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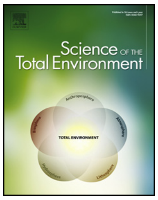

Enhanced nitrogen loss from rivers through coupled nitrification-denitrification caused by suspended sediment

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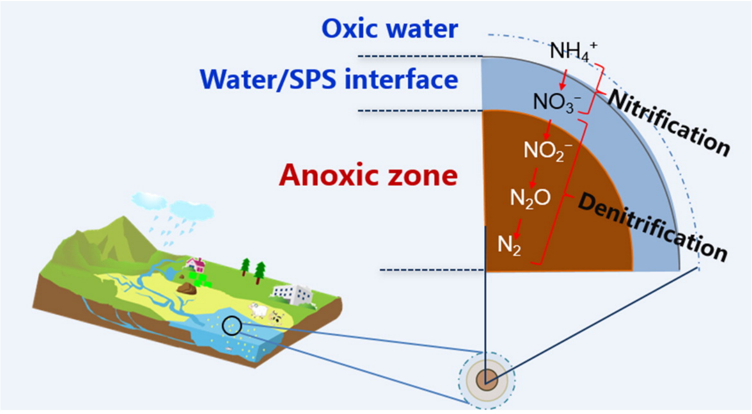
HIGHLIGHTS

- Coupled nitrification-denitrification can occur around SPS in oxic waters.
- $^{15}N_2$ production rate from $^{15}NH_4^+$ increased with SPS concentration as a power function.
- Nitrifying and denitrifying bacteria population increased with SPS as a power function.
- $1\ g\ L^{-1}$ SPS will lead to N-loss enhancement by approximately 25–120%.
- N-loss enhancement caused by SPS increased with organic carbon content of SPS.

GRAPHICAL ABSTRACT



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ABSTRACT

Present-day estimations of global nitrogen loss (N-loss) are underestimated. Commonly, N-loss from rivers is thought to be caused by denitrification only in bed-sediments. However, coupled nitrification-denitrification occurring in overlying water with suspended sediments (SPS) where oxic and anoxic/low oxygen zones may coexist is ignored for N-loss in rivers. Here the Yellow and Yangtze Rivers were taken as examples to investigate the effect of SPS, which exists in many rivers of the world, on N loss through coupled nitrification-denitrification with nitrogen stable (^{15}N) isotopic tracer simulation experiments and *in-situ* investigation. The results showed even when SPS was surrounded by oxic waters, there were redox conditions that transitioned from an oxic surface layer to anoxic layer near the particle center, enabling coupled nitrification-denitrification to occur around SPS. The production rate of $^{15}N_2$ from $^{15}NH_4^+$-N ($R_{15N2\text{-production}}$) increased with increasing SPS concentration ($[SPS]$) as a power function ($R_{15N2\text{-production}} = a \cdot [SPS]^b$) for both the SPS-water and bed sediment-SPS-water systems. The power-functional increase of nitrifying and denitrifying bacteria population with [SPS] accounted for the enhanced coupled nitrification-denitrification rate in overlying water. SPS also accelerated denitrification in bed-sediment due to increased NO_3^- concentration caused by SPS-mediated nitrification. For these two rivers, $1\ g\ L^{-1}$ SPS will lead to N-loss enhancement by approximately 25–120%, and the enhancement increased with

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organic carbon content of SPS. Thus, we conclude that SPS in overlying water is a hot spot for nitrogen loss in river systems and current estimates of in-stream N-loss are underestimated without consideration of SPS; this may partially compensate for the current imbalance of global nitrogen inputs and sinks.

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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, 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, 2009, 2013). 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 $^{15}NO_3^-$-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 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} $^{15}NH_4^+$-N as $^{15}NH_4^+Cl$ (99.0 atm% ^{15}N, 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

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