Enhanced Nitrogen Loss from Rivers through Coupled Nitrification-denitrification Caused by Suspended Sediment

Xinghui Xia^{a,*}, Ting Liu^a, Zhifeng Yang^{a,*}, Greg Michalski^{b,*}, Shaoda Liu^c,

Zhimei Jia^a, Sibao Zhang^a

^a *School of Environment, Beijing Normal University / State Key Joint Laboratory of Environmental Simulation and Pollution Control, Beijing, 100875, China;*

^b *Department of Earth, Atmospheric and Planetary Sciences, Purdue University, West Lafayette, Indiana 47907, United States;*

^c *Department of Geography, National University of Singapore, 1 Arts Link, Kent Ridge, 117570, Singapore*

*To whom correspondence should be addressed. E-mail: xiakh@bnu.edu.cn (Xia, X.), zfyang@bnu.edu.cn (Yang, Z.), gmichals@purdue.edu (Michalski, G.).

1 **Abstract**

2 Present-day estimations of global nitrogen loss (N-loss) are underestimated. Commonly, N-loss
3 from rivers is thought to be caused by denitrification only in bed-sediments. However, coupled
4 nitrification-denitrification occurring in overlying water with suspended sediments (SPS) where
5 oxic and anoxic/low oxygen zones may coexist is ignored for N-loss in rivers. Here the Yellow and
6 Yangtze Rivers were taken as examples to investigate the effect of SPS, which exists in many rivers
7 of the world, on N loss through coupled nitrification-denitrification with nitrogen stable (^{15}N)
8 isotopic tracer simulation experiments and *in-situ* investigation. The results showed even when SPS
9 was surrounded by oxic waters, there were redox conditions that transitioned from an oxic surface
10 layer to anoxic layer near the particle center, enabling coupled nitrification-denitrification to occur
11 around SPS. The production rate of $^{15}\text{N}_2$ from $^{15}\text{NH}_4^+\text{-N}$ ($R_{^{15}\text{N}_2\text{-production}}$) increased with increasing
12 SPS concentration ($[SPS]$) as a power function ($R_{^{15}\text{N}_2\text{-production}}=a\cdot[SPS]^b$) for both the SPS-water
13 and bed sediment-SPS-water systems. The power-functional increase of nitrifying and denitrifying
14 bacteria population with $[SPS]$ accounted for the enhanced coupled nitrification-denitrification rate
15 in overlying water. SPS also accelerated denitrification in bed-sediment due to increased NO_3^-
16 concentration caused by SPS-mediated nitrification. For these two rivers, 1 g L^{-1} SPS will lead to
17 N-loss enhancement by approximately 25-120%, and the enhancement increased with organic
18 carbon content of SPS. Thus, we conclude that SPS in overlying water is a hot spot for nitrogen loss
19 in river systems and current estimates of in-stream N-loss are underestimated without consideration
20 of SPS; this may partially compensate for the current imbalance of global nitrogen inputs and sinks.

21

22 *Keywords: coupled nitrification-denitrification; suspended sediment; nitrogen loss; rivers; nitrogen*
23 *budget*

1 Introduction

Nitrogen (N), a major element required by all organisms, is widely applied in industry, agriculture, and domestic activities, but excess N can cause eutrophication and hypoxia in water bodies, destruction of habitats for resident organisms, and reduction of species diversity (Lenihan and Peterson, 1998; Mallin et al., 2006; Liu et al., 2013a; Roberts et al., 2014). Therefore, understanding the processes controlling N budget, such as the N fixation, assimilation, nitrification, and denitrification is vital. Yet, despite decades of research, the global N budget remains out of balance, with inputs exceeding losses (Galloway, 1998; Gruber and Galloway, 2008; Schlesinger, 2009). This imbalance indicates that some unknown processes might contribute to N losses, thus resulting in major uncertainties in model simulations and limiting the accuracy of forecasts of future river N export caused by climate change, urbanization, and human population growth.

Rivers are subject to high loads of nitrogen and can convert approximately 40% of terrestrial nitrogen (N) runoff (~47 Tg per year) to biologically unavailable dinitrogen gas (Galloway et al., 2004). Nitrogen loss from rivers plays an important role for N delivery to coastal ecosystems (Donner and Kucharik, 2008), which often controls eutrophication and the development of pelagic “dead zones” (Turner and Rabalais, 1994; Rabalais, 2002; Diaz and Rosenberg, 2008). Studies of N loss from river systems have mainly focused on bed-sediment denitrification which is currently considered as the main in-stream N loss process (Boyer et al., 2006; Johannsen et al., 2008; Li et al., 2010; Liu et al., 2013b). Nitrification, the aerobic oxidation of NH_4^+ to NO_3^- via NO_2^- performed primarily by ammonia-oxidizing bacteria and archaea, is an important nitrate supply that is subsequently transported to anaerobic zones in bed-sediments and reduced by denitrification in river systems (Seitzinger, 1988; Pina-Ochoa and Álvarez-Cobelas et al., 2006; Mulholland et al., 2008; Liu et al., 2013b). This suggests that nitrification and denitrification in rivers might be tightly coupled, however the timescale of nitrate diffusion at the water-sediment interface is thought to limit the role of bed-sediment denitrification in river systems (Venterink et al., 2003; O’Connor and Hondzo, 2007).

Although several studies suggested that coupled nitrification-denitrification (CND) might occur in water column of some estuaries with high turbidity (Abril et al., 2000; Sebilo et al., 2006), N loss

in river systems by *CND* occurring in water column containing suspended sediments (SPS) where possible anoxic/low oxygen microsites may exist has been largely ignored. SPS exists in many rivers around the world (Mulder and Syvitski, 1995; Sivakumar, 2002; Billi and Ali, 2010; Water Conservancy Committee of the Yellow River, 2013), and it has been shown that nitrification rates increase with SPS concentration (Xia et al., 2004; Xia et al., 2009). Additionally, it has been proven that denitrification rate increases linearly with SPS concentration in oxic waters according to the results of incubation experiment with added $^{15}\text{NO}_3^-$ -N (Liu et al., 2013b), and Reisinger et al (2016) found denitrification occurring in the water column of certain rivers. The anoxic/low oxygen microsites were assumed to exist in suspended particles surrounded by oxidized waters according to the theories of transport limitations and multispecies biofilms (Lamontag.Ra et al., 1973; Bianchi et al., 1992; Michotey and Bonin, 1997; Falkowski et al., 2008). Building on these recent findings, we hypothesized that a gradient of oxic and anoxic/low oxygen conditions exists within SPS particles with oxic conditions at the surface layer and anoxic/low oxygen conditions near the center of particle. Then *CND* could occur in oxic waters in the presence of SPS, with nitrification occurring at the surface layer and denitrification occurring in the inner layer of SPS. In addition to providing oxygen-limited microsites for denitrification, SPS will also accelerate river denitrification rates by generating more NO_3^- from SPS, which increases NO_3^- concentration gradients between overlying water and bed-sediment and thus enhances diffusion rates, promoting bed-sediment denitrification in river systems.

Therefore, we hypothesized that there will be enhanced N-loss through *CND* and other nitrogen transformation processes in river systems where there are significant amounts of SPS. The influences of SPS concentration on N loss in the Yellow River and the Yangtze River were investigated using isotopic and chemical techniques; the SPS concentration of these two rivers ranged from 0.01 to 54.8 g L⁻¹ and from 0.01 to 10.5 g L⁻¹, respectively (Changjiang Water Resource Committee, 2014; Xia et al., 2016). In detail, the present study aimed to: 1) examine whether *CND* could occur at SPS particles, and explore the relevant mechanisms; 2) investigate and model the relationship between *CND* rate and SPS concentration; 3) estimate the impacts of enhanced *CND* by SPS on N-loss from river systems.

2 Materials and Methods

2.1 Sample collection

Sediment and water samples used in incubation experiments were collected from five sites including Longmen (LM, 110°36'06.4"E, 35°39'33.9"N), Huayuankou (HYK, 113°41'07.7"E, 34°54'16.8"N), and Aishan (AS, 116°16'39.7"E, 36°13'44.4"N) Stations in the Yellow River and 37-Dock (37-Dock, 114°20'09.5"E, 30°36'30.5"N) and Wanzhou (WZ, 108°22'56.30"E, 30°48'39.85"N) Stations in the Yangtze River (Fig.1). To avoid being contaminated by surface pollutants, water samples at 0.2 m below the water surface were collected with a TC-Y sampler (TECH Instrument in Shenyang, China), and analyzed for properties including the content of organic carbon and nitrogen species (see below). The top 10-cm bed-sediment samples, which are more easily re-suspended during flow fluctuation, were collected with a sediment grab sampler. In addition, SPS and water samples were collected from 17 sites of the Yellow River to study the effect of SPS concentration on in-situ nitrifying and denitrifying bacteria abundances (Fig.1). Then all the above samples were kept under 4°C in a cooler and shipped to laboratory for further analysis. Incubation experiments began within 48 h after sampling. The sediment characteristics at the five sampling sites of the Yellow River and the Yangtze River are shown in Table 1.

2.2 Incubation of SPS-water systems with various SPS concentrations

Taking the samples collected from site AS as an example to examine whether CND could occur on SPS particles, a set of chambers containing SPS and water but no bed-sediment were designed. The experimental design was similar to our previous study (Liu et al., 2013b). A series of chambers containing 800-ml artificial overlying water with 5 mgL⁻¹ ¹⁵NH₄⁺-N as ¹⁵NH₄⁺Cl (99.0 atm% ¹⁵N, Shanghai Research Institute of Chemical Industry, China) were designed to obtain a 20-cm-deep water column. The artificial overlying water contained indigenous bacteria in the Yellow River and its preparation is shown in Supplementary Section S1. Triplicate experiments were conducted for each incubation set. A certain amount (0, 0.8, 2, 6.4, 12 and 16 g) of homogenized bed-sediment was respectively added into each chamber, which were then incubated at 25 °C. All the added sediment was suspended by setting agitation rates at 50, 150, 200, 400, 550, and 700 r min⁻¹, obtaining SPS

107 concentrations of 0, 1, 2.5, 8, 15, and 20 g L⁻¹, respectively. The chemical conditions of water in each
108 chamber were the same (Supplementary Table S1), and they are similar to that of river water. At
109 pre-determined intervals (every three days), gas from headspace produced during each incubation
110 period was sampled using a gas-tight syringe for the measurement of $\delta^{15}\text{N-N}_2$, and then the
111 concentration excess $^{15}\text{N-N}_2$ was calculated. The remaining $^{15}\text{N}_2$ produced during previous
112 incubation period was purged by aerating the water phase for 30 min, and the aeration also helped
113 keep oxygen level at saturation. The dissolved oxygen concentration was determined after each
114 sampling and it was approximately 8.2 mg L⁻¹ during experiment. At the end of experiment, sediment
115 and water were separated by letting the sediment settle out, and the density of denitrifying bacteria in
116 the SPS and water samples was measured using the most-probable-number-PCR (MPN-PCR)
117 method (section 2.6 and Supplementary Section S3). The control sets were performed using
118 sterilized sediment and water samples, and mercuric chloride was added to the control sets with a
119 final concentration of 0.5% to inhibit the microbial activities. The production of $^{15}\text{N}_2$ caused by
120 microbial activities was calculated by subtracting $^{15}\text{N}_2$ produced in the control systems from that in
121 each incubation set.

122 In addition, the mechanisms accounting for the occurrence of CND on SPS particles were
123 analyzed by examining the O₂ flux and profile around the SPS particle. To do this, one more set of
124 chambers containing 800 ml solution with 5 mg L⁻¹ NH₄⁺-N and 20 g L⁻¹ SPS of different sizes (< 20
125 μm , 20-50 μm , 50-100 μm , and >100 μm), which were separated by wet screening and sedimentation
126 methods using samples collected from the site AS, were incubated at 25°C. The detection of O₂ flux
127 and profile around SPS particles was performed using Non-invasive Micro-test Technology and
128 microelectrode technology (Jaffe and Nuccitelli, 1974; Revsbech, 1989; Smith, 1995), respectively.
129 The details for the determination are described in Supplementary Section S2.

130 2.3 Incubation of bed sediment-SPS-water system with various SPS concentrations

131 A 10-cm-deep sediment column was constructed by adding a certain amount of homogenized
132 bed-sediment into the gastight polymethyl methacrylate (PMMA) incubation chambers, as described
133 by Liu et al.(2013b). Then the sediment was covered by 800 ml artificial overlying water with 5 mg
134 L⁻¹ $^{15}\text{NH}_4^+$ -N. Triplicate experiments were conducted for each incubation set. The SPS concentrations

were set at 20, 15, 8, 2.5, 1, and 0 g L⁻¹ by adjusting the agitation rates at 700, 550, 400, 200, 150, and 0 r min⁻¹ respectively, simulating the real rivers containing different concentrations of SPS. The variation of measured SPS concentration in each chamber was less than 5% of the pre-set one. The particle size of SPS in the systems containing different concentrations of SPS was similar to that of SPS in real rivers (Supplementary Table S2). All the chambers were incubated at 25 °C. At pre-determined intervals (every one to three days), water samples were sucked out of the chamber using a columnar sampler as described in Liu et al.(2013b); the other procedures including gas sampling and aeration were the same as that mentioned at Section 2.2. At the end of incubation, the water phase containing SPS was decanted; SPS and water were separated by standing for 12 h. The denitrifying and nitrifying bacteria densities in the water phase, bed-sediment, and SPS samples were measured using MPN-PCR method. The CND rate was calculated according to the production of ¹⁵N₂, while nitrification rate was calculated according to the variation of NH₄⁺-N concentration in the systems. To distinguish the contribution of SPS itself and agitation to the increased N loss through CND caused by the presence of SPS, another experiment set was carried out for systems containing bed-sediment and water (BS-WS) without agitation using samples collected from site AS. The control sets were conducted as the same as that mentioned at Section 2.2.

2.4 Incubation of bed sediment-SPS-water system with various nitrate concentration

To investigate the influencing mechanisms of SPS concentration on CND, the effect of initial NO₃⁻ concentration ([NO₃⁻]) on denitrification was investigated by using samples collected from the site HYK. The 10-cm-deep sediment columns were constructed using the same method as Section 2.3, then 800 ml artificial overlying water containing 10, 6, 4.5, 3 and 1 mg L⁻¹ NO₃⁻-N as KNO₃ were respectively added into each chamber. These concentrations are within the range of NO₃⁻ concentration detected in the Yellow River (Xia et al., 2002). Triplicate experiments were conducted for each incubation set. The SPS concentration in each column was set at 8 g L⁻¹ by adjusting agitation rate, which occurred in the temperate seasons (October-November and March-June) in the Yellow River. Then all the chambers were incubated at 25 °C. At pre-determined intervals (every two to four days), bed-sediment, SPS, and water samples were collected by using columnar samplers, and the samples were then put into tubes to separate sediment (including SPS and bed-sediment) and

163 water phases by centrifuge; sediment and water samples were subsequently analyzed for the
164 concentrations of N species. The denitrification rate was calculated according to the variation of
165 $[\text{NO}_3^-]$ in the systems including SPS, bed-sediment, and water phases. The other experimental
166 procedures were the same as that in *Section 2.3*. All the above mentioned experimental conditions
167 are summarized in **Table 2**.

168 **2.5 Chemical analysis**

169 Water sample was filtrated through 0.45 μm filters (Millipore corp. USA), and analyzed for
170 NO_3^- -N, NO_2^- -N, and NH_4^+ -N concentrations immediately. The NO_3^- -N, NO_2^- -N, and NH_4^+ -N
171 contents in sediment were analyzed after extraction with KCl solution (2M). Dissolved nitrogen
172 (NO_3^- -N, NO_2^- -N, and NH_4^+ -N) concentrations were detected using an Autoanalyser-3 (Bran &
173 Luebbe, France). Organic nitrogen contents in the sediment and water were measured after Kjeldahl
174 digestion. Analytical reagents were used throughout the chemical analysis. The standard deviations
175 for standards and replicate analysis were less than 4.4%, 2.8%, 5.4%, 5.0% for NH_4^+ -N, NO_2^- -N,
176 NO_3^- -N and organic nitrogen, respectively. The $^{15}\text{N}_2$ in gas samples was detected through using an
177 isotopic ratio mass spectrometer (Thermo Delta V Advantage), in line with an automated gas PreCon
178 unit. Nitrogen isotope ratios (including 29/28 and 30/28) of the samples were expressed as standard δ
179 notation (‰) relative to atmospheric N_2 . The standard deviation for standards and replicate analysis
180 of the $\delta^{15}\text{N}$ measurement was better than 0.2 ‰.

181 The SPS concentration in each water sample was obtained by filtering a certain amount of water
182 through a Pall membrane (0.45 μm) and weighing the filter residue after drying at 60 °C for 7~8
183 hours. A Laser Particle Sizer (Microtrac, S3500) was used to measure the size characteristics of SPS.
184 A TOC analyzer (Shimadzu TOC-500) was used to detect the total organic carbon (TOC) in water
185 and sediment samples. An oxygen meter (Mettler Toledo, SG9-FK2) was used to monitor dissolved
186 oxygen concentration of water. Redox potential, temperature, and pH of water were detected using a
187 pH meter (Mettler Toledo, SG23).

188 **2.6 Biological and statistical analysis**

189 To investigate the abundances of denitrifying and nitrifying bacteria in SPS particles,

bed-sediment, and overlying water samples, DNA from the water and/or sediment were extracted using an Ultra Clean Soil DNA Isolation Kit (Mo-bio Laboratories, Inc., Carlsbad, CA, USA). The extracted DNA were stored at -20°C until analysis by most-probable-number (MPN)-PCR (Cochran, 1950; Rotthauwe et al., 1997; Liu et al., 2003). The detailed procedures on MPN-PCR are shown in Supplementary Section S3.

SPSS 18.0 for windows (SPSS Inc., Chicago IL., USA) was used for all statistical analyses in this research, including correlation analysis, nonlinear fitting, and variance analysis. The significance of correlation between each two variables was tested by calculating the Pearson correlation coefficient, and the significant differences among groups were tested with Duncan's multiple range test.

3 Results and Discussion

3.1 Effect of SPS concentration on CND in oxic SPS-water system

3.1.1 Dependence of CND rate on SPS concentration

The presence of CND in SPS-water system (SPS-WS) was assessed by measuring the $^{15}\text{N}_2\text{-N}$ production from a $^{15}\text{NH}_4^+\text{-N}$ addition, which can only occur by $^{15}\text{NO}_3^-$ production through nitrification of $^{15}\text{NH}_4^+\text{-N}$ followed by its reduction to $^{15}\text{N}_2$ during denitrification or anammox. According to our research (submitted to *Biogeochemistry*), the $^{15}\text{N}_2$ production from anammox was very low in SPS-water system of the Yellow River, thus the $^{15}\text{N}_2$ production was primarily from CND in the present research. The average CND rate, expressed as $^{15}\text{N}_2\text{-N}$ production, followed a power function with SPS concentration, and jumped from 25 to $264\ \mu\text{g-N m}^{-2}\text{d}^{-1}$ when the concentration of SPS was elevated from 0 to $20\ \text{g L}^{-1}$ (Fig. 2a). Even a low concentration of SPS ($1\ \text{g L}^{-1}$) resulted in an 8-fold increase in the $^{15}\text{N}_2\text{-N}$ production relative to water without SPS. In addition, both nitrifying and denitrifying bacteria populations increased with increasing SPS concentration (Fig. 2b) as power functions respectively ($p < 0.05$), and they were positively correlated with the average CND rate (Fig. S1). This data suggest SPS was providing conditions for CND to occur.

3.1.2 Conditions provided by SPS for CND

The activity of SPS particle was investigated by determining the O_2 flux and dissolved O_2 (DO)

217 around the particle. The observed high O₂ influx around SPS particles and decrease in DO
218 concentrations (Fig. 3, Fig. S2 and S3) suggest that oxygen was being consumed near SPS particle
219 surfaces by nitrification and/or microbial respiration. The DO concentration in the bulk solution was
220 approximately 8.2 mg L⁻¹ (100% air saturated), but it decreased to less than 53% O₂ saturation near
221 the surface for particles with size <100 μm, and the decrease was the sharpest near the surface of
222 particles. Similarly, maximum increases in the O₂ influx (-2.073 to -0.612 pmol cm⁻² s⁻¹) occurred
223 near particle surface regardless of particle size. Particles with diameters smaller than 20 μm had the
224 largest overall increase in O₂ influx (Fig. S3); this is likely caused by the higher organic carbon
225 content (%) and larger specific surface area of smaller particles compared with particles with larger
226 sizes (Table S3). Despite differences in O₂ influxes among particles of different sizes, the above
227 results indicate a gradient of oxic and low oxygen conditions exists within SPS particles with oxic
228 conditions at the surface layer and low oxygen conditions near the center of particle. It infers that
229 SPS particles can act as activated redox sites for microbial nitrification and denitrification because
230 some bacteria can conduct denitrification under limited/low oxygen conditions. According to our
231 previous research (Liu et al., 2013b), most of the denitrifying bacteria in the Yellow River are
232 facultative bacteria and can conduct denitrification under limited/low oxygen conditions (oxygen
233 level <53% and <70% of the saturated O₂ concentration, respectively). Therefore, the presence of
234 SPS in the Yellow River can provide microsite redox conditions needed for CND. In addition,
235 because the SPS used in our incubation experiment had lower organic carbon contents than other
236 rivers (Table S4), the SPS in other rivers might provide low/limited oxygen even anoxic
237 micro-environments for denitrification.

238 Therefore, the mechanisms about the influence of SPS on CND in oxic waters can be analyzed
239 as follows. The nitrifying bacteria distribute on the outer layer of particles and perform nitrification
240 under high oxygen conditions, and the denitrifying bacteria reduced nitrate to N₂ at the deep layer of
241 particles under low oxygen conditions. That is to say, firstly, the oxic conditions and NH₄⁺-N
242 adsorbed on the surface layer of suspended particles favor nitrification, and the nitrification rate rises
243 with SPS concentration as a power function due to the fact that the percentage of NH₄⁺-N sorbed on
244 SPS increases with SPS concentration following a power function (Xia et al., 2009). Secondly, not

only respiration by heterotrophic bacteria but nitrification at SPS particles lead to the presence of microsites with limited/low oxygen around/inside SPS particles, providing conditions for denitrification. The existence of both oxygen-limited microsites and denitrifying bacteria within the SPS particles promotes denitrification. These relationships between nitrification and denitrification at SPS particles lead to the occurrence of CND at SPS particles, as well as the accelerated CND rate with SPS concentration.

The positive correlation of nitrification with SPS concentration has been observed for river systems (Xia et al., 2009; Hsiao et al., 2014), and such a correlation has been reported in the relatively turbid region of estuaries with higher nitrification activities (Helder and De Vries, 1983; Owens, 1986; Berounsky and Nixon, 1993). In addition, although denitrification is most commonly observed in anoxic sediments, this process has also been reported for oxic environments where anoxic micro environments are thought to be associated with suspended particle surfaces and/or biofilms (Rao et al., 2008; Liu et al., 2013b; Reisinger et al., 2016). Furthermore, for the high-turbidity river mouth, the *amoA* and *nirS* genes abundances of the particle-associated ($> 3 \mu\text{m}$) were found to be significantly higher than the free-living communities ($0.2 \sim 3 \mu\text{m}$) (Zhang et al., 2014). These research results suggest that the suspended particles and biofilms can promote both nitrification and denitrification in freshwater and seawater systems, which is probably due to the fact that the particles and biofilms can provide microsite redox conditions for both nitrification and denitrification.

3.2 Influence of SPS concentration on CND in bed sediment-SPS-water system

3.2.1 Increased $^{15}\text{N}_2$ production from $^{15}\text{NH}_4^+$ with SPS concentration

The production of $^{15}\text{N}_2$ produced by CND increased with incubation time and SPS concentration for the bed sediment-SPS-water system (BS-SPS-WS) using samples collected from five sites of the Yellow and Yangtze Rivers (Fig. 4 and Fig. S4). The average $^{15}\text{N}_2$ production rate increased dramatically with SPS concentration at low SPS concentrations and the increase tapered off at higher SPS concentrations. For the HYK sample, the $^{15}\text{N}_2$ production rate increased by $0.1 \text{ mg-N m}^{-2} \text{ d}^{-1}$ when SPS concentration increased from 0 to 1 g L^{-1} , but only increased by an additional $0.008 \text{ mg-N m}^{-2} \text{ d}^{-1}$ when SPS increased from 1 to 20 g L^{-1} .

273 The average $^{15}\text{N}_2$ production rate ($R_{15\text{N}_2\text{-production}}$, $\text{mg-N m}^{-2} \text{ d}^{-1}$) during incubation in BS-SPS-WS
274 increased with SPS concentration ($[\text{SPS}]$, g L^{-1}) as a power function for all of the five study sites (Fig.
275 5):

$$276 \quad R_{15\text{N}_2\text{-production}} = a \cdot [\text{SPS}]^b \quad (1)$$

277 This indicates the universality of the relationship between N-loss rate and SPS concentration. As
278 shown in Fig. S5, the constant a , reflecting the nitrogen loss rate with 1 g L^{-1} SPS, was positively
279 related to the TOC content of SPS and the percentage of particles smaller than $20 \mu\text{m}$ ($p < 0.05$). The
280 constant b , reflecting the dependence of N-loss on SPS concentration, was also positively related to
281 the TOC content of SPS ($p < 0.05$). This was probably due to the fact that high TOC content favored
282 the growth of heterotrophic denitrifying bacteria. Smaller particles might provide more favorable
283 conditions for CND due to the sharper decrease of O_2 concentration near the particles and provided
284 more sites for bacteria to grow due to larger specific surface areas. According to our previous
285 research, the denitrification rate was negatively related to particle size of SPS in oxic waters (Jia et
286 al., 2016). The enhancement of the N-loss rate by the presence of SPS differed among samples
287 collected from various sites, indicating local biogeochemical conditions can control N-loss. For
288 example, the sediments at site WZ of the Yangtze River that had the highest fine particle ($< 20 \mu\text{m}$)
289 percentage and TOC content among the five study sites also had the highest N-loss rate.

290 3.2.2 Influence of SPS concentration on nitrogen transformation

291 The presence of SPS accelerated the transformation of $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ (Fig. 6 and Fig. 7).
292 For the HYK sample, NH_4^+ concentration ($[\text{NH}_4^+]$) decreased nearly to zero by the 11th day in
293 systems when SPS concentrations were between 8 and 20 g L^{-1} , while it remained over 1.5 mg L^{-1} in
294 the system without SPS after 31 days of incubation. The average $\text{NH}_4^+\text{-N}$ loss rate at the first nine
295 days ascended with SPS concentration by a power function ($r = 0.921$, $p < 0.01$, Fig. 7).
296 Correspondingly, the NO_3^- concentration ($[\text{NO}_3^-]$) increased and the $^{15}\text{N}_2$ evolved from each column
297 along with the decrease in $[\text{NH}_4^+]$ (Fig. 6), supporting the hypothesis of coupled
298 nitrification-denitrification. However, a certain amount of $\text{NO}_3^-\text{-N}$ was accumulated in systems,
299 which meant not all of the $\text{NO}_3^-\text{-N}$ produced from nitrification was denitrified immediately.

300 In the incubation experiments of bed sediment-SPS-water system, ammonification, anammox,
 301 dissimilatory nitrate reduction to ammonium (DNRA), and inorganic nitrogen uptake may occur
 302 simultaneously in addition to nitrification and denitrification processes. Overall, approximately 1.8%,
 303 3.6%, 6.4%, 12%, and 20% of the added ^{15}N in the form of $\text{NH}_4^+\text{-N}$ has been transformed into N_2
 304 after incubation for 31 d for the bed sediment-SPS-water system containing 20 g L^{-1} SPS collected
 305 from HYK, LM, AS, 37 Dock, and WZ Stations, respectively. In detail, taking the HYK for example
 306 (Table S3), approximately 1.80% and 71.8% of the added ^{15}N in the form of $\text{NH}_4^+\text{-N}$ has been
 307 transformed into N_2 and NO_3^- for the incubations with 20 g L^{-1} SPS, respectively; approximately 5.09%
 308 remained as NH_4^+ and 21.3% assimilated by organisms based on the mass balance of added $\text{NH}_4^+\text{-N}$.

309 The variation of NH_4^+ concentration was affected by ammonification, nitrification, anammox,
 310 DNRA, and its uptake by heterotrophic organisms. As shown in Fig. 6 for the HYK sample, NH_4^+
 311 concentration increased during the first three days of the incubation systems with SPS concentration
 312 less than 2.5 g/L ; this might be ascribed to the ammonification. After that, NH_4^+ concentration
 313 decreased significantly, suggesting that nitrification, anammox, and uptake by heterotrophic
 314 organisms controlled the variation of NH_4^+ . Although Lansdown et al. (2016) found that anammox
 315 can represent an important nitrogen loss pathway in permeable river sediments, some researchers
 316 reported that the contribution of anammox process, in the riverine sediments, to the N-loss was
 317 approximately 10% (Dale et al., 2009; Wang et al., 2012; Zhu et al., 2015). The anammox process
 318 might be less significant than nitrification in the present research because the sediment was different
 319 from that studied by Lansdown et al. In addition, the fraction of NH_4^+ transformed to NO_3^-
 320 (55.6%-89.5%) was much higher than the assimilation (3.71%-28.9%) (Table S5). The variation of
 321 NO_3^- concentration would be affected by nitrification, DNRA, and denitrification. DNRA has been
 322 shown as significant in many organic rich sediments and for instance during oxic-anoxic oscillations
 323 (Abril et al., 2010). As less than 23% of the added NH_4^+ remained as NH_4^+ , the DNRA must be much
 324 less significant than nitrification in the present research. In addition, the TOC content of the sediment
 325 sample was low (Table 1), therefore, the DNRA might be not significant in the incubations of the
 326 present research. However, SPS might provide oxic-anoxic oscillations for DNRA in other rivers,
 327 and more research should be conducted in this regard.

328 According to the above analysis, the variations of NH_4^+ and NO_3^- in the incubation systems
 329 resulted from several processes. However, the transformation of added NH_4^+ was mainly controlled
 330 by nitrification and denitrification. Therefore, the processes were simplified as nitrification and
 331 denitrification to study the nitrogen transformation kinetics of the present research. The following
 332 logistic model was used to analyze NH_4^+ -N variation during nitrification:

$$333 \quad \left(-\frac{dS_{\text{NH}_4^+}}{dt} \right)_{\text{nitrification}} = \frac{\mu_{\text{max-nitrifier}} S_{\text{NH}_4^+} (S_{\text{NH}_4^+0} + X_{0\text{-nitrifier}} - S_{\text{NH}_4^+})}{k_{s\text{-nitrifier}}} \quad (2)$$

334 the integral form of equation (2) is:

$$335 \quad S_{\text{NH}_4^+} = \frac{S_{\text{NH}_4^+0} + X_{0\text{-nitrifier}}}{1 + (X_{0\text{-nitrifier}} / S_{\text{NH}_4^+0}) \cdot e^{k_{\text{nitrification}} (S_{\text{NH}_4^+0} + X_{0\text{-nitrifier}}) t}} \quad (3)$$

336 where $S_{\text{NH}_4^+}$ represents the $[\text{NH}_4^+]$ at time t (mg L^{-1}); $S_{\text{NH}_4^+0}$ represents the $[\text{NH}_4^+]$ in the beginning
 337 (mg L^{-1}); $X_{0\text{-nitrifier}}$ represents the bacterial population quota in the beginning (mg L^{-1}); $k_{\text{nitrification}}$
 338 represents the nitrification rate constant ($\text{d}^{-1} (\text{mg L}^{-1})^{-1}$), $k_{\text{nitrification}} = \mu_{\text{max-nitrifier}} / K_{s\text{-nitrifier}}$; $\mu_{\text{max-nitrifier}}$
 339 represents the maximum specific growth rate of nitrifying bacteria (d^{-1}); $K_{s\text{-nitrifier}}$ represents the
 340 half-saturation growth constant for nitrifying bacteria (mg L^{-1}). The NH_4^+ -N variation in systems
 341 containing various SPS concentrations fit well with the Logistic model shown in equation (2) ($p <$
 342 0.01), and the nitrification rate constant ($k_{\text{nitrification}}$) increased with SPS concentration conforming to
 343 a power function ($p < 0.01$, Fig. 7 and Table 3).

344 The variation of nitrate during CND depends on relative nitrification and denitrification rates,
 345 and the latter was described by the Monod kinetics:

$$346 \quad \frac{dS_{\text{NO}_3^-}}{dt} = \frac{d(S_{\text{NH}_4^+0} - S_{\text{NH}_4^+})}{dt} - \left(\frac{\mu_{\text{max-denitrifier}} S_{\text{NO}_3^-} (S_{\text{NO}_3^-0} + X_{0\text{-denitrifier}} - S_{\text{NO}_3^-})}{(K_{s\text{-denitrifier}} + S_{\text{NO}_3^-})} \right) \quad (4)$$

347 where $S_{\text{NO}_3^-}$ represents the $[\text{NO}_3^-]$ at time t (mg L^{-1}); $S_{\text{NO}_3^-0}$ represents the initial $[\text{NO}_3^-]$ (mg L^{-1});
 348 $\mu_{\text{max-denitrifier}}$ represents the maximum specific growth rate of denitrifying bacteria (d^{-1}); $X_{0\text{-denitrifier}}$
 349 represents the denitrifying bacterial population quota in the beginning (mg L^{-1}); $K_{s\text{-denitrifier}}$ represents
 350 the half-saturation growth constant for denitrifying bacteria (mg L^{-1}). As shown in Fig. 6, the kinetics

of nitrate in system could be divided into two stages. At the first stage, $[\text{NO}_3^-]$ increased with time; the denitrifying bacteria was much higher than $[\text{NO}_3^-]$ at time zero ($X_{0\text{-denitrifier}} \gg S_{\text{NO}_3\text{-}0}$) and the $K_{s\text{-denitrifier}}$ was much higher than $[\text{NO}_3^-]$ as well ($K_{s\text{-denitrifier}} \gg S_{\text{NO}_3}$), accordingly formula (4) could be simplified as equation (4-1). At the second stage, $[\text{NO}_3^-]$ decreased with time while $[\text{NH}_4^+]$ kept constant at $\sim 0.3 \text{ mg L}^{-1}$ (i.e., $\frac{d(S_{\text{NH}_4^+0} - S_{\text{NH}_4^+})}{dt} = 0$); $K_{s\text{-denitrifier}}$ was much higher than nitrate concentration ($K_{s\text{-denitrifier}} \gg S_{\text{NO}_3}$), accordingly formula (4) could be simplified as equation (4-2) as follows:

$$\frac{dS_{\text{NO}_3^-}}{dt} = \begin{cases} \frac{d(S_{\text{NH}_4^+0} - S_{\text{NH}_4^+})}{dt} - \frac{\mu_{\text{max-denitrifier}} S_{\text{NO}_3^-} X_{0\text{-denitrifier}}}{K_{s\text{-denitrifier}}} & (\text{First Stage}) \end{cases} \quad (4-1)$$

$$\frac{dS_{\text{NO}_3^-}}{dt} = \begin{cases} \frac{\mu_{\text{max-denitrifier}} S_{\text{NO}_3^-} (S_{\text{NO}_3^-0} + X_{0\text{-denitrifier}} - S_{\text{NO}_3^-})}{K_{s\text{-denitrifier}}} & (\text{Second Stage}) \end{cases} \quad (4-2)$$

358

the integral forms of Equations (4-1) and (4-2) are:

$$S_{\text{NO}_3^-} = \begin{cases} \frac{S_{\text{NH}_4^+0} + X_{0\text{-nitrifier}}}{1 + (X_{0\text{nitrifier}} / S_{\text{NH}_4^+0}) \cdot e^{k_{\text{nitrification}} (S_{\text{NH}_4^+0} + X_{0\text{-nitrifier}})t}} \cdot (1 - e^{k_{\text{denitrification-1}}t}) & (\text{First Stage}) \end{cases} \quad (5-1)$$

$$S_{\text{NO}_3^-} = \begin{cases} \frac{S_{\text{NO}_3^-0} + X_{0\text{-denitrifier}}}{1 + (X_{0\text{-denitrifier}} / S_{\text{NO}_3^-0}) \cdot e^{k_{\text{denitrification-2}} (S_{\text{NO}_3^-0} + X_{0\text{-denitrifier}})t}} & (\text{Second Stage}) \end{cases} \quad (5-2)$$

where $k_{\text{denitrification-1}}$ is the denitrification rate constant during the first stage (d^{-1}),

$k_{\text{denitrification-1}} = \frac{\mu_{\text{max-denitrifier}} \cdot X_{0\text{-denitrifier}}}{K_{s\text{-denitrifier}}}$; $k_{\text{denitrification-2}}$ is the denitrification rate constant during the second

stage ($\text{d}^{-1} (\text{mgL}^{-1})^{-1}$), $k_{\text{denitrification-2}} = \mu_{\text{max-denitrifier}} / K_{s\text{-denitrifier}}$.

Based on the variations of $[\text{NO}_3^-]$ shown in Fig. 6, the kinetics of nitrate fit the equations (5-1) and (5-2) very well ($p < 0.01$). According to the results shown in Table 3, the denitrification rate constant ($K_{\text{denitrification-1}}$) ascended with SPS concentration conforming to a power function during the first stage (Fig. S6), coinciding with the relationship between nitrification rate constant ($k_{\text{nitrification}}$) and SPS concentration as mentioned before. It implied that the denitrification rate might be tightly related to nitrate available produced by nitrification. For the system containing 0 g L^{-1} SPS, the second stage did not occur obviously; the denitrification rate constant ($k_{\text{denitrification-2}}$) in systems

371 containing 1, 2.5, 8, 15, and 20 g L⁻¹ SPS also ascended with SPS concentration conforming to a
372 power function ($R^2=0.72$) during the second stage, indicating the enhanced effect of SPS on
373 denitrification.

374 **3.3 Mechanisms about the influence of SPS on CND in bed sediment-SPS-water system**

375 ***3.3.1 Influence of SPS concentration on bacterial abundances***

376 The SPS's nitrifying bacteria populations increased with SPS concentration also following a
377 power function ($R^2=0.93$, Fig. 8A), which was similar to the nitrification rate dependence on the SPS
378 concentration. Nitrification rates were positively correlated to nitrifying bacteria populations in SPS
379 ($p<0.01$, Fig. 8B). The nitrifying bacteria population in the bed sediment, however, was smaller than
380 that in the SPS and did not show an obvious relationship with nitrification rate (Fig. S7). This
381 implies that NH_4^+ -N loss in the system was mainly caused by nitrification occurring on SPS, not in
382 the bed-sediments.

383 For both SPS and bed-sediment, the denitrifying bacteria population increased with SPS
384 concentration as a power function (Fig. 8C). When SPS concentration was elevated from 15 to 20 g
385 L⁻¹, the denitrifying bacteria in SPS jumped from 1.6×10^4 to 2.1×10^6 gene copies while the
386 bed-sediment decreased from 4.9×10^5 to 3.0×10^4 gene copies. This might have been caused by
387 abrupt increase in oxygen-limited microsites at SPS phase due to cohesions of SPS particles and the
388 increased oxygen diffusion into the sediment caused by increasing agitation rate. Overall, when SPS
389 concentration increased from 0 to 20 g L⁻¹, the total denitrifying bacteria in system (bacteria in SPS
390 plus that in bed-sediment) increased with SPS concentration and was positively related to
391 denitrification rate ($p<0.05$, Fig. 8D). Therefore, it suggests that denitrification in systems was
392 related to denitrifying bacteria in not only SPS but also bed-sediment.

393 ***3.3.2 Influence of SPS concentration on denitrification in bed-sediment***

394 Some of the NO_3^- produced by nitrifying bacteria on SPS was subsequently reduced to N_2 in
395 oxygen-limited microsites of SPS and the remaining NO_3^- diffused into bed-sediment where it was
396 denitrified. Therefore, the NO_3^- produced by nitrification at SPS would exert influences on the
397 denitrification process occurring not only in SPS but also in bed-sediment phases. As shown in Fig.

398 **S8**, the average removal rate of NO_3^- (including denitrification and other process) increased linearly
399 with initial NO_3^- concentration ($p < 0.01$, Supplementary Section S4) in bed sediment-SPS-water
400 system, suggesting that denitrification rate was influenced by nitrate availability. According to the
401 results shown in **Fig. S9**, the average CND rates were 248, 634, 1515 $\mu\text{g m}^{-2} \text{d}^{-1}$ in systems
402 containing only 8 g L^{-1} SPS, containing bed-sediment but without SPS, containing both 8 g L^{-1} SPS
403 and bed-sediment, respectively. Based on our estimations (**Supplementary Section S5**), compared to
404 the system with bed-sediment covered by static water, roughly 28% of the increased N-loss in bed
405 sediment-SPS-water system was ascribed to N-loss at SPS and 72% to N-loss in bed-sediment
406 accelerated by elevated nitrate supply produced by overlying-water nitrification which was enhanced
407 by the presence of SPS. Because agitation, simulating the river flow and causing the presence of SPS,
408 would pose effects on the diffusion of nitrate from water to bed-sediment, the increased nitrate
409 supply in bed-sediment was also caused by the agitation conditions in addition to the presence of
410 SPS itself which accelerated the production of nitrate. Since SPS concentration also depends on
411 hydrodynamic conditions including river flow velocity in natural rivers (Xia et al., 2016), the results
412 obtained under different agitation rates in this study can reflect the effect of SPS concentration on
413 N-loss from natural rivers.

414 **3.4 The *in-situ* nitrifying and denitrifying bacteria abundances in the Yellow River**

415 Both the *in-situ* nitrifying and denitrifying bacteria populations in water (including aqueous and
416 SPS phases) in the Yellow River rose with SPS concentration following a power function (Fig. 9,
417 despite approaching linearity, a power function still fit these data better), which was in accordance
418 with the relationship between bacteria population and SPS concentration observed in the lab
419 experiments. This implies that the N-loss rate in natural rivers will increase with SPS concentration
420 as we have observed in the incubation experiments.

421 **3.5 Importance of SPS to N loss from river systems**

422 As the N-loss in the incubation systems might also be caused by anammox processes in addition
423 to denitrification, the N-loss caused by coupled nitrification-denitrification might be lower than the
424 emission of $^{15}\text{N}_2$ observed in the incubation experiments of the present research. However, our data

show that SPS enhances the nitrification and denitrification processes as well as N-loss from rivers. NH_4^+ and NO_3^- are the main inorganic nitrogen species in the Yellow River and Yangtze River, and they mainly come from nitrogenous fertilizer application and wastewater discharge (Xia et al., 2002; Li et al., 2010; Liu et al., 2013a), with a relatively low atmospheric deposition contribution. This is significantly different from the ocean where up to one third of the external nitrogen supply comes from the atmospheric anthropogenic fixed nitrogen (Duce et al., 2008). NH_4^+ and NO_3^- can be removed through CND, anammox, and denitrification processes in natural rivers. In the present research, although the initial NH_4^+ concentration was much higher while NO_3^- concentration was much lower than their concentrations in natural rivers, respectively, the results showed that the SPS can enhance CND as well as N-loss from rivers if N exists as NH_4^+ . In addition, our previous study showed that if N exists as NO_3^- , N loss through denitrification increases with the SPS concentration as well (Liu et al., 2013b). Therefore, according to the results of this study and our previous study, it is suggested that the total N-loss in the Yellow River and the Yangtze River will increase with the SPS concentration. However, more research should be carried out to study the effect of SPS on N-loss in natural river systems.

Based on the $^{15}\text{N}_2$ emission rate in the SPS-water system obtained in this study, the CND rate is estimated at $730 \text{ kg-N/km}^2\cdot\text{yr}$ ($\frac{200 \text{ mg/m}^2\cdot\text{d} \times 365\text{d} \times 200\text{cm}}{20\text{cm}} = 730 \text{ kg N/km}^2 \cdot \text{yr}$) for rivers with 1 g L^{-1} SPS and a depth of two meters. Previous study reported that denitrification occurring in river networks (including continental shelf, terrestrial, oceanic oxygen minimum zones, groundwater, rivers, lakes, and estuaries globally) has an average denitrification rate of 0 to $2173 \text{ kg-N/km}^2\cdot\text{yr}$ (Seitzinger et al., 2006). This is at comparable scales to the N-loss caused by CND occurring on SPS in the present study, suggesting that CND on SPS can not be neglected in river N removal.”

We defined the increased proportion of N-loss ($\Delta\%_{\text{Nloss}}$) caused by SPS as follows:

$$\Delta\%_{\text{Nloss}} = \frac{N_{\text{loss}} \text{ in BS-SPS-WS containing SPS} - N_{\text{loss}} \text{ in BS-WS without SPS}}{N_{\text{loss}} \text{ in BS-WS without SPS}} \times 100 \quad (6)$$

If SPS concentration was assumed to be 1 g L^{-1} , the $\Delta\%_{\text{Nloss}}$ in the samples collected from the HYK, LM, AS, WZ, and 37-Dock location were 25%, 53%, 53%, 80%, and 122%, respectively; they were positively related to the TOC of SPS (Fig. S10). The above results indicate that the accelerated effect

of SPS on N-loss was impacted by both concentration and properties of SPS. In addition, according to the above results, the increased proportion of N loss caused by SPS reached 100% when SPS concentration increased to 1 g L⁻¹ in the Yangtze River (TOC ≈ 0.64%). Approximately 46% of the rivers around the world has SPS concentration higher than 1 g L⁻¹ (Xia et al., 2016), and the TOC content in most of these rivers are higher than that in the Yangtze River (Table S4), thus the nitrogen loss in these rivers might increase at least by one time due to the presence of SPS.

The importance of water column processes becomes increasingly remarkable with the increase of stream size according to the river continuum concept (Vannote et al., 1980), and Reisinger et al. (2015) also reported that the water column nutrient uptake including assimilation and dissimilatory transformation increase with stream size. Therefore, for the large rivers of the world, where the interaction between the water column and the bed sediment is limited, coupled nitrification-denitrification and other nitrogen transformation processes in the overlying water containing SPS will act as a crucial role in nitrogen loss in river systems. Traditional frameworks of global N cycle have not considered coupled nitrification-denitrification and other processes occurring on SPS, leading to an underestimation of N-loss from rivers, especially for the large rivers. The observation of the present research is consistent with recent research that the estimated global N budget is out of balance, with inputs exceeding losses (Galloway et al., 2004; Schlesinger, 2009). Therefore, the enhanced effect of SPS on N-loss from rivers need to be factored into the new generation model of N cycle. Because spatio-temporal and vertical variations in SPS concentration and composition exist for each river (Bouchez et al., 2011a, 2011b; Xia et al., 2016), more research should be conducted to estimate the contribution of water column containing SPS to N-loss in rivers by considering the SPS concentration and composition as well as other environmental conditions and climatic factors including temperature. In addition, the linkage between SPS and emission of N₂O, an important greenhouse gas produced by nitrification/denitrification, should be examined to determine the role of SPS in nitrogen cycle under the context of climate change.

4 Conclusion

Taking the Yellow and Yangtze Rivers as examples, this study demonstrates that SPS, a major component of rivers, is a hot spot for both nitrification and denitrification through simulation

experiments and *in-situ* investigation. CND could occur on SPS in oxic waters because of the presence of oxic and anoxic/low oxygen microsites around SPS; the nitrogen loss rate (R_{N-loss}) increased with SPS concentration ($[SPS]$) as a power function ($R_{N-loss} = a*[SPS]^b$). The influencing mechanisms of SPS on CND rate have been analyzed; the nitrifying and denitrifying bacteria population was elevated with SPS concentration conforming to a power function for both the lab simulation experiments and *in-situ* investigation of the Yellow River. This study suggests that 1 g L⁻¹ SPS will lead to an enhancement in nitrogen loss by approximately 25~120% in the Yellow and Yangtze Rivers, and the enhancement increases with organic carbon content (TOC) of SPS. Thus N-loss from rivers in previous studies might be underestimated due to non consideration of the presence of SPS; this may partially compensate for the current disequilibrium between N inputs and N sinks. The future N research regarding SPS needs to aim at estimating the role of SPS more accurately by considering other nitrogen transformation processes, and the SPS concentration and composition as well as other environmental conditions.

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Table 1 Summary of sediment characteristics at the five sampling sites of
the Yellow River and the Yangtze River

Site name	River	Particle Size (%)				TOC	Total organic nitrogen
		< 20μm	20-50 μm	50-100 μm	> 100μm	(%)	(%)
Huayuankou	the Yellow River	2.9	22.8	37.5	19.9	0.167	0.02
Longmen	the Yellow River	4.0	24.9	50.8	15.8	0.186	0.01
Aishan	the Yellow River	23.2	46.4	25.1	4.3	0.215	0.02
37-Dock	the Yangtze River	23.0	18.6	35.9	16.0	0.599	0.05
Wanzhou	the Yangtze River	55.7	17.4	12.7	6.1	0.668	0.06

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Table 2 Incubation experiment conditions

Research purpose	System composition	$^{15}\text{NH}_4^+\text{-N}$ (mg L^{-1})	$\text{NO}_3^-\text{-N}$ (mg L^{-1})	SPS concentration (g L^{-1})	Particle Size of SPS (μm)
Occurrence of CND at SPS	SPS + water (samples from site AS)	5	0	0	Homogenized sediment
				1	
				2.5	
				8	
				15	
				20	< 20 20~50 50~100 >100
Effect of SPS concentration on CND in BS-SPS-WS	bed-sediment + SPS +water (samples from sites HYK, LM, AS, 37-Dock, and WZ)	5	0	0	homogenized sediment
				1	
				2.5	
				8	
				15	
Effect of initial nitrate concentration on denitrification in BS-SPS-WS	bed-sediment + SPS + water (samples from site HYK)	0	1	8	homogenized sediment
			3		
			4.5		
			6		
			10		
CND in BS-WS	bed-sediment + water (samples from site AS)	5	0	0	homogenized sediment

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List of Figure Captions

Figure 1 The sampling sites of the Yellow River and the Yangtze River. Red triangles mark sampling sites for simulation experiments (Y8: Longmen =LM, Y11: Huayuankou = HYK, Y14: Aishan = AS; C1: Wanzhou = WZ, C2: 37-Dock), and black circles represent locations for investigation of *in-situ* bacteria abundances (Y1: Lanzhou, Y2: Xiaheyan, Y3: Qingtongxia, Y4: Shizuishan, Y5: Sanhuhekou, Y6: Toudaoguai, Y7: Wubao, Y9: Tongguan, Y10: Sanmenxia, Y12: Kaifeng, Y13: Gaocun, Y15: Luokou, Y16: Lijin, Y17: Kenli).

Figure 2 Influence of suspended sediment concentration on $^{15}\text{N}_2$ emission (A), population of nitrifying and denitrifying bacteria (B) in suspended sediment-water system at the end of incubation (26 days). Data represent the average of three replicates $\pm$ SD; the a, b, c and a', b', c' in (B) mean the significant differences among the 6 groups based on Duncan's multiple range test, respectively.

Figure 3 Net O_2 flux (A) and O_2 gradient (B) around suspended sediment particles in relation to the distance from near the surface of SPS particle. Note: J indicates the flux ($\text{pmol cm}^{-2} \text{s}^{-1}$); negative values of net O_2 flux in (A) indicate net influxes; negative values of distance in (B) indicate the distance away from particle surface in bulk solution, and positive values of distance in (B) indicate the distance away from particle surface within particle. Oxygen levels of 53% and 70% air saturation represented the levels where facultative bacteria, which were identified as *Alcaligenes faecalis* and *Paracoccus denitrificans* on the SPS in the Yellow River, can conduct denitrification (Liu et al., 2013).

Figure 4 Effect of suspended sediment concentration on the $^{15}\text{N}_2$ production from $^{15}\text{NH}_4^+\text{-N}$ in bed sediment-suspended sediment-water system (BS-SPS-WS) for samples collected from site HYK of the Yellow River. Data represent the average of three replicates, and the standard deviation is less than 10%. Note: The effect of SPS concentration on $^{15}\text{N}_2$ production for samples from site AS, WZ, LM, 37-Dock is shown in Figure S4.

Figure 5 Power functional relationship between suspended sediment concentration and production rate of $^{15}\text{N}_2$ from $^{15}\text{NH}_4^+\text{-N}$ in bed sediment-suspended sediment-water

system (BS-SPS-WS) for samples collected from different sites of the Yellow River and the Yangtze River, respectively. Data represent the average of three replicates $\pm$ SD.

Figure 6 Variations of NH_4^+ , NO_3^- , NO_2^- , and N_2 in bed sediment-suspended sediment-water system (BS-SPS-WS) containing different concentrations of suspended sediment for samples collected from site HYK of the Yellow River. Data represent the average of three replicates, and the standard deviation is less than 10%.

Figure 7 Variation of NH_4^+ -N concentration with time and its fitting with the Logistic model (A), and the relation of nitrification rate constants with suspended sediment concentration (B) in bed sediment-suspended sediment-water system (BS-SPS-WS) for samples collected from site HYK. Data represent the average of three replicates $\pm$ SD.

Figure 8 Relationship between SPS concentration and nitrifying bacteria population (A), between nitrification rate constant and nitrifying bacteria population (B), between suspended sediment concentration and denitrifying bacteria population (C), and between average denitrification rate and denitrifying bacteria population (D) in bed sediment-suspended sediment-water system (BS-SPS-WS) for samples collected from site HYK.

Figure 9 The *in-situ* nitrifying and denitrifying bacteria population in water samples (containing suspended sediment) collected from the Yellow River (15 sites)

Figure 1 The sampling sites of the Yellow River and the Yangtze River. Red triangles mark sampling sites for simulation experiments (Y8: Longmen =LM, Y11: Huayuankou = HYK, Y14: Aishan = AS; C1: Wanzhou = WZ, C2: 37-Dock), and black circles represent locations for investigation of *in-situ* bacteria abundances (Y1: Lanzhou, Y2: Xiaheyan, Y3: Qingtongxia, Y4: Shizuishan, Y5: Sanhuhekou, Y6: Toudaoguai, Y7: Wubao, Y9: Tongguan, Y10: Sanmenxia, Y12: Kaifeng, Y13: Gaocun, Y15: Luokou, Y16: Lijin, Y17: Kenli).

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744 Figure 6 Variations of NH₄⁺, NO₃⁻, NO₂⁻, and N₂ in bed sediment-suspended sediment-water system
 745 (BS-SPS-WS) containing different concentrations of suspended sediment for samples collected from
 746 site HYK of the Yellow River. Data represent the average of three replicates, and the standard
 747 deviation is less than 10%.

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