

# Mechanisms of Nitrate Concentration Effects on N<sub>2</sub>O and N<sub>2</sub> Fluxes Across Different River Widths

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## Abstract

This study systematically investigated the mechanisms by which nitrate concentrations influence N<sub>2</sub>O and N<sub>2</sub> fluxes in rivers of varying widths (20–800 m) within the Haihe River Basin, Luanhe River Basin, and independent rivers flowing into the sea in the Beijing-Tianjin-Hebei region. Through a combination of field sampling and laboratory analysis, nitrate concentrations and gas fluxes at the sediment-water interface were measured, while qPCR technology was employed to analyze the abundance of nitrogen-functioning microbial genes. Results indicate that nitrate concentration significantly influences N<sub>2</sub>O and N<sub>2</sub> fluxes by providing denitrification substrates and regulating microbial community structure. River width further modulates nitrate influence intensity and pathways by altering water dilution capacity and microbial habitat. Narrow rivers exhibit heightened sensitivity to nitrate, yielding higher gas fluxes, whereas wide rivers promote nitrate conversion to N<sub>2</sub> due to dilution effects and stable environments. This study provides microbial mechanism-level insights into understanding river nitrogen cycling and its environmental effects.

## Keywords

Nitrate Concentration; River Width; N<sub>2</sub>O Flux; N<sub>2</sub> Flux; Denitrifying Microorganisms; Nitrogen Cycle; Benthic Niche; qPCR.

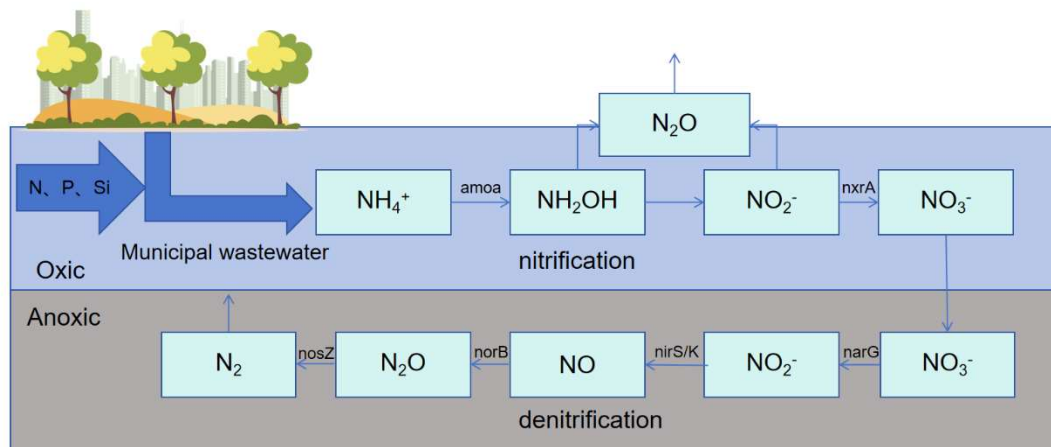
## 1. Introduction

Nitrogen is a vital biogenic element in aquatic ecosystems, with its transformation processes directly impacting water quality and greenhouse gas emissions. As a common nitrogen form in water bodies, nitrate can be converted into N<sub>2</sub>O and N<sub>2</sub> through microbially driven nitrification and denitrification. Among these, N<sub>2</sub>O, a potent greenhouse gas, significantly influences climate change through its emissions. While preliminary studies exist on nitrate concentration effects on riverine N<sub>2</sub>O and N<sub>2</sub> fluxes, most focus on single rivers or small-scale systems, lacking systematic comparisons of nitrate pathways across rivers of varying widths.

River width, as a key geomorphological feature, influences physical mixing, nutrient dilution capacity, and microbial habitats, potentially modulating nitrate transformation efficiency and gas release ratios. However, the underlying microbial mechanisms governing how nitrate concentration and river width synergistically regulate N<sub>2</sub>O and N<sub>2</sub> fluxes remain unclear.

Therefore, this study systematically conducted nitrate concentration measurements, gas flux monitoring, and functional microbial gene analysis in rivers of varying widths (20–800 m) within the Beijing-Tianjin-Hebei region. The objectives were to reveal: (1) the direct impact of nitrate concentration on N<sub>2</sub>O and N<sub>2</sub> fluxes; (2) the role of river width in modulating nitrate effects; (3) the response mechanisms of key nitrogen-functioning microorganisms (e.g., denitrifying bacteria and ammonium-oxidizing bacteria) in gas production and reduction

processes. These findings contribute to a deeper understanding of riverine nitrogen cycling and provide scientific basis for managing nitrogen pollution and greenhouse gas emissions in regional water bodies.



**Figure 1.** Schematic Diagram of Nitrogen Cycling in Rivers

## 2. Materials and Methods

### 2.1. Study Area and Sampling Design

Select rivers flowing through the Haihe River system in the Beijing-Tianjin-Hebei region (such as the North Canal, Yongding River, Daqing River, Ziya River, and South Canal), the Luan River system, and rivers flowing directly into the sea (e.g., Dou River) as the study areas, covering rivers of varying widths (20 to 800 meters). Multiple sampling points were established within each river system to ensure coverage of rivers with different widths, hydrological conditions, and land use types.

### 2.2. Nitrate, Gas Flux, and Concentration Determination

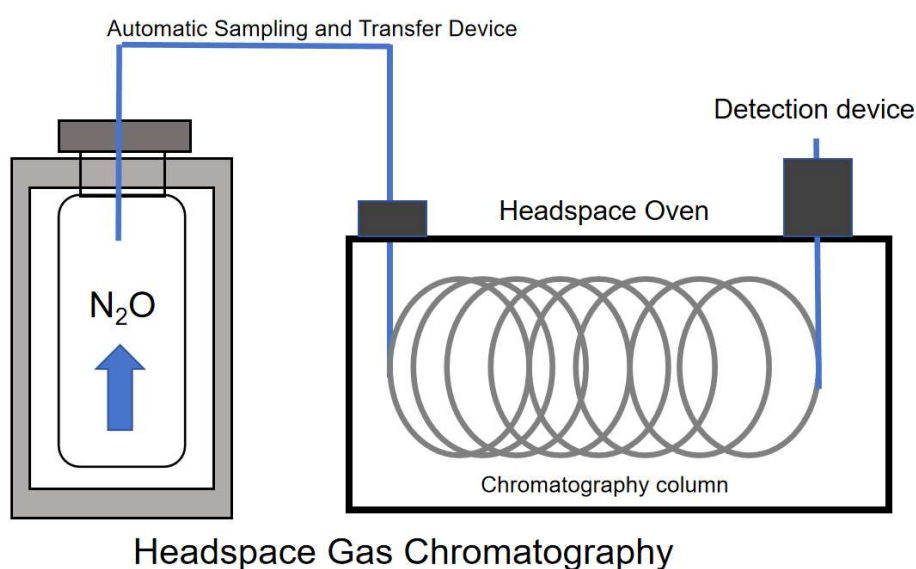
Nitrate concentration data were obtained through a two-tier process of “field rapid screening followed by laboratory precision determination” to ensure data reliability. During the field sampling phase, sampling points were established at 20–800 m river width gradients along the Haihe River system, Luanhe River system, and independent rivers flowing into the sea (e.g., Douhe River) within the Beijing-Tianjin-Hebei region. Surface water samples were collected using clean plastic bottles, with simultaneous recording of river width, water temperature, and flow velocity (using the simple float method) at each sampling point. Nitrate test strips were used for preliminary concentration estimation, enabling rapid identification of high-concentration (deep blue strips) and low-concentration (light blue strips) areas. This prioritized laboratory analysis targets, enhancing research efficiency.

During the laboratory analysis phase, precise nitrate concentration determination was performed using the cadmium reduction spectrophotometric method, following this procedure:

- ① Water sample pretreatment: Filter water samples through a 0.45 $\mu$ m membrane filter to remove suspended particulate matter interference;
- ② Cadmium column reduction: Pass the filtered water sample through a cadmium column to reduce nitrate to nitrite;
- ③ Colorimetric reaction: Add sulfanilamide and N-(1-naphthyl)-ethylenediamine hydrochloride reagent to react with nitrite, forming azo compounds;
- ④ Photometric measurement: Measure absorbance at 540 nm using a spectrophotometer;
- ⑤ Concentration calculation: Determine actual nitrate concentration by substituting the water sample's absorbance value into the standard curve

(absorbance-concentration relationship) plotted using potassium nitrate standard solution [1-3].

To assess  $N_2O$  and  $N_2$  fluxes at the sediment-water interface, an open-bottomed sediment chamber method was employed. At each site, three cylindrical sediment chambers (inner diameter 30 cm, inner height 20 cm) made of transparent acrylic plastic were gently deployed onto the sediment. Each chamber had an area of  $700\text{ cm}^2$ , a total volume of approximately 14 liters, and a 5 cm lip for sediment insertion. Sealing integrity was verified using the fluorescent dye Rhodamine WT (RWT), with no significant dye leakage observed during testing. Each chamber was equipped with three Teflon membranes. Ports: Two ports facilitated water circulation and mixing via a miniature pump (WKY1000, China), while one port enabled water sampling using a separate miniature pump. Prior to sampling, chambers were filled with water to displace air and remained open on the sediment for 15–30 minutes to minimize disturbance from insertion. Water samples were collected hourly from the chambers using the designated miniature pump during the 6-hour incubation period. After each sampling, an equal volume of surface water is added back to the chamber to maintain a constant volume.  $N_2O$  and  $N_2$  concentrations are determined in the samples using the same procedure as for surface water samples. Additionally, samples of ammonium-oxidizing bacteria and denitrifying bacteria from the sediment were collected for subsequent DNA extraction and microbial community analysis. Dissolved  $N_2O$  concentrations were measured in situ using headspace equilibration. 10 milliliters of ultra-high-purity helium gas was injected into glass vials alongside an equal volume of water. The vials were vigorously shaken for 10 minutes to ensure gas equilibrium under ambient conditions [4]. Equilibrated headspace gas was extracted from each vial using a sealed syringe and transferred into pre-evacuated 30-milliliter aluminum foil gas canisters. In the laboratory, gas samples were analyzed using a gas chromatograph equipped with an electron capture detector. Chromatographic peak areas corresponding to different concentration standard solutions were recorded. Standard curves were plotted based on the chromatographic peak areas and concentrations of  $N_2O$  and  $N_2$  in the samples. The corresponding concentration values were then determined from the curve to obtain the concentrations of  $N_2O$  and  $N_2$ .



**Figure 2.** Schematic Diagram of Headspace Gas Chromatography Analysis

### 2.3. Data Analysis

qPCR technology was employed to determine the gene abundance of nitrogen-functioning microorganisms in sediments from each sampling site, including denitrifying bacteria (nirS and nirK genes), ammonium-oxidizing bacteria (amoA gene), and N<sub>2</sub>O-reducing microorganisms (nosZ gene). Linear regression models were used to analyze the relationship between nitrate concentration and nitrogen-functioning microbial abundance[5]: The significant influence of nitrate concentration on sediment N<sub>2</sub>O and N<sub>2</sub> fluxes was attributed to the finding that higher nitrate concentrations correlated with increased denitrifying bacterial abundance (nirS+nirK genes) ( $p < 0.05$ ). Denitrifying bacterial abundance showed a negative correlation with temperature ( $r = -0.3$ ,  $p < 0.05$ ), primarily involving denitrifying bacteria (nirS, nirK genes), ammonia-oxidizing bacteria (amoA gene), and N<sub>2</sub>O-reducing microorganisms (nosZ gene). This confirms the pattern: “Low nitrate concentrations dominate nitrification, while high concentrations promote denitrifying bacterial proliferation but inhibit N<sub>2</sub>O reductase activity.” In rivers, nitrate is progressively reduced to N<sub>2</sub>O and N<sub>2</sub> through microbially driven denitrification, serving as the primary source of both gases. Nitrate concentration directly influences denitrification intensity, thereby altering the magnitude and ratio of N<sub>2</sub>O and N<sub>2</sub> fluxes[6-7].

### 3. Conclusion

Results indicate that nitrate concentration influences N<sub>2</sub>O and N<sub>2</sub> fluxes by directly supplying denitrification substrates and indirectly regulating the abundance of nitrogen-functioning microorganisms. River width modulates the intensity and pathways of nitrate effects by altering water dilution capacity and microbial community structure-- Nitrate influence is more pronounced, sensitivity higher, and critical thresholds lower in narrow rivers, with the opposite observed in wide rivers.

Wider rivers possess greater water volume, diluting nitrate concentrations. Their slower flow rates provide a stable environment for microbial denitrification, promoting efficient conversion of nitrate into gases like N<sub>2</sub> that escape. Narrower rivers, often tributaries, experience significant agricultural runoff, resulting in high nitrate inputs and more active denitrification, which in turn increases gas fluxes.

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