, implying that anammox had different biogeochemical controls from denitrification and DNRA. In addition, the multivariate analyses showed that denitrification and DNRA processes were driven by the same sediment parameters in the same direction while anammox had different optimal conditions, strongly suggesting that the denitrification and DNRA should have a dependent correlation, whereas anammox can easily occur less related with the denitrification and DNRA.

At the regional or local scales, dissimilatory nitrate/nitrite reduction processes show a significant seasonal change, supporting the importance of temperature to these nitrogen reduction processes (Kreiling et al., 2011; Tomasek et al., 2017; Xiong et al., 2017; Zhao et al., 2015). Dissimilatory nitrate/nitrite reduction processes were strongly influenced by climatic and sediment variables in river environments because nitrogen cycling processes are mediated by their microbial niche preferences (Kim et al., 2016). However, denitrification, anammox, and DNRA exhibit different sensibilities in response to temperature changes. Previous studies reported that there was no significant seasonal difference in anammox rates, although the high anammox rates were observed in winter (Lin et al., 2017; Lisa et al., 2014). Anammox was not detected even in tropical

Table 2
Partial Correlation Analyses of Mean Annual Temperature With Denitrification, Anammox, and DNRA Via Controlling for Sediment pH, TOC, DOC, NO$_3^-$, and NO$_2^-$ (n = 48)

|                | Partial correlation analysis controlling for |
|----------------|--------------------------------------------|
|                | pH         | TOC         | DOC         | NO$_3^-$     | NO$_2^-$     |
|                | r   | p    | r   | p    | r   | p    | r   | p    | r   | p    |
| Denitrification | 0.72 | <0.001 | 0.66 | <0.001 | 0.65 | <0.001 | 0.63 | <0.001 | 0.66 | <0.001 |
| Anammox         | 0.09 | 0.54  | 0.31 | 0.035 | 0.44 | 0.002  | 0.33 | 0.024 | 0.31 | 0.036 |
| DNRA            | 0.65 | <0.001 | 0.53 | <0.001 | 0.57 | <0.001 | 0.54 | <0.001 | 0.59 | <0.001 |

Figure 5. Abundance of nosZ, hzsB, and nrfA genes in the sediments. The sampling sites listed increase high to low latitudes. Bars above columns indicate the standard deviation of the triplicate values.
Table 3
Direct and Partial (Controlling for Mean Annual Temperature) Correlation Analyses of Dissimilatory Nitrate/Nitrite Reduction (Denitrification, Anammox, and DNRA) Rates With Sediment Geochemical Parameters and Gene Abundance (n = 48)

|            | Denitrification | Anammox | DNRA          |
|------------|-----------------|---------|---------------|
|            | Direct correlation | Partial correlation | Direct correlation | Partial correlation | Direct correlation | Partial correlation |
| pH         | 0.19             | 0.20   | 0.18          | 0.23          | -0.55               | <0.001             | -0.49               | <0.001             | 0.07               | 0.65               | 0.26               | 0.076              |
| TOC        | 0.41             | 0.004  | 0.14          | 0.34          | 0.05               | 0.74               | -0.10               | 0.52               | 0.44               | 0.002              | 0.23               | 0.11               |
| TN         | -0.02            | 0.901  | -0.06         | 0.68          | -0.23              | 0.112              | -0.25               | 0.08               | -0.07              | 0.639              | -0.12              | 0.44               |
| DOC        | 0.54             | <0.001 | 0.39          | 0.007         | -0.23              | 0.11               | -0.41               | 0.005              | 0.31               | 0.031              | 0.08               | 0.59               |
| LOC        | 0.29             | 0.048  | 0.15          | 0.32          | -0.05              | 0.74               | -0.14               | 0.36               | 0.04               | 0.81               | -0.16              | 0.27               |
| NH₄⁺       | 0.68             | <0.001 | 0.73          | <0.001        | -0.39              | 0.006              | -0.51               | <0.001             | 0.57               | <0.001             | 0.54               | <0.001             |
| NO₃⁻       | 0.62             | <0.001 | 0.48          | 0.001         | 0.01               | 0.94               | -0.15               | 0.33               | 0.38               | 0.008              | 0.14               | 0.35               |
| NO₂⁻       | 0.40             | 0.005  | 0.10          | 0.49          | 0.06               | 0.70               | -0.10               | 0.51               | 0.25               | 0.093              | -0.06              | 0.67               |
| Fe²⁺       | 0.69             | <0.001 | 0.59          | <0.001        | -0.37              | 0.01               | -0.58               | <0.001             | 0.52               | <0.001             | 0.34               | 0.02               |
| Fe³⁺       | -0.26            | 0.08   | -0.26         | 0.08          | 0.03               | 0.86               | 0.06                | 0.68               | -0.26              | 0.08               | -0.24              | 0.10               |
| C/N        | 0.41             | 0.004  | 0.13          | 0.40          | 0.28               | 0.052              | 0.17                | 0.25               | 0.52               | <0.001             | 0.34               | 0.02               |
| nosZ       | 0.75             | <0.001 | 0.37          | 0.01          | 0.19               | 0.19               | -0.11               | 0.47               | 0.50               | <0.001             | -0.03              | 0.82               |
| hzsB       | 0.05             | 0.724  | 0.01          | 0.70          | 0.68               | <0.001             | 0.70                | <0.001             | 0.02               | 0.92               | -0.03              | 0.86               |
| nrfA       | 0.55             | 0.00   | 0.23          | 0.12          | 0.01               | 0.84               | -0.21               | 0.17               | 0.72               | <0.001             | 0.56               | <0.001             |

Thus, anammox rates in this study showed no latitude gradient change at the continental scale. Denitrification is highly favored by the increasing temperature because denitrifiers are found to have a great affinity for NO₃⁻/NO₂⁻ even from low- to high-temperature changes (Ogilvie et al., 1997). In addition, the heterotrophic respiration of organic carbon is stimulated by the increasing temperature, thereby affecting denitrification rates (Greaver et al., 2016). Xiong et al. (2017) reported that denitrification in Han River exhibited a seasonal order of January < November < April < August, which was consistent with the increasing temperature tendency among these months. DNRA generally occurs strongly in high-temperature condition, mostly due to the thermodynamic favorability of DNRA (Dong et al., 2011; Reisinger et al., 2016; Wang et al., 2018). High organic carbon provides more respiration energy yield, and thus stimulates denitrification activity (Ström et al., 2007). In addition, intense bacterial respiration under the high organic carbon condition can lead to oxygen depletion and form a more reducing condition, which further favors the denitrification (Stelzer & Scott, 2018). In addition, a strong relationship between denitrification and NO₃⁻ was observed throughout the rivers (Table 3), because NO₃⁻ was the terminal electron receptor of the denitrification process (Xiong et al., 2017). However, previous studies reported that NO₃⁻ had a weak influence on denitrification in other rivers (Tomasek et al., 2017; Xiong et al., 2017). An explanation for this weak relationship is the largely seasonal fluctuations of nitrate concentrations in these rivers (Tomasek et al., 2017; Xiong et al., 2017). Another reason is that when the covariant relationship between NO₃⁻ and sediment water content was considered, denitrification was significantly related to sediment water content (Xiong et al., 2017), which likely masked the effect of NO₃⁻ on denitrification. Our partial correlation analysis controlling sediment organic carbon and nitrate/nitrite indicated a significant relationship between mean annual temperature and denitrification (Table 2). Temperature sensitivity (Q₁₀) of microbial activity increases with the increasing temperature (Nottingham et al., 2019), which is favorable for the estuarine sediments (Dong et al., 2011), further suggesting a limited effect of temperature on anammox.
Denitrification  

| Dependent variable | Independent variable | Statistics |  
|--------------------|----------------------|------------|  
| MAT                | $R^2 = 0.52$         | $t = 7.11$   | $p < 0.0001$  
| pH                 | $R^2 = 0.035$        | $t = -1.29$  | $p = 0.20$    
| TOC                | $R^2 = 0.17$         | $t = 3.03$   | $p = 0.0040$  
| TN                 | $R^2 = 0.0003$       | $t = -0.125$ | $p = 0.90$    
| DOC                | $R^2 = 0.29$         | $t = 4.31$   | $p < 0.0001$  
| LOC                | $R^2 = 0.083$        | $t = 2.04$   | $p = 0.04$    
| NH$_4^+$           | $R^2 = 0.46$         | $t = 6.23$   | $p < 0.0001$  
| NO$_3^-$           | $R^2 = 0.39$         | $t = 5.40$   | $p < 0.0001$  
| NO$_2^-$           | $R^2 = 0.16$         | $t = 2.98$   | $p = 0.0046$  
| Fe$^{3+}$          | $R^2 = 0.47$         | $t = 6.38$   | $p < 0.0001$  
| Fe$^{2+}$          | $R^2 = 0.066$        | $t = -1.81$  | $p = 0.077$   
| C/N                | $R^2 = 0.17$         | $t = 3.02$   | $p = 0.0041$  
| nrfA               | $R^2 = 0.33$         | $t = 1.53$   | $p < 0.0001$  
| hzaB               | $R^2 = 0.0051$       | $t = 0.48$   | $p = 0.63$    
| nosZ               | $R^2 = 0.73$         | $t = 7.17$   | $p < 0.0001$  

Anammox  

| Dependent variable | Independent variable | Statistics |  
|--------------------|----------------------|------------|  
| MAT                | $R^2 = 0.088$        | $t = 1.17$   | $p = 0.040$  
| pH                 | $R^2 = 0.30$         | $t = -3.78$  | $p < 0.0001$  
| TOC                | $R^2 = 0.0024$       | $t = 0.16$   | $p = 0.74$    
| TN                 | $R^2 = 0.054$        | $t = -2.24$  | $p = 0.11$    
| DOC                | $R^2 = 0.051$        | $t = -2.37$  | $p = 0.12$    
| LOC                | $R^2 = 0.0024$       | $t = -0.53$  | $p = 0.74$    
| NH$_4^+$           | $R^2 = 0.15$         | $t = -1.38$  | $p = 0.0058$  
| NO$_3^-$           | $R^2 = 0.00011$      | $t = 0.072$  | $p = 0.94$    
| NO$_2^-$           | $R^2 = 0.0032$       | $t = 0.48$   | $p = 0.70$    
| Fe$^{2+}$          | $R^2 = 0.14$         | $t = -0.86$  | $p = 0.10$    
| Fe$^{3+}$          | $R^2 = 0.00071$      | $t = 0.088$  | $p = 0.86$    
| C/N                | $R^2 = 0.079$        | $t = 2.63$   | $p = 0.053$   
| hzaB               | $R^2 = 0.57$         | $t = 7.88$   | $p < 0.0001$  
| nosZ               | $R^2 = 0.00086$      | $t = -0.24$  | $p = 0.84$    
| nrfA               | $R^2 = 0.0035$       | $t = -0.31$  | $p = 0.69$    

DNRA  

| Dependent variable | Independent variable | Statistics |  
|--------------------|----------------------|------------|  
| MAT                | $R^2 = 0.38$         | $t = 2.27$   | $p < 0.0001$  
| pH                 | $R^2 = 0.0047$       | $t = -0.21$  | $p = 0.64$    
| TOC                | $R^2 = 0.19$         | $t = -0.82$  | $p = 0.0018$  
| TN                 | $R^2 = 0.0048$       | $t = 0.58$   | $p = 0.64$    
| DOC                | $R^2 = 0.094$        | $t = 1.60$   | $p = 0.334$   
| LOC                | $R^2 = 0.0014$       | $t = 1.09$   | $p = 0.80$    
| NH$_4^+$           | $R^2 = 0.33$         | $t = 0.95$   | $p < 0.0001$  
| NO$_3^-$           | $R^2 = 0.14$         | $t = 0.49$   | $p = 0.0081$  
| NO$_2^-$           | $R^2 = 0.061$        | $t = -0.77$  | $p = 0.092$   
| Fe$^{2+}$          | $R^2 = 0.27$         | $t = -0.46$  | $p = 0.00017$ 
| Fe$^{3+}$          | $R^2 = 0.065$        | $t = 0.0067$ | $p = 0.080$   
| C/N                | $R^2 = 0.27$         | $t = -2.04$  | $p = 0.00015$ 
| nosZ               | $R^2 = 0.49$         | $t = 4.61$   | $p < 0.0001$  
| hzaB               | $R^2 = 0.027$        | $t = 0.48$   | $p = 0.26$    
| nrfA               | $R^2 = 0.64$         | $t = 2.34$   | $p < 0.0001$  

Final models were achieved using stepwise model selection, following AIC. MAT, mean annual temperature.

Temperature preference of heterotrophic denitrification (Reisinger et al., 2016). Interestingly, DOC and NO$_3^-$ increased with the increasing mean annual temperature from the high to low latitude (Table 1). High temperature can increase the organic carbon decomposition to produce more dissolved organic carbon for heterotrophic bacterial respiration (Wang et al., 2016). In addition, high temperature generally increases the soil NO$_3^-$ leaching, which is an important source for the rising concentration of NO$_3^-$ in subtropical and tropical rivers (Greever et al., 2016; Reisinger et al., 2016). The increasing concentrations in DOC and NO$_3^-$ likely further enhanced the cumulative effect of temperature and led to a favorable latitude toward denitrification in our study. Therefore, the high spatial variation of climatic and sediment parameters

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indicated an interactive effects of temperature, DOC, and NO$_3^-$ on the magnitude and latitude gradient of denitrification throughout the rivers. Fe$^{2+}$ was found to have an important influence on denitrification, which may suggest that chemodenitrification process can proceed through abiotic reactions coupled to redox cycling of iron (Buchwald et al., 2016). Specifically, Fe$^{2+}$, especially mineral or surface-bound Fe$^{2+}$, is an effective catalyst of NO$_2^-$ reduction under the anaerobic and reducing conditions (Picardal, 2012). Although direct report on the effect of Fe$^{2+}$ on denitrification is limited, many studies have revealed that the presence of mineral surfaces and elevated levels of Fe$^{2+}$ can enhance the reduction of NO$_3^-$ to NO$_2^-$ and increase N$_2$O yield (Buchwald et al., 2016; Jones et al., 2015). Low redox extent in the environments driven by the strong respiration and oxygen depletion can favor the form of Fe$^{2+}$ (Buchwald et al., 2016), which further fuels the denitrification. The nosZ gene plays a key role in the reduction of N$_2$O to N$_2$ as the final step of denitrification (Henry et al., 2006), and is usually used to indicate the denitrification capacity. We observed that nosZ gene abundance had an important influence on denitrification. Meanwhile, nosZ gene abundance was significantly affected by organic carbon and nitrate/nitrite (Table S3). However, a recent study reported that denitrifying gene abundance cannot predict denitrification potential in the channels and riparian areas because intermittent oxygen saturation strongly alters the suitable condition of denitrifying community, especially the varying hydrologic regimes in the rivers (Tomasek et al., 2017). The mean annual temperature had a significant influence on nosZ gene abundance, which may suggest a crucial effect of temperature on denitrification throughout the rivers. Overall, denitrification in the rivers was affected by both climatic temperature regime and sediment variables. Mean annual temperature was the most important predictor of denitrification variance, and individual sediment characteristic played a minor role in the variation along the climate gradient.

4.2.2. Anammox

It has been reported that anammox occurs strongly in high concentrations of NH$_4^+$ and NO$_x^-$ in the rivers, because anammox bacteria have a dense and rigid membrane that can hinder the excess transportation of NH$_4^+$ and NO$_x^-$ (Zhu et al., 2015). However, we observed a negative correlation between anammox and NH$_4^+$ in our study. The high anammox rates were found in the rivers where DOC contents were generally lower, which indicates that low organic carbon condition likely favors the anammox (Plummer et al., 2015). It has been reported that anaerobic respiration can tend to more accumulation of sulfide in organic matter-enriched environments (Giblin et al., 2013; Jensen et al., 2008). Therefore, the inverse relationships of anammox with DOC and NH$_4^+$ in our study may be attributed to the higher sulfide inhibition on anammox in the high DOC and NH$_4^+$ content environments (Jensen et al., 2008; Lin et al., 2017). The partial correlation analysis controlling for sediment pH indicated that mean annual temperature had no significant influence on anammox rates, which in turn reflected the dominant role of pH in regulating anammox rates throughout the rivers. In addition, anammox is not sensitive to temperature change (Zhu et al., 2015), which could lead to no significant difference in anammox rates along the climate gradient in our study. Although previous studies reported that anammox bacteria can adapt to a wide range of pH from 4 to 9 (Zhu et al., 2015), the optimal condition for anammox is neutral pH (Tomaszewski et al., 2017). Higher
climate zones, respectively. DNF denotes denitrification, while anammox and DNRA contributed 2.1–21.9% and 2.3–33.4%, respectively. DNF accounted for 60.3–89.9% of the total nitrate/nitrite removal (Kreiling et al., 2011). Recently, many studies have reported that anammox can contribute up to 10% of total nitrogen removal in freshwater environments (Landsdown et al., 2016; Wang et al., 2012; Wang et al., 2018; Zhang et al., 2017; Zhu et al., 2013). Our results showed that denitrification accounted for 60.3–89.9% of the total nitrate/nitrite reduction, while anammox and DNRA contributed 2.1–33.4% and 3.3–21.9%, respectively. DNRA contributed most largely to total nitrate/nitrite reduction in the tropical climate zone than in other climate zones, indicating an increasing importance of DNRA to nitrogen cycling in tropical climate zone. Interestingly, we observed that the nitrogen conservation performed by DNRA could roughly compensate a temperature preference and respiration energy demand pathway (Hardison et al., 2015), which is favored in tropical climate because high temperature can enhance the organic carbon decomposition and leaching, providing more delivery of DOC and LOC for DNRA. NH$_4^+$ was found to affect DNRA rates because NO$_2^-$ was generated by aerobic NH$_4^+$ oxidation (nitrification) and delivered to the DNRA. However, organic matter mineralization can in situ produce large parts of NH$_4^+$ and tend to more accumulation in tropical climate, leading to a spurious correlation between NH$_4^+$ and DNRA rates. Thus, NH$_4^+$ may be not the important factor affecting the DNRA rates in our study. Fe$^{2+}$ plays an important role in mediating DNRA rates, which has been reported in aquatic sediments (Robertson et al., 2016). Actually, Fe$^{2+}$ is an effective catalyst which can enhance the reduction capacity of NO$_3^-$. More Fe$^{2+}$ is generally produced in the low reducing environments which are also favorable for the DNRA occurrence (Giblin et al., 2013). Overall, the mean annual temperature played an important role in affecting the sediment characteristics, further mediating the DNRA rates in the rivers along the climate gradient. Therefore, mean annual temperature explained mostly of the total DNRA variance along the latitude gradient, while individual sediment factor played a minor role.

### 4.3. Environmental Implications and Future Outlook

Denitrification and anammox can export nitrogen mainly through N$_2$, N$_2$O, and NO emissions (Reisinger et al., 2016; Stelzer et al., 2014; Stelzer & Bartsch, 2012; Xiong et al., 2017), while DNRA retains nitrogen as a more bioavailable ammonium for recycling in the rivers (Kim et al., 2016; Lin et al., 2017; Shelley et al., 2017). In many inland aquatic environments, denitrification is the dominant pathway of nitrate/nitrite reduction, which has been found to account for over 80% of total NO$_3^-$ removal (Kreiling et al., 2011). Recently, many studies have reported that anammox can contribute up to 10% of total nitrogen removal in freshwater environments (Landsdown et al., 2016; Wang et al., 2012; Wang et al., 2018; Zhang et al., 2017; Zhu et al., 2013). Our results showed that denitrification accounted for 60.3–89.9% of the total nitrate/nitrite reduction, while anammox and DNRA contributed 2.1–33.4% and 3.3–21.9%, respectively. DNRA contributed most largely to total nitrate/nitrite reduction in the tropical climate zone than in other climate zones, indicating an increasing importance of DNRA to nitrogen cycling in tropical climate zone. Interestingly, we observed that the nitrogen conservation performed by DNRA could roughly compensate...
the nitrogen loss caused by anammox throughout the rivers. Therefore, as the previously overlooked pathway, DNRA had a potential importance in the regulation of nitrogen recycling in river environments.

In our study, nitrogen loss and retention were estimated to reveal the ecological implications of nitrogen cycling in river environments. Assuming that the mean sediment density of the examined rivers was 1.3 g/cm³ (Table S1), it is conservatively estimated that about 77.5 g N m⁻² yr⁻¹ could be removed from the river sediments through denitrification. Anammox can also reduce the nitrogen at a rate of 10.0 g N m⁻² yr⁻¹, occupying roughly 13% of denitrification capacity. This comparison suggests that anammox also plays an important role in nitrogen removal from the river environments. Nitrogen retention by DNRA in the rivers was approximately at a rate of 12.6 g N m⁻² yr⁻¹, which is comparable to the nitrogen loss caused by anammox. The nitrogen removal capacity through both denitrification and anammox was totally 87.5 g N m⁻² yr⁻¹. However, there may be an overestimation of nitrogen loss and retention in our study. In the ¹⁵N tracing approach, the concentration of ¹⁵NO₃⁻ addition substrate (100 µmol/L) is excessively higher than in situ field, which could contribute substantially to the high potential rates in slurry incubation (Risgaard-Petersen et al., 2003; Xiong et al., 2017). In addition, the preincubation can lead to a large depletion of oxygen and form a more anoxic condition, which could also intensify nitrate/nitrite reduction processes. The potential rates of nitrate/nitrite reduction from slurry incubation are thus likely to be overestimated because river sediments in nature environments are often oxic, which will influence nitrogen cycling processes. In addition, the dissimilatory nitrate/nitrite reduction rates were just measured in summer, without considering the seasonal variation. Actually, dissimilatory nitrate/nitrite reduction rates in the rivers are highly variable and generally higher in summer than other seasons (Xiong et al., 2017), thereby resulting in an overestimation throughout a whole year.

Nitrogen pollution is currently increased and anticipated to have a large influence on ecological health of river environments. Although the rivers are the hot spots of denitrification and anammox, the nitrogen removal capacity of both processes is likely overwhelmed by the excess nitrogen loading, and large part of nitrogen is therefore retained within the rivers (Reisinger et al., 2016; Stelzer & Scott, 2018; Tomasek et al., 2017; Zhao et al., 2015). In this case, controlling exogenous nitrogen transported into the rivers is an effective management strategy to reduce the nitrogen loading and keep balance between input and export of nitrogen in the rivers. However, Tomasek et al. (2017) reported that the intensive agriculture has resulted in the excess nitrogen in surface water and groundwater, which exceeded the upper bound threshold of nitrogen removal capacity and negatively affected human health and aquatic ecosystems. China is a country with intensive agricultural activity that excessively uses chemical nitrogen fertilizer for enough food production, and agricultural activity is thus a primary factor of nitrogen pollution of the terrestrial rivers in China (Strokal et al., 2016; Zhao et al., 2015). Thereby, chemical nitrogen fertilizer in the agricultural activities should be reasonably fertilized or replaced of farm manure, which can effectively reduce the nitrogen discharge into the rivers. In addition, Greaver et al. (2016) reported that nitrogen cycling in rivers are highly variable under the fluctuations of temperature and precipitation, suggesting an important effect of climatic regime. The results of our study also indicated that climatic temperature regime was a strong factor affecting sediment characteristics and dissimilatory nitrate/nitrite reduction processes. Therefore, we should concern that the increasing temperature as a result of global climate change would have a potential influence on nitrogen cycling processes, which can lead to a more complicate dynamic of nitrogen fate in terrestrial rivers.

5. Conclusions

This study indicated that potential rates of denitrification and DNRA increased from temperate to tropical climatic zones, but there was no significant difference in anammox rates along the climate gradient. Redundancy analyses revealed that mean annual temperature together with sediment parameters explained 72.3–84.6% of total variances of denitrification, anammox, and DNRA. Mean annual temperature explained mostly 54% and 40% of denitrification and DNRA variances, respectively, while sediment pH was responsible for 26% of anammox variance along the climate gradient. Sediment DOC, pH, NH₄⁺, NO₃⁻, Fe²⁺, and functional gene abundances showed significant correlations with denitrification, anammox, and DNRA rates. These results evidence that dissimilatory nitrate/nitrite reduction processes were influenced by both biogeochemical controls and climatic temperature regime in rivers along the climate gradient.
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