Supporting information

Relative contributions of denitrification and anammox to nitrogen removal in riverine wetlands across China

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Determination of nitrogen removal potential

The denitrification and anammox rates of channel sediments, riparian rhizosphere soils, and depth-selected riparian bulk soils (0–20, 60–80, and 160–180 cm in depth) were quantified using the ^{15}N isotope pairing method [1,2]. Briefly, three 5 g replicates of homogenized sediment or soil samples were weighed into 12-mL cylindrical glass vials containing helium-purged distilled water [3]. Subsequently, all vials were pre-incubated in a horizontal shaker (180 rpm) at 24°C for 3 days to remove the background NO_3^- and NO_2^-, as well as residual oxygen [4]. All vials were then divided into three groups and injected with 100 μL helium-purged stock solution: $^{15}NH_4Cl$ (98 at.% ^{15}N), $^{15}NH_4Cl + K^{14}NO_3$, or $^{14}NH_4Cl + K^{15}NO_3$ (99.99

at.% ^{15}N). This resulted in a final total N concentration of 100 μmol in each vial [5]. Additions of $^{15}NH_4^+$ solutions were used to verify that background NO_3^--N and NO_2^--N have been consumed, while additions of $^{15}NH_4^+$-N + $^{14}NO_3^-$-N solutions were used to confirm the presence of anammox process. Additions of $^{14}NH_4^+$-N + $^{15}NO_3^-$-N solutions were used to determine denitrification and anammox rates. The incubation was terminated at five-time intervals (0, 1, 2, 4, and 8 h) by injecting 200 μL of 7 M $ZnCl_2$ solution. The isotopic abundance of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ in the glass vials were measured using isotope ratio mass spectrometry (Gasbench II and Delta V Advantage, Thermo Finnigan, Germany).

Analysis of abundance of denitrifying and anammox bacteria

DNA was extracted from replicate soil and sediment samples using a PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., CA, USA), and DNA concentrations were determined using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, MA, USA). The abundance (i.e., copy number) of *nirK*, *nirS*, and *hzsB* genes was measured in triplicate by using a Roche LightCycler480 real-time PCR system (Roche Diagnostics, Mannheim, Germany) with the fluorescent dye SYBR green qPCR method. The reduction of NO_2^--N to nitric oxide (NO) is the rate-limiting step of denitrification, which is catalyzed by either a copper-containing enzyme encoded by the *nirK* gene or a cytochrome-cd1 enzyme encoded by the *nirS* gene[6]. The *hzsB* gene encoded for hydrazine synthase β-subunit which catalyzes the production of hydrazine from NH_4^+-N and NO_2^--N during the anammox process [4]. Detailed procedures of qPCR were described previously[7]. In brief, the abundance (i.e., copy number) of *nirS*, *nirK*, and *hzsB* genes was determined in triplicate using a Roche LightCycler480 real-time PCR System (version 1.5.0; Roche Diagnostics, Mannheim, Germany) with the fluorescent dye SYBR green quantitative PCR method. Primer sets of nirSCd3aF/nirSR3cdR, nirKF1aCu/nirKR3Cu, and hzsB396F/hzsB742R were applied for the *nirS*, *nirK*, and *hzsB* genes, respectively (Table S2). The 20-μL quantitative PCR mixture contained 10 μL of SyberGreen qPCR Master Mix (2×), 1 μL of primers (10 μM), and 2 μL of DNA template. The primers and qPCR protocol are listed in Supplementary Table S2. Standard curves were constructed with serial plasmid dilutions of a known amount of plasmid DNA containing a fragment of the *nirS*, *nirK*, and *hzsB* genes.

Measurements of soil, sediment, and water physicochemical properties

Soil pH was measured at a soil-to-water ratio of 1:5 (v/v) using a pH meter (Hanna Instruments, Padova, Italy), whereas soil moisture content was determined gravimetrically by drying 20 g of fresh soil at 105℃

for 24 hours. T The soil TN, TC, and TOC concentrations of air-dried soil samples were determined by an elemental analyzer. (Vario TOC cube, Hanau, Germany). Soil or sediment physicochemical properties, including moisture, pH, total N (STN), NH_4^+-N, NO_3^--N, total carbon (STC) and organic carbon (SOC) were determined following published analytical methods [8,9]. Soil Fe^{2+} and AFe concentrations were measured using the phenanthroline spectrophotometric method [10]. The concentrations of water TN, NH_4^+-N, NO_3^--N, TC, and TOC were measured in the laboratory, as described previously[11]. The concentrations of NH_4^+ and NO_3^- in water were analyzed by a continuous flow analyzer (EasyChem plus, Systea, Italy). The water TN, TC, and TOC concentrations of samples were determined by a Total Organic Carbon Analyzer Analyzer (Liquid). (Vario TOC cube, Hanau, Germany).

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