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Using ^{15}N, ^{17}O, and ^{18}O To Determine Nitrate Sources in the Yellow River, China

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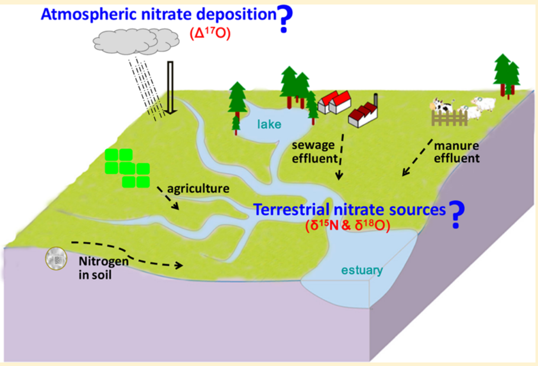
Supporting Information

ABSTRACT: Many previous studies have used δ^{15}N and δ^{18}O of nitrate ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) to determine the nitrate sources in rivers but were subject to substantial uncertainties and limitations, especially associated with evaluating the atmospheric contribution. The Δ^{17}O of nitrate ($\Delta^{17}O_{NO3}$) has been suggested as an unambiguous tracer of atmospheric NO_3^- and may serve as an additional nitrate source constraint. In the present study, triple nitrate isotopes ($\delta^{15}N_{NO3}$, $\Delta^{17}O_{NO3}$, and $\delta^{18}O_{NO3}$) were used for the first time to assess the sources and sinks of nitrate in the Yellow River (YR) basin, which is the second longest river in China. Results showed that the $\Delta^{17}O_{NO3}$ of the water from the YR ranged from 0‰ to 1.6‰ during two normal-water seasons. This suggested that unprocessed atmospheric nitrate accounted for 0–7% of the total nitrate in the YR. The corrected $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values with atmospheric imprints being removed indicated that the main terrestrial sources of nitrate were sewage/manure effluents in the upstream of the YR and manure/sewage effluents and ammonium/urea-containing fertilizer in the middle and lower reaches which made comparable contributions to the nitrate. In addition, there was a significant positive relationship between $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values of river water ($p < 0.01$) which may signal the presence of denitrification. This study indicates that the triple nitrate isotope method is useful for assessing the nitrate sources in rivers, especially for the measurements of atmospheric nitrate contribution.



■ INTRODUCTION

Annual fixed nitrogen (N) production by humans is currently more than double the natural fixation rate,[1,2] and its release into the environment is associated with a range of negative impacts. Human N fixation is mainly due to the synthetic fertilizer production for agriculture, but a significant fraction is from incidental generation of NO_x ($NO+NO_2$) during fossil fuel combustion that can be emitted to the atmosphere, converted to nitric acid, and subsequently deposited to the ground.[2,3] A fraction of the anthropogenic N finds its way into water bodies, where excess N can lead to eutrophication and hypoxia, destroying habitats for resident organisms and enhancing their susceptibility to disease, which often results in the deaths of fish and vertebrates, reduction in species diversity, and overall degradation of ecosystem services.[4,5] Furthermore, high concentrations of nitrogen threaten drinking water supplies because of its potential toxicity to humans and livestock, as well as the taste/odor and toxicity of compounds produced by cyanobacteria, algae, and other plants whose growth is enhanced by N fertilization.[6−8] Therefore, understanding the sources of excess N in aquatic ecosystems, including delineating the relative amount of N from fertilizer or the atmosphere, is important from economic, societal, and ecosystem perspectives and for developing effective mitigation strategies. This is particularly relevant because if unabated by policy changes, fertilizer N and NO_x production rates are expected to double every two decades.[9]

However, it is usually unclear what the sources of fixed N are in a given river. River systems, serving as the linkage between nitrate sources and sinks, play an important role in nitrogen transportation and transformation.[8] For many rivers, one of the major species of fixed N is NO_3^--N whose source mainly includes atmospheric deposition, sewage NO_3^- discharge, and NO_3^- fertilizer application.[2,10,11] However, there are few reports about the relative contribution of these sources to riverine NO_3^- budget.

Stable isotope technique has been proved useful for discriminating sources and sinks of nitrate in various ecosystems. A dual isotope method with simultaneous analysis of δ^{15}N and δ^{18}O values of nitrate ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) has been widely used to determine the nitrate sources and constrain the nitrogen cycling in aquatic environments.[12−17] It is based on the distinct isotopic characteristics of nitrate from different

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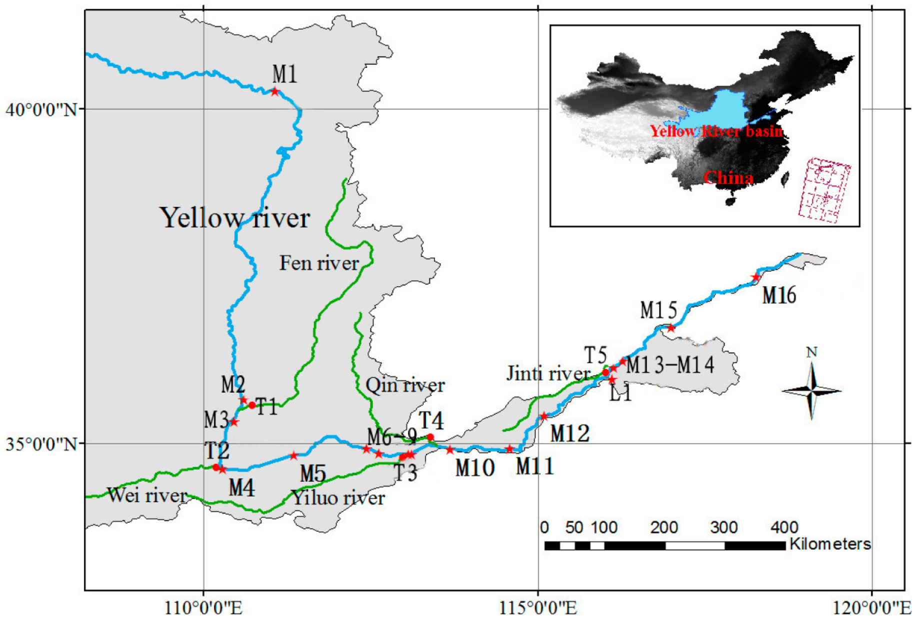

Figure 1. The Yellow River and locations of sampling sites.

sources which can potentially be preserved through the nitrogen cycle. For example, $\delta^{15}N_{NO3}$ values range from −15‰ to +15‰ for atmospheric nitrate, from −4‰ to +4‰ for synthetic fertilizer, from +2‰ to +5‰ for soil organic nitrogen, and from +5‰ to +25‰ for manure/sewage effluent.[7,10,16,18] Likewise, $\delta^{18}O_{NO3}$ values range from +25‰ to +80‰ in atmospheric nitrate, from +18‰ to +22‰ for nitrate-containing fertilizer, and from −10‰ to +10‰ for nitrate produced by microbial nitrification.[8,18,19] Furthermore, $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ can also provide clues about denitrification (or assimilation) which causes the enrichment of $\delta^{15}N$ and $\delta^{18}O$ of the remaining nitrate in roughly a 1:1−2:1 (or 1:1) ratio through kinetic isotopes effects.[15,18,20−22] Therefore, the combination of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ can provide good constraints for the sources and transformation processes of nitrate in water samples. However, due to the presence of the wide ranges of observed $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ from atmospheric deposition and other sources, which were further expanded by the enrichment process of denitrification, it is difficult to accurately quantify different sources using only the dual isotope method.

Recent oxygen isotope studies have shown that atmospheric nitrate is labeled with an anomalous ^{17}O enrichment, which is radically different from terrestrial nitrate and can be used to distinguish atmospheric contribution.[23−28] The majority of terrestrial biogeochemical processes such as nitrification and denitrification follow normal kinetic or equilibrium isotope effects involving oxygen to produce oxygen isotope enrichments (depletions) depending on the relative isotopic mass differences that is termed mass-dependent fractionation ($\delta^{17}O \sim 0.52\cdot\delta^{18}O$). In contrast, photochemical production of nitrate in the atmosphere can result in an anomalous ^{17}O excess and a deviation from mass-dependent fractionation that can be quantified by $\Delta^{17}O = \delta^{17}O - 0.52\cdot\delta^{18}O$, where $\delta = (R_{sample}/R_{standard}-1)*1000$ and R is the $^{17}O/^{16}O$ or $^{18}O/^{16}O$ ratio of the sample and the standard.[2] Therefore, $\Delta^{17}O$ of nitrate ($\Delta^{17}O_{NO3}$) can be used as an unambiguous tracer of atmospheric NO_3^- deposition.[2,11,19,29] The $\Delta^{17}O_{NO3}$, in combination with $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$, can then provide more accurate assessment of sources and sinks of nitrate in waters, and this has been used in groundwater systems.[19] However, the triple nitrate isotope approach, to our knowledge, has been rarely applied in surface waters, especially for large rivers, in which nitrogen transportation and transformation are complicated and nitrate sources are difficult to be traced.

The Yellow River (YR), the second longest river in China, is a major source of N to the Bohai Bay; one of the dominant forms of N in the YR is NO_3^--N,[30] which has been suspected to account for the eutrophication in the Bohai Bay of China.[31] However, in the YR, where nitrate concentrations ($[NO_3^-]$) have been increasing since 1980,[30] it is unclear which NO_3^- sources are controlling the YR's NO_3^- budget. Furthermore, there have been few isotopic studies of NO_3^- in the YR to identify the sources of nitrate.

In the present study, a triple isotope mixing model ($\delta^{15}N_{NO3}$, $\Delta^{17}O_{NO3}$, $\delta^{18}O_{NO3}$) was established to analyze the relative importance of different nitrate sources in rivers. The triple isotopic compositions of nitrate ($\delta^{15}N$, $\Delta^{17}O$, $\delta^{18}O$) in the water samples of the YR were determined for the first time, and the relative importance of each source including atmospheric deposition, fertilizer, and sewage/manure was estimated using the model. Additionally, variations of nitrate sources along the main-channel of the YR and the tributaries were analyzed, and the sources of nitrate delivered by the YR to the Bohai Bay were estimated as well.

■ EXPERIMENTAL SECTION

Study Site. The YR originates from the Bayan Har Mountains and flows into the Bohai Bay, draining a basin area of 7.5×10^5 km^2 (Figure 1). The landscape for the whole basin of the YR consists of 26.6% agricultural land, 13.0% woodland, 47.9% grassland, and 8.7% unused land, and the land use varies greatly between different stream segments.[32] The upper reach from the river source to Hekou Town of Inner Mongolia mainly supports animal production with an area of 202,793 km^2 for grassland (52.5%, mainly for livestock farming), 30,013 km^2 for agricultural land (7.8%), and 22,826 km^2 for woodland (5.9%). In contrast, the middle and lower reaches of the Yellow River (MLRs-YR) from Hekou Town to the river's mouth have land use areas of 88,240 km^2 for agricultural land (24.0%), 77,580 km^2 for woodland (21.1%), and 63,560 km^2 for grassland (17.3%). The average rate of nitrogenous fertilizer application in China is approximately 1.9×10^4 kg km^{-2} yr^{-1}.[9] The nitrogenous fertilizer are mainly in the forms of ammonium and urea, which accounts for approximately 98%.[33] It is suggested that ~7% of ammonium/urea-containing fertilizer is lost through nitrification followed by leaching or runoff.[34] Consequently, there is an estimated 2.6×10^8 kg ($=1.9 \times 10^4$ kg-N km^{-2} yr^{-1} * 98% * 7.5×10^5 km^2 * 26.6% * 7%) of nitrate from nitrification of ammonium/urea-containing fertilizer discharged into the YR annually. Lü and Tian[35] estimated the amount of nitrogen deposited from the atmosphere in the YR to be ~2,000 kg-N km^{-2} yr^{-1}, indicating that atmospheric nitrogen inputs is approximately 1.5×10^9 kg annually (=2,000 kg-N km^{-2} yr^{-1} * 7.5×10^5 km^2). Assuming a similar efficiency (7%) of atmosphere-sourced N in the YR basin ending up in the river, the atmospheric contribution to riverine nitrate would be about 1.1×10^8 kg, which was comparable to the contribution of nitrogenous fertilizer. However, the two values might be overestimates of nitrogen loads because of ignoring denitrification within the river. Additionally, on the one hand, the atmospheric N might be taken up by plants within the watershed leading to a minor contribution of atmospheric nitrate to river water; on the other hand, N saturation might have been reached due to long-term N deposition in the watershed,[36] resulting in a major contribution of nitrate deposition to the N budget in the YR. To make these estimations clear, a more accurate quantification of the contribution of different nitrate sources (atmospheric deposition, sewage/manure, and agriculture) in the YR is necessary.

The annual average discharges at the major gauging stations in the MLRs-YR range from 517 to 910 m^3 s^{-1} (Supporting Information (SI), Table S1).[37] The discharge at Toudaoguai-Station (M1), the end point station of the upper reach of the Yellow River (UR-YR), is the lowest with 517 m^3 s^{-1}; and then with the input of waters from Fen River (FR, T1), Wei River (WR, T2), and other tributaries, the discharge at Tongguan-Station (M4) increases to 823 m^3 s^{-1}; the site at Huayuankou-Station (M10) receives waters from its upper reach and Yiluo River (YLR, T3) and has an annual average discharge of 910 m^3 s^{-1}; the site at Lijin-Station has a mean annual discharge of 584 m^3 s^{-1} due to small water input and large water demand for the economy development. The YR transports a large amount of nitrate annually. The annual average $[NO_3^-]$ was 196, 220, 214, 235, and 309 μmol L^{-1} at Toudaoguai-, Longmen-, Tongguan-, Huayuankou-, and Lijin-Station, respectively.[30] The estimated annual NO_3^--N load at the corresponding sites would be 4.5×10^7 kg for Toudaoguai-Station, 5.2×10^7 kg for Longmen-Station, 7.8×10^7 kg for Tongguan-Station, 9.4×10^7 kg for Huayuankou-Station, and 8.0×10^7 kg for Lijin-Station.

In addition, approximately 97.8 million people live within the YR basin, averaging 123 people km^{-2}. Population densities are highest in the Shandong province with ~636 people km^{-2}, and lowest in some regions of the upper reach with less than 1 people km^{-2}. The climate is temperate semiarid with annual average temperatures of 1−8 °C in the northwest and 12−14 °C in the southeast, and annual precipitation ranging from 300 mm in the northwest to 700 mm in the southeast.[32] As a result, it seems that nitrate sources in the MLRs-YR are more complex including being subject to more precipitation and anthropogenic sources. Therefore, we put emphasis on investigating the nitrate sources and sinks in the MLRs-YR. The major tributaries in the MLRs-YR are FR, WR, YLR, Qin River (QR, T4), Jindi River (JDR, T5), and Dawen River (DWR). The DWR flows into the Dongping Lake, which is the only natural lake in the MLRs-YR, and finally drains from the lake northward into the YR. The characteristics of these tributaries are summarized in SI Table S2.

Sampling and Sample Analysis. Two rounds of water samples were collected using a TC-Y sampler (TECH Instrument in Shenyang, China), which can open and close at any desired depth, from the main-channel and major tributaries in the MLRs-YR, as well as Dongping Lake (Figure 1) during 10/21/2011−10/31/2011 and 9/3/2013−9/22/2013. The two samplings were both conducted within the normal-water season of the YR which is referred to as the period with relatively moderate precipitation. During the sampling trip in October 2011, the mean monthly discharges at Toudaoguai-, Longmen-, Tongguan-, and Huayuankou-Station were 485, 560, 933, and 1195 m^3 s^{-1} respectively, which were comparable to the mean annual discharges shown in SI Table S1.[37] Water samples were filtered through 0.2-μm polyether sulfone membrane (Pall, U.S.A.) into polyethylene bottles. A portion of each water sample was used in the field to measure approximate $[NO_3^-]$ using DR/890 Portable Colorimeter (Hach Company, U.S.A.) and determine how much water to collect for isotopic analysis; then all the water samples (water volumes of 50−300 mL) were kept under 4 °C in a cooler, transported to the laboratory within 24 h, and kept frozen in a freezer for future analysis.

In the laboratory, the inorganic N concentrations were determined colorimetrically with an Autoanalyser-3 (Bran&-Luebbe, Germany) using the salicylate method for NH_4^+-N (ISO/DIS 11732), the hydrazine reduction method for NO_3^--N, and the sulfanilamide/N-1-naphthylethylene method for NO_2^--N (ISO/DIS 13395) (details shown in the SI). Isotopic analysis of nitrate in water samples was performed using a denitrifier method, by which nitrate was converted to N_2O or to N_2 and O_2 after a further gold tube thermal reduction of N_2O.[38,39] The $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values were determined by Delta V Plus isotope ratio mass spectrometry (IRMS) at the Purdue Stable Isotope facility that was normalized to multiple laboratory working standards that were previously calibrated to international standards USGS32, USGS34, and USGS35. The precisions for $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values were ±0.4‰, ±1.0‰, and ±0.3‰, respectively, based on replicate analysis of the working standards and calibrations.

Quantification of Nitrate Sources in Rivers. In China, the sewage/industrial wastewater discharge and nitrogenous fertilizer application rate increased by a factor of about 2.5 between 1980 and 2000;[9] during the same time period, an increasing discharge of atmospheric N has been matched by a doubling of NO_x emissions (estimated ~4.5×10^9 to 1.1×10^{10} kg), mainly as a byproduct of coal combustion.[35,40] Therefore, for many rivers in China, three sources are likely to play important roles in the nitrate budget: atmospheric deposition ($NO_3^-{}_{atm}$), nitrification of ammonium/urea-containing fertilizer ($NO_3^-{}_{fer}$), and nitrification of NH_4^+ from sewage/manure ($NO_3^-{}_{sew}$). If each source has a unique isotopic composition, when nitrate from these three sources is mixed together, the relative importance of each source can then be determined using an isotope mixing model as shown by the following formulas

Table 1. Isotopic Compositions of the Water Samples and Nitrate Sources in the Yellow River, China

sample	river, location	date	$\delta^{15}N_{NO3}$ (‰)	$\delta^{18}O_{NO3}$ (‰)	$\Delta^{17}O_{NO3}$ (‰)	$\delta^{18}O_{H2O}$ (‰)	NO_3^- (μmol L^{-1})
M1	Yellow River, Toudaoguai	10/21/2011	8.1	−1.2	0.9	-[a]	140
M2	Yellow River, Longmen	10/23/2011	7.3	−2.7	0.0	−9.1	244
M2′	Yellow River, Longmen	9/3/2013	12.3	−2.3	0.0	-	176
M3	Yellow River, Yuncheng	10/24/2011	−1.4	−2.9	0.7	−9.3	584
M4	Yellow River, Tongguan	10/24/2011	2.9	−2.1	0.0	−9.8	378
M4′	Yellow River, Tongguan	9/5/2013	12.1	−1.2	0.0	-	343
M5	Yellow River, Sanmenxia	10/25/2011	4.5	−2.7	0.0	−9.5	355
M6	Yellow River, Xiaolangdi	10/25/2011	3.4	−1.8	0.0	−9.6	319
M6′	Yellow River, Xiaolangdi	9/8/2013	12.8	0.1	0.0	-	232
M7	Yellow River, Luoyang	10/25/2011	3.9	−1.5	0.0	−9.7	308
M8	Yellow River, Jiaozuo	10/26/2011	4.5	−1.4	0.0	−9.7	308
M9	Yellow River, Gongyi	10/26/2011	7.2	0.2	0.9	−9.3	397
M10	Yellow River, Huayuankou	10/27/2011	5.0	−1.6	0.6	−9.5	324
M10′	Yellow River, Huayuankou	9/14/2013	12.5	0.1	0.3	-	285
M11	Yellow River, Xinxiang	10/27/2011	4.1	−1.7	0.9	−9.5	324
M12	Yellow River, Gaocun	10/27/2011	5.1	−1.6	0.0	−9.5	333
M13	Yellow River, ShanDong	10/28/2011	4.7	−1.6	0.0	−9.5	329
M14	Yellow River, Aishan	10/28/2011	4.8	−1.5	0.0	−9.4	327
M14′	Yellow River, Aishan	9/18/2013	12.8	0.7	0.0	-	224
M15	Yellow River, ShanDong	10/29/2011	4.5	−1.9	0.5	−9.4	325
M16	Yellow River, Lijin	10/30/2011	4.5	−1.3	1.6	−9.3	319
M16′	Yellow River, Lijin	9/22/2013	12.9	4.7	0.6	-	217
T1	Fen River, Shanxi	10/23/2011	−1.3	−3.3	0.7	−9.2	599
T1′	Fen River, Shanxi	9/3/2013	4.5	−9.0	0.0	-	462
T2	Wei River, Shaanxi	10/24/2011	0.9	−2.5	0.0	−9.9	411
T2′	Wei River, Shaanxi	9/6/2013	-	−17.0	0.0	-	212
T3	Yiluo River, Henan	10/25/2011	7.5	−0.1	0.0	−9.1	392
T3′	Yiluo River, Henan	9/12/2013	10.9	0.1	0.0	-	231
T4	Qin River, Henan	10/26/2011	10.3	0.7	1.2	−9.1	397
T5	Jindi River, Henan	10/28/2011	8.8	1.5	1.0	−7.8	69
L1	Dongping Lake, Taian	10/28/2011	15.0	7.5	0.8	−5.5	20
rainwater	Yellow River, Lijin	7/10/2013	-	46.3	23.6	-	9
NO_3^- from soil organic nitrogen[b]			+4 ± 4	−9−10	0.0	−25−4	
NO_3^- from sewage/manure effluent[b]			+15 ± 10	−9−10	0.0	−25−4	
NO_3^- from complete turnover of ammonium/urea-containing fertilizer[c]			−0.6 ± 1.8	−9−10	0.0	−25−4	
newly formed NO_3^- from nitrification of ammonium/urea-containing fertilizer[c]			−8 ± 4	-	0.0	-	

[a] Stands for not detected. [b] Data from Kendall et al.[11] and Xue et al.[53] [c] Data from Hübner[59] and Feigin et al.[54]

$$\delta^{15}N_{mix} = f_{atm}\delta^{15}N_{atm} + f_{fer}\delta^{15}N_{fer} + f_{sew}\delta^{15}N_{sew} + \varepsilon_1 \tag{1}$$

$$\delta^{18}O_{mix} = f_{atm}\delta^{18}O_{atm} + f_{fer}\delta^{18}O_{fer} + f_{sew}\delta^{18}O_{sew} + \varepsilon_2 \tag{2}$$

$$f_{atm} + f_{fer} + f_{sew} = 1 \tag{3}$$

where f_{atm}, f_{fer}, and f_{sew} are the mole fractions of nitrate from atmosphere, fertilizer, and sewage/manure, respectively; $\delta^{15}N_{mix}$ ($\delta^{18}O_{mix}$), $\delta^{15}N_{atm}$ ($\delta^{18}O_{atm}$), $\delta^{15}N_{fer}$ ($\delta^{18}O_{fer}$), and $\delta^{15}N_{sew}$ ($\delta^{18}O_{sew}$) are the $\delta^{15}N$ ($\delta^{18}O$) values of nitrate from river/lake water, atmosphere, fertilizer, and sewage/manure, respectively; ε_1 and ε_2 are the enrichment factors (theoretically $\varepsilon_1 \sim 2\varepsilon_2$) for nitrogen and oxygen during denitrification, respectively, and they were found to be much smaller in the YR (<0.029‰ day^{-1}, details in "Nitrate Removal by Denitrification" section) compared to $\delta^{15}N$ ($\delta^{18}O$) of samples and would thus be assumed as zero for simplicity to reduce uncertainties in the calculation using formula 1.

The atmospheric nitrate contribution could be determined by assessing any ^{17}O isotope anomaly in the riverine nitrate. Previous observations have shown that atmospheric nitrate $\Delta^{17}O$ values range from 20 to 40 ‰,[23,25,27,41] in contrast with $\Delta^{17}O$ of ~zero in terrestrial nitrate (including nitrate from sewage and fertilizer) as mentioned before. Therefore, we could determine the relative contribution of atmospheric deposition and terrestrial nitrate to the nitrate mixture based on a two-component mixing model as developed in formula 6 by Dejwakh et al.[19]

$$\Delta^{17}O_{mix} = f_{terr} \times \Delta^{17}O_{terr} + f_{atm} \times \Delta^{17}O_{atm} \tag{4}$$

where $\Delta^{17}O_{mix}$ is the $\Delta^{17}O$ values of nitrate in water samples; f_{terr} is the mole fraction of terrestrial nitrate, $f_{terr} = f_{fer} + f_{sew}$; $\Delta^{17}O_{terr}$ is the $\Delta^{17}O$ values of terrestrial nitrate, here $\Delta^{17}O_{NO3terr} \sim 0‰$; $\Delta^{17}O_{atm}$ is the $\Delta^{17}O$ values of atmospheric nitrate. There are seasonal and spatial oscillations in $\Delta^{17}O_{atm}$, which depends on the effects of atmospheric circulation, and atmospheric chemistry involving oxidized N species.[24,42] However, these seasonal variations in $\Delta^{17}O_{atm}$ tend to average

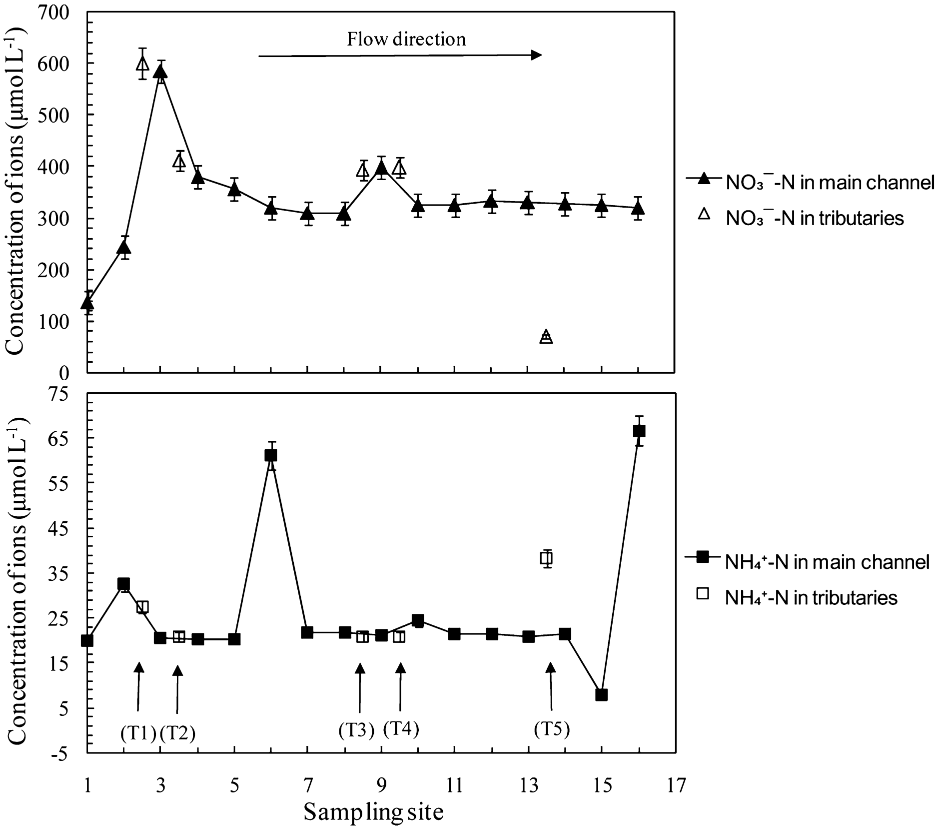

Figure 2. Variations of NO_3^--N and NH_4^+-N concentrations in the Yellow River samples collected in October, 2011. Data represent the mean values of three replicates ± SD.

out over long periods (e.g., years or decades). Because of the lack of atmospheric nitrate oxygen isotope measurements in different atmospheric environments, an average $\Delta^{17}O_{atm}$ value of 23‰ with a standard deviation of 3‰,[23,41] which is consistent with the measured $\Delta^{17}O_{NO3}$ of 23.6‰ for a rainwater sample in Lijin station during July of 2013, has hereby been used.

The f_{NO3atm} determined according to the $\Delta^{17}O_{mix}$ value and formula 4 could be used to transform the $\delta^{18}O$ and $\delta^{15}N$ values of nitrate in water sample based on the formulas as follows

$$\delta^{15}N_{NO3terr} = (\delta^{15}N_{NO3mix} - f_{NO3atm} \times \delta^{15}N_{NO3atm}) / f_{NO3terr} \quad (5)$$

$$\delta^{18}O_{NO3terr} = (\delta^{18}O_{NO3mix} - f_{NO3atm} \times \delta^{18}O_{NO3atm}) / f_{NO3terr} \quad (6)$$

where $\delta^{15}N_{NO3terr}$ and $\delta^{18}O_{NO3terr}$ are the corresponding isotopic values of the transformed terrestrial nitrate in water samples after the removal of atmospheric imprint; $\delta^{15}N_{NO3mix}$ and $\delta^{18}O_{NO3mix}$ are the isotopic values of nitrate in water samples; $\delta^{15}N_{NO3atm}$ is the $\delta^{15}N$ values of atmospheric nitrate, $\delta^{15}N_{NO3atm} \sim 0‰$;[11] $\delta^{18}O_{NO3atm}$ is the $\delta^{18}O$ values of atmospheric nitrate, $\delta^{18}O_{NO3atm} = (2.287 \times \Delta^{17}O_{NO3atm} - 10.1) \pm 10$ according to Dejwakh et al.[19]

Uncertainty Estimation. The uncertainty propagated from the water sampling and isotopic analysis in this study was estimated using the method reported by Phillips and Gregg[43] which assumes a simple direct mixing approach of two sources as shown in formulas 3 and 4. The variance of atmospheric nitrate fractions (f_{atm}) can be calculated using the following formula

$$\sigma^2_{f_{atm}} = \frac{1}{(\Delta^{17}O_{atm} - \Delta^{17}O_{terr})^2} \times [\sigma^2_{\Delta^{17}O_{mix}} + f^2_{atm}\sigma^2_{\Delta^{17}O_{atm}} + (1 - f_{atm})^2\sigma^2_{\Delta^{17}O_{terr}}] \quad (7)$$

where $\sigma^2_{\Delta^{17}O_{mix}}$, $\sigma^2_{\Delta^{17}O_{atm}}$, and $\sigma^2_{\Delta^{17}O_{terr}}$ represent variances of the mean $\Delta^{17}O$ signatures for the nitrate from water samples, atmospheric sources, and terrestrial sources, respectively. The standard deviation of $\Delta^{17}O$ signatures from each individual water sample was ±0.3‰. The standard deviations of $\Delta^{17}O$ signatures were assumed to be ±0.3‰ for nonatmospheric sources based on laboratory experience that nonatmospheric test materials always return values of 0 for $\Delta^{17}O$ and ±3‰ for atmospheric nitrate as mentioned above.[23,29] The atmospheric proportions and their variances could be calculated using an Excel spreadsheet available at http://www.epa.gov/wed/pages/models.htm.

RESULTS AND DISCUSSION

Water Chemistry and Isotopic Compositions of Nitrate. A wide range of $[NO_3^-]$ was observed in the two rounds of water samples collected from the main-channel and tributaries of the YR basin. The $[NO_3^-]$ in the main-channel of the MLRs-YR ranged from 136 to 584 μmol L^{-1}, with an average concentration of 309 μmol L^{-1} (Table 1). The $[NO_3^-]$ in the tributaries spanned an even wider range from 69 to 599 μmol L^{-1}, with a mean value of 347 μmol L^{-1} (Table 1). The $[NO_3^-]$ in water samples collected in October 2011 was slightly higher than that collected in September 2013 at each sampling site (Table 1), which was likely due to the intense fertilizer application for winter wheat growth during early and middle October (10/5−10/15) each year.

The $[NO_3^-]$ increased quickly from 136 to 584 μmol L^{-1} (Figure 2) when the YR flowed through Jinshan Valley into the Shanxi province (M1-M3), along which the YR drains areas where population density (>180 person km^{-2}) is much higher than that in the upper reach (<100 person km^{-2}).[44] The $[NO_3^-]$ then decreased to 378 μmol L^{-1} at the M4 site probably because of the dilution effect of WR, relatively small N inputs from areas with only 2.6% urban land, and/or denitrification occurring in the YR. There was an increase in $[NO_3^-]$ between M8 and M10 probably owing to the influx of the YLR; and then $[NO_3^-]$ did not change significantly downstream in the YR after M10, which was due to the small runoff of the converging tributaries (i.e., T4-T5, discharge <50 m^3 s^{-1}) compared with that in the main-channel (discharge >500 m^3 s^{-1}). The $[NH_4^+]$ in water samples were generally lower compared with $[NO_3^-]$, ranging from 8 to 67 μmol L^{-1}; it was probably due to relatively low $[NH_4^+]$ in its reaches draining these source regions, adsorption of NH_4^+ on suspended particles and sediment, and/or nitrification process in the YR basin that led to rapid depletion of NH_4^+-N (<70 μmol L^{-1}). Nitrite (NO_2^-) concentrations in most of the water samples were lower than the detection limit of 0.2 μmol L^{-1}, and the only detectable $[NO_2^-]$ was 1.4 μmol L^{-1} at M10 site, much smaller than the $[NO_3^-]$ (324 μmol L^{-1}). The low $[NO_2^-]$ could be attributed to the unstable chemical characteristics of nitrite in certain conditions,[7] and therefore, nitrite would have nominal impact on isotope signatures of nitrate in this study. Obviously, nitrate was the dominant N species in the water samples from the YR, suggesting the importance of studying nitrate sources and sinks in the YR.

The $\delta^{15}N_{NO3}$ values in the two rounds of water samples throughout the main-channel and tributaries of the MLRs-YR ranged from −1.4‰ to +12.9‰ (Table 1), with an average value of +6.6‰; and the $\delta^{18}O_{NO3}$ values had a range from −3.3‰ to +4.7‰ with an average value of −1.1‰. The average $\delta^{15}N_{NO3}$ value in this work was lower than those (11.5 to 17.5‰) of the underground water of the Yellow River delta reported by Chen et al.[45] but was comparable to those rivers with similar land-use patterns reported by other studies;[15,22] the $\delta^{18}O_{NO3}$ values were generally lower than those in other research,[8,12] and the reason is discussed below ("Identification of Terrestrial Nitrate Sources" section). The overall $\delta^{15}N_{NO3}$ values in water samples collected during October 2011 (−1.4−10.3‰, mean value ~4.7‰) were much smaller than those collected during September 2013 (10.9−12.9‰, mean value ~12.3‰), but the average $\delta^{18}O_{NO3}$ values showed no significant differences between the two sets of water samples. This might be caused by the routine application of ammonium/urea-containing fertilizers with low $\delta^{15}N_{NO3}$ (<0‰) activities happening right before our October 2011 sampling trip, which is in line with the higher $[NO_3^-]$ in water samples collected in October 2011.Therefore, the samples collected in October 2011 might have a relatively larger contribution of nitrate from chemical ammonium/urea fertilizer. For all the two rounds of water samples, $\Delta^{17}O_{NO3}$ values at each site were all small (0−1.6‰) indicating the overall minor contributions from atmospheric nitrate in the YR basin.

As shown in Figure 3, for both the $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values from upstream to downstream in the main-channel of the YR, there were two distinct turning points between M2 and M4, between and M8 and M10. The former demonstrated an abrupt decrease probably due to the FR influx and agricultural land area drainage, while the latter showed an increase likely owing to the inputs from the YLR and surround urban land area.

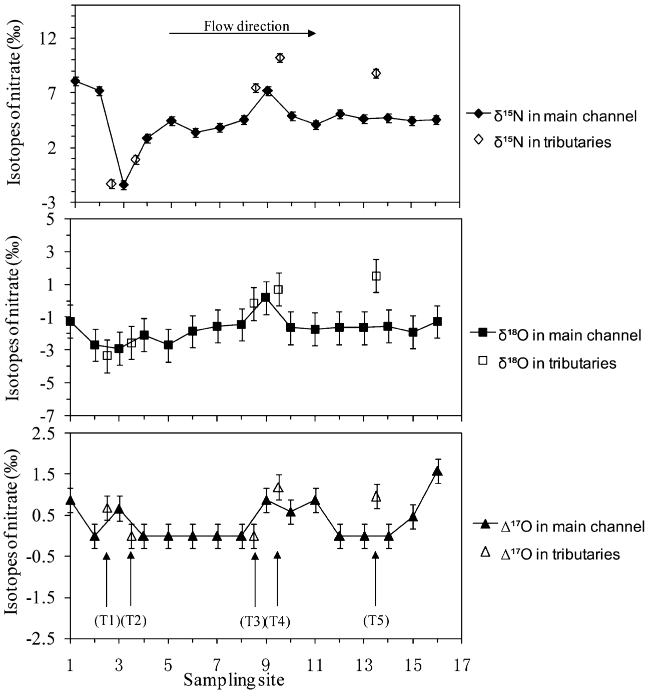

Figure 3. Variations of isotopic compositions of nitrate along the Yellow River samples collected in October, 2011. Data represent the mean values of three replicates ± SD.

Contribution of Atmospheric Sources to Nitrate.

According to formula 4, the $\Delta^{17}O_{NO3mix}$ values (0−1.6‰) in the two rounds of water samples suggested that 0−7% of the river water nitrate was unprocessed atmospheric nitrate, which refers to the nitrate directly from atmospheric deposition without going through the N cycle. The observed atmospheric proportions were relatively small (SI Figure S1) but comparable to the contribution (5−10%) of atmospheric deposition to the YR Cl^- inventory calculated by Zhang et al.,[46] and this indicated that $\Delta^{17}O_{NO3}$ is a practical tool for assessing atmospheric deposition. Additionally, if atmospheric nitrate proportion was estimated based on the $\delta^{18}O_{NO3mix}$ that were mostly negative at our sampling sites, there would be no atmospheric deposition considering the commonly high $\delta^{18}O_{NO3}$ values of atmospheric nitrate.[41] This suggested that, apart from less variation in the endmembers, $\Delta^{17}O_{NO3}$ is more sensitive in detecting atmospheric nitrate of small fractions when compared to $\delta^{18}O_{NO3}$.

Despite the overall minor atmospheric contributions in the YR basin, the fractions of atmospheric nitrate were noticeable at some sites. These sites mainly distributed in two river sections along the main-channel: between M9 and M11 and between M15 and M16, as well as in the Dongping Lake (L1). The above three regions are all located within the high-rainfall zones of the MLRs-YR with annual rainfall >700 mm,[44] indicating that wet deposition plays an important role in the atmospheric nitrate deposition in these regions. This also suggests an enhanced significance of assessing the contribution of atmospheric deposition to river water in the YR.

At the Toudaoguai site (M1), the end point station in the UR-YR, the riverine nitrate had a relatively large $\Delta^{17}O_{NO3}$ value of 0.9‰, implying that 4 ± 1.5% (±1.5% was the uncertainty of atmospheric nitrate fraction calculated using formula 7) of the nitrate delivered from the upper reach to middle reach was unprocessed atmospheric nitrate. However, water samples at the sites M2 and M4-M8 along the middle reach of the YR showed no positive $\Delta^{17}O_{NO3}$ values (Figure 3), and these abrupt decreases of $\Delta^{17}O_{NO3}$ value from M1 to M2 and from M3 to M4 implied a fast transformation of atmospheric NO_3^- → organic-N → NH_4^+-N → biological NO_3^-. At the end point station of middle reach of the YR (M10), the $\Delta^{17}O_{NO3}$ value of 0.6‰ indicated that unprocessed atmospheric nitrate accounted for approximately 3 ± 1.4% of the nitrate delivered from middle reach to the lower reach. Although the turnover of atmospheric nitrate is very intense along the YR in the present study, the $\Delta^{17}O_{NO3}$ at site M16 which is located before the river enters the estuarine transition zones between fresh water and salt water was 1.6‰, suggesting that the atmospheric contribution of nitrate delivered by the YR to the Bohai Bay was at least 7 ± 1.5%, considering the atmospheric nitrate fraction calculated here does not include atmospheric nitrate that has entered the microbial N cycle.

Identification of Terrestrial Nitrate Sources. The f_{NO3atm} determined from the $\Delta^{17}O$ data was then used to transform the $\delta^{18}O$ and $\delta^{15}N$ values of nitrate in water samples based on the formulas 5 and 6. The $\delta^{18}O_{NO3atm}$ value in the formula 6 was calculated to be 42.5 ± 10‰ using average $\Delta^{17}O_{NO3atm}$ of 23‰, and herein the average $\delta^{18}O_{NO3atm}$ value (42.5‰) was used in the subsequent calculations. Although this $\delta^{18}O_{NO3atm}$ value is lower than the range of 60−80‰ reported by some studies,[47,48] the $\delta^{18}O_{NO3}$ value (~46.3‰) detected in one rainwater sample of the YR was just within the range (42.5 ± 10‰). Additionally, the difference of $\delta^{18}O_{NO3atm}$ values (42.5‰ and 80‰) would only lead to a variation of approximately 0.7‰ in average $\delta^{18}O_{NO3terr}$ value of all the YR samples, which is smaller than the analytical error. Removal of $\delta^{18}O_{NO3atm}$ fraction would help us analyze other terrestrial sources or biogeochemical processes which might have affected the nitrate isotopic values of water samples.

The $\delta^{15}N_{NO3terr}$ and $\delta^{18}O_{NO3terr}$ values of water samples, with atmospheric imprint being removed, were used to analyze the relative importance of different terrestrial nitrate sources, such as synthetic nitrate fertilizer, microbial nitrate originated from soil nitrogen species, ammonium/urea fertilizers, and manure/sewage effluent. The $\delta^{18}O_{NO3terr}$ can be more useful than $\delta^{15}N_{NO3terr}$ for identifying nitrate from synthetic nitrate fertilizer and microbial nitrification due to the presence of large differences between the $\delta^{18}O_{NO3}$ values of these two sources.[11] Synthetic nitrate fertilizer had $\delta^{18}O_{NO3}$ values of +18‰ to +22‰, which is similar to that of atmospheric oxygen of ~+23.5‰. Theoretically, the $\delta^{18}O_{NO3}$ values of nitrate produced by microbial nitrification could be calculated because approximately one-third of the oxygen in NO_3^- should be derived from oxygen in the air, while two-thirds should be derived from ambient water at the site of nitrate formation.[8,15,49] As a result, using the $\delta^{18}O_{H2O}$ values of riverine water in the YR shown in Table 1, the $\delta^{18}O_{NO3}$ values of nitrate derived from in-stream microbial nitrification would be narrowed to the range of +1.2‰ to +1.8‰ at most sites, while at T5 and L1, the $\delta^{18}O_{NO3}$ would be as high as +2.6‰ and +4.2‰, respectively, because of the higher $\delta^{18}O_{H2O}$. Additionally, nitrate produced by soil microbial nitrification would have $\delta^{18}O_{NO3}$ values ranging from −9‰ to +10‰ if adopting $\delta^{18}O_{H2O}$ ≈ −25 ~ +4‰ for the soil/precipitation water.[11]

In the YR basin, the direct input of nitrate fertilizer can be small because nitrate fertilizers (e.g., KNO_3 and NH_4NO_3) are not commonly used (only 2%) in China.[8,33] The $\delta^{18}O_{NO3terr}$ values (−4.6‰ to +0.2‰, mean value ~ −2.2‰) of water samples except from the L1 site (Figure 4) were generally lower than the theoretically expected values for the nitrate from in-stream nitrification (+1.2‰ ~ +1.8‰). This might be caused by the mixing of nitrate from in-soil nitrification ($\delta^{18}O_{NO3}$ = −9 ~ +10‰) which may also be important to export nitrate into the YR considering the easy adsorption of NH_4^+ on soil, fast water-nitrite oxygen isotopic exchanges during nitrification,[38,50] and/or the shift of O_2/H_2O ratio.[51] Besides, sewage/manure effluent ($\delta^{18}O_{NO3}$ = −9 ~ +10‰) is another potential source of nitrate in the YR. Therefore, nitrification might be one of the most important nitrogen transformation processes existing in the YR basin.[52] Instead, the $\delta^{15}N_{NO3terr}$ values of riverine nitrate ranged from −1.4‰ to +12.9‰ in the MLRs-YR, suggesting that nitrate in this river had come from the nitrification of multiple nitrogen sources including chemical fertilizer, manure/sewage effluent, and soil ammonium and organic nitrogen (Figure 4).

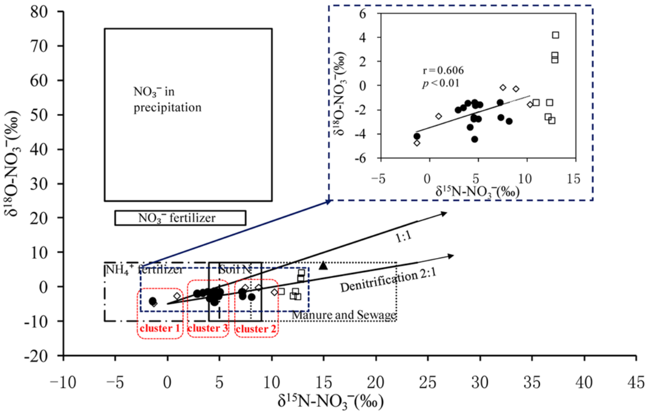

Figure 4. Cross-plot of $\delta^{15}N_{NO3}$ versus $\delta^{18}O_{NO3}$ after removing atmospheric imprint (circles: mainstream; diamonds: tributaries; triangles: lakes; square: river samples (Sep. 2013)). The isotope compositions of various sources in the diagram are summarized from refs 9,14, and 15. Also shown is the expected trend for the isotopic composition of residual nitrate undergoing microbial denitrification, assuming that the initial nitrate was derived from nitrification of ammonium/urea-containing fertilizer.

The importance of these sources has been found to vary between different sampling sites during the same sampling trip. Although the ranges of $\delta^{15}N_{NO3terr}$ and $\delta^{18}O_{NO3terr}$ values of water samples collected in October 2011 were wide, the sites with various isotopic composition of riverine nitrate could be grouped into three different clusters based on Ward's method by using cluster analysis of SPSS 20.0 software (SPSS Inc., Chicago II, USA) (Figures 4 and S2). Samples from the FR and WR (cluster 1: Figure 4), two major tributaries of the YR, had low $\delta^{15}N_{NO3terr}$ (<+0.9‰) and $\delta^{18}O_{NO3terr}$ (<−2.3‰) values, indicating that nitrate nitrified from ammonium/urea-fertilizer was likely their major source; this was consistent with the wide application of ammonium/urea-containing fertilizers in these regions, where cropland was the main form of land use.[32] While for the YLR, QR, and JDR (cluster 2: Figure 4), mean

$\delta^{15}N_{NO3terr}$ and $\delta^{18}O_{NO3terr}$ values were +8.2‰ and −1.4‰, respectively, suggesting that manure/sewage effluent was likely the main source of nitrate. Along the main-channel of the YR, nitrate at Toudaoguai (M1) and Longmen (M2) sites was mainly from manure/sewage effluent, and this might be caused by the livestock farming in the UR-YR, where 52.5% of the land is used as grassland. With the input of river water from the FR, the major nitrate source at Yuncheng (M3) was fertilizer which had low $\delta^{15}N_{NO3terr}$ and $\delta^{18}O_{NO3terr}$ values. Riverine nitrate in the downstream area after M3 (cluster 3: Figure 4) had average $\delta^{15}N_{NO3terr}$ and $\delta^{18}O_{NO3terr}$ values of +4.3‰ and −2.2‰, respectively. These sites may have contained a mixing of nitrate from soil organic nitrogen ($\delta^{15}N_{NO3} \approx +4 \pm 4$‰[53]), newly formed nitrate from nitrification of ammonium/urea-containing fertilizer ($\delta^{15}N_{NO3} \approx -8 \pm 4$‰[54]), and sewage/manure effluent ($\delta^{15}N_{NO3} \approx +15 \pm 10$‰[11,53]). The contributions of the latter two sources to riverine nitrate were comparable according to formula 1 and the isotope compositions of different nitrate sources shown in Table 1 (calculation details shown in the SI). The contributions of different sources were also different at the same sampling sites during different sampling trips. The $\delta^{15}N_{NO3terr}$ values of water samples collected in September 2013 had a relatively narrow range from 10.9‰ to 12.9‰, indicating that sewage/manure effluent was the major nitrate source (>75%) at the time right before intense fertilizer application activities (details shown in the SI).

The sources of riverine nitrate delivered by the YR into the Bohai Bay could be estimated according to the isotopic signatures of nitrate at M16 before the YR enters the estuarine transition zones between fresh water and salt water. The data from this site suggested that river discharge delivered nitrate with moderate $\delta^{15}N_{NO3terr}$ (+4.5‰) and low $\delta^{18}O_{NO3terr}$ (−1.3‰) to the Bohai Bay; it suggested that the terrestrial nitrate inputs from the YR to Bohai was mainly composed of the nitrified product of ammonium/urea-containing fertilizer and manure/sewage effluent, and the contributions of these two sources to the terrestrial nitrate were comparable.

Nitrate Removal by Denitrification. Microbial denitrification, the reduction of nitrate to N_2O and N_2 when oxygen is limited and labile organic carbon is available,[55] can cause increases in $\delta^{15}N$ and $\delta^{18}O$ values of residual nitrate with decreasing $[NO_3^-]$.[18] We analyzed the data of water samples collected from the YR basin, including both the main-channel and tributaries of the YR, in October 2011, and there were clear trends of increasing $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values with decreasing $[NO_3^-]$ ($p < 0.01$ for $\delta^{15}N_{NO3}$, and $p < 0.05$ for $\delta^{18}O_{NO3}$, $n = 21$) (SI Figure S3). Because it is difficult for algae/phytoplankton to grow in the YR due to high concentrations of suspended sediment (SPS), assimilation would not be the major reason for the above relationships. Therefore, the nitrate transformation might be mainly influenced by microbial denitrification within the YR basin. Many studies showed that denitrification produces a distinctive positive correlation between $\delta^{15}N_{NO3}$ versus $\delta^{18}O_{NO3}$ values with slopes of about 2.[11,56] A significant positive relationship with a slope of ∼4 was found between $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values ($p < 0.01$) in this study (Figure 4), indicating denitrification may be partially responsible for the isotopic composition of nitrate within the YR basin, though our slope was actually bigger than that reported for denitrification (≈1−2).[15,18,20−22] The water sample from Dongping Lake (L1), the only natural lake in the MLRs-YR, had $\delta^{15}N_{NO3terr}$ and $\delta^{18}O_{NO3terr}$ values of 15.0‰ and 6.1‰, respectively, which were distinctly higher than those detected in riverine samples. Algae tend to grow in this lake (a relatively closed system compared with rivers) and may prefer to uptake light isotopes of nitrate, leading to enrichment with heavy isotopes in the residual nitrate. Assimilation might cause 1:1 changes in the $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$,[11] while denitrification causing the increase of isotopic values of residual nitrate with $\delta^{15}N_{NO3}:\delta^{18}O_{NO3} \approx 1.3-2.1$ might occur in the sediment as well. Both assimilation and denitrification might then have been responsible for the nitrate ^{15}N and ^{18}O isotope enrichment in Dongping Lake.

For the YR, denitrification might occur in both the SPS and bed-sediment based on our previous study.[57] The bed-sediment is an important zone for denitrification because of the great likelihood of anoxic conditions, while SPS plays an additional impact on denitrification due to the presence of low-oxygen microsites in SPS which creates suitable conditions for denitrifying bacteria to thrive and denitrify NO_3^-. The denitrification rate in the YR was about 18 mg-N m^{-2} day^{-1};[57] according to the Rayleigh equation using enrichment factors of 5−40‰,[18] the $\delta^{15}N$ values of residual nitrate in the YR resulting from denitrification increased by approximately 0.004−0.029‰ (assuming 25 g-N m^{-2} for the YR) per day (details shown in the SI). Despite the uncertainties inherent in the above calculation, the results indicated that denitrification occurring in the YR may partially explain the increasing trends of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values with decreasing $[NO_3^-]$ in the water samples. The $\delta^{15}N$ and $\delta^{18}O$ values of riverine nitrate in the present study plotted along a slope >2, which might be caused by the co-occurrence of denitrification with (1) enzymatically catalyzed O exchange between NO_2^- and water;[11,38] (2) the process of "aerobic denitrification" because denitrifiers in the YR are facultative and can keep denitrifying abilities at oxygen level of 70% according to our previous results;[57] and (3) concomitant mixing of nitrate from manure/sewage effluent which has high $\delta^{15}N_{NO3}$ and low $\delta^{18}O_{NO3}$ values and might produce high $\delta^{15}N_{NO3}/\delta^{18}O_{NO3}$ ratio as well, especially for large rivers that have more complex nitrate sources along the rivers.

Interestingly, in the $\delta^{18}O_{NO3}$ vs $\delta^{15}N_{NO3}$ plot, the samples from relative small tributaries (T3, T4, T5) had heavier $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values compared to those from the other two large tributaries (T1, T2) and main-channel of the YR (Figure 4), indicating that stronger denitrification might exist in small streams. This difference could be explained from two aspects. First, an intensive denitrification process occurred in small streams since aquatic nitrate in small rivers or lakes has more opportunities to interact with the bed-sediment than in larger rivers,[58] and this would result in strong nitrogen and oxygen isotope fractionation during microbial denitrification in small tributaries like YLR, QR, and JDR. Second, large rivers such as the YR, FR, and WR with the corresponding area of 7.5 × 10^5, 3.9 × 10^4, and 1.3 × 10^5 km^2 might receive N inputs from a larger variety of sources along the river, which would mask any isotopic signals of denitrification in these rivers. However, since a river is not a closed system with a single nitrate source, it might be not possible to determine the size and extent of denitrification by only analyzing the water samples collected from several sites. Systematic experiments conducted both in situ and in-laboratory, combined with monitoring data, would be warranted to well understand microbial denitrification in large rivers.

In summary, the sources and sink dynamics of nitrate in the YR were investigated for the first time using triple nitrate isotopes in the present study. The results suggested that unprocessed atmospheric nitrate accounted for 0−7% of the total nitrate of the YR in the normal-water season. The main terrestrial nitrate source in the UR-YR was sewage/manure effluents, while sewage/manure effluents and ammonium/urea-containing fertilizer made comparable contributions to the nitrate inventory in the MLRs-YR. Additionally, denitrification might play an important role in the nitrogen cycle of the YR. This study suggests that the analysis of triple nitrate isotopes ($\delta^{15}N_{NO3}$, $\Delta^{17}O_{NO3}$, $\delta^{18}O_{NO3}$), combined with mixing models based on an isotopic mass balance, can provide good estimations for the proportional contributions of nitrate sources in surface water, especially for the fractions of atmospheric nitrate contribution. The calculation about proportional contributions of nitrate sources needs to include an analysis of uncertainties caused by the broad ranges of $\delta^{15}N$, $\Delta^{17}O$, and $\delta^{18}O$ signatures for specific nitrate sources. Further constraints of the isotopic compositions of potential NO_3^- sources within the study area may be useful for more accurate estimates of their relative contributions to river water.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental details: details about analytical method and calculations; characteristics of the YR; hierarchical dendrogram for $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values; relationships between $\delta^{15}N_{NO3}$ ($\delta^{18}O_{NO3}$) and [NO_3^-]. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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