

Article

Spatio-Temporal Variations in Nitrate Sources and Transformations in the Midstream of the Yellow River Determined Based on Nitrate Isotopes and Hydrochemical Compositions

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Abstract: Nitrate pollution is a major environmental problem threatening rivers, and nitrogen and oxygen isotopes have proved to be an effective means of analyzing the sources and transformations of nitrate in rivers. However, a low monitoring frequency cannot accurately reflect the changes in nitrate. In this study, the sources and transformations of nitrate in the middle reaches of the Yellow River and its tributaries during the dry season and the wet season were analyzed based on water quality parameters and nitrate isotopes. Stable isotope analysis conducted using the R (SIAR) model was used to estimate the proportions of different nitrate sources. The results showed that the main nitrate sources in the main stream were soil nitrogen (40.95–45.83%) and domestic sewage and manure (30.93–32.60%), respectively, with little variation between the dry season and wet season because of the large flow of the Yellow River. During the dry season, the nitrate sources of the two tributaries were mainly domestic sewage and manure (45.23–47.40%), followed by soil nitrogen (31.35–34.00%). However, the primary nitrate source of T2 (Qin River) became soil nitrogen (40.05%) during the wet season, a phenomenon that was mainly caused by the significant increase in river discharge and in soil erosion in the basin. During the wet season, the concentrations of total nitrogen (TN) and nitrate (NO_3^-) significantly decreased in the main stream and tributaries, and nitrification and denitrification processes occurred in both the main stream and tributaries of the Yellow River. In addition, the T2 tributary (Qin River) was also significantly affected by mixed dilution. High-frequency sampling can reflect the isotopic information of nitrate in the river more comprehensively, which helps us to understand the conversion process of nitrate more accurately.

Keywords: the midstream of the Yellow River; nitrate source; dual nitrate isotopes; nitrification; denitrification



Citation: Su, C.; Su, Y.; Zhang, R.; Xu, X.; Li, J. Spatio-Temporal Variations in Nitrate Sources and Transformations in the Midstream of the Yellow River Determined Based on Nitrate Isotopes and Hydrochemical Compositions. *Water* **2024**, *16*, 1173. <https://doi.org/10.3390/w16081173>

Academic Editor: Christos S. Akrotas

Received: 23 March 2024

Revised: 12 April 2024

Accepted: 18 April 2024

Published: 20 April 2024



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1. Introduction

Due to the influence of industrial and agricultural activities, the concentration of nitrogen pollutants in rivers has increased, significantly impacting river ecological environments and human health [1]. The sources of nitrogen pollutants are complex, and this is related to the population distribution and land use type of a basin. The content of nitrogen pollutants in rivers is also related to nitrogen transformation processes [2]. Therefore, it is of great significance to study the source and transformation rules of nitrogen pollutants for the formulation of effective prevention and control programs for nitrogen pollutants.

Nitrate is the main form of nitrogen in rivers, generally accounting for over 80% of the total nitrogen in rivers [3]. The dual isotope ($\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$) technique has been shown to be an effective method for analyzing the sources of nitrate [4–8]. Studies have shown that NO_3^- in water mainly comes from domestic sewage, animal manure,

synthetic fertilizers, soil organic nitrogen, and atmospheric precipitation. Nitrate from different sources has different isotopic characteristics: the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ range from 4–25‰ and –5–10‰ for domestic sewage and manure, –6–6‰ and 17–25‰ for synthetic fertilizers, 0–8‰ and –10–15‰ for soil organic nitrogen, and –13–13‰ and 23–75‰ for atmospheric deposition. [2,9,10]. In order to quantitatively analyze nitrate sources, stable isotope analysis in R (SIAR) conducted according to the Bayesian isotopic mixing model [11] has been successfully applied in the study of several rivers [12–15]. Compared with traditional methods, SIAR has superior accuracy and has proved to be an effective analysis method [16].

The isotopic characteristics of nitrate are not only affected by its sources but also the biological transformation processes of nitrogen. Assimilation, nitrification, and denitrification processes all cause isotope fractionation, and ^{14}N and ^{16}O are more easily utilized, thus enriching ^{15}N and ^{18}O in the residues. Therefore, based on the isotopic characteristics, nitrogen transformation processes can also be identified [5,17–19]. However, nitrogen and oxygen isotopes' characteristics are also affected by land use, hydrological conditions, human activities, and other factors and change with time and space. Nitrate sources and the mixing of river water can sometimes mask the isotopic evidence of denitrification in a river [20–22], and sampling frequency is considered another factor that increases the uncertainties in determining transformations [15,23]. Recently, combined methods consisting of using dual isotopes of nitrate together with hydro-chemical compositions (e.g., $\text{NO}_3^-/\text{Cl}^-$) [24,25] have proved to be helpful for identifying nitrate transformation and sources.

The Yellow River is the second-largest river in China. It is affected by the natural environment, population, and economic factors, and the sources of nitrogen pollutants in the upper and middle-lower reaches of the Yellow River are different. Yue et al. [3] found that nitrate in the upper reaches of the Yellow River mainly came from soil organic nitrogen, while in the middle-lower reaches, the presence of nitrate was influenced by soil nitrogen, domestic sewage, chemical fertilizer, and other sources, which were mainly related to the inflow of tributaries. Liu et al. [21] found that nitrates in the upper reaches of the Yellow River mainly came from domestic sewage and animal manure, while in the middle-lower reaches, the contributions of domestic sewage, animal manure, and fertilizers were comparable. The difference in the conclusions arrived at by Yue [3] and Liu [21] is mainly due to the fact that the sampling times were different, resulting in different characteristics of nitrogen and oxygen isotopes. In addition, a small number of samples also increased the uncertainty of the isotope analysis results [16]. The Zhengzhou section of the Yellow River is part of the middle reaches of the Yellow River. It is located in a densely populated area with a high land utilization rate and has two tributaries, the Yiluo River and the Qin River. Studying the sources and transformations of nitrogen pollutants in this area has significant value for the protection of the Yellow River. To ensure the accuracy of the results, in this study, we adopted the method of multiple sampling analysis. Based on the hydrochemical characteristics of the main stream and tributaries of the Yellow River during the wet season and dry season, as well as the $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ isotope values, the sources and transformation pathways of nitrate in the middle reaches of the Yellow River were analyzed. The contributions of different nitrate sources were estimated using the SIAR model.

2. Materials and Methods

2.1. Study Area

The Yellow River, located at $32^\circ 10'\text{--}41^\circ 50'$ N and $95^\circ 53'\text{--}119^\circ 10'$ E, with a total length of 5464 km and a basin area of $7.95 \times 10^5 \text{ km}^2$, is the second-largest river in China, providing irrigation and drinking water for 140 million people. The upper reaches of the Yellow River are dominated by grasslands, mainly used for animal husbandry, and the middle and lower reaches are dominated by agricultural and forestry land, which are greatly affected by human activities. The study area is located in the middle reaches of

the Yellow River, which is in a warm temperate continental monsoon climate. The annual average rainfall in this region is 542.2 mm, occurring mainly from June to September. The distributions of farmland and cities in this area are relatively dense, and human activities are frequent. Urea and ammonia fertilizer are the main fertilizers applied to farmland, and the fertilization periods are generally from February to March and from June to July. From 2018 to 2022, the average annual runoff of the Yellow River was 44.488 billion cubic meters, that of the Yiluo River was 2.29 billion cubic meters, and that of the Qin River was 0.937 billion cubic meters.

2.2. Sample Collection and Analysis Methods

There were four sections (Figure 1), namely, the M1 ($34^{\circ}50'29''$ W, $113^{\circ}3'17''$ E) and M2 ($34^{\circ}55'1''$ W, $113^{\circ}40'5''$ E) sections in the main stream of the Yellow River; the T1 ($34^{\circ}48'55''$ W, $113^{\circ}3'37''$ E) section, where the Yiluo River enters the Yellow River; and T2 ($35^{\circ}0'44''$ W, $113^{\circ}25'41''$ E) section, where the Qin River enters the Yellow River.

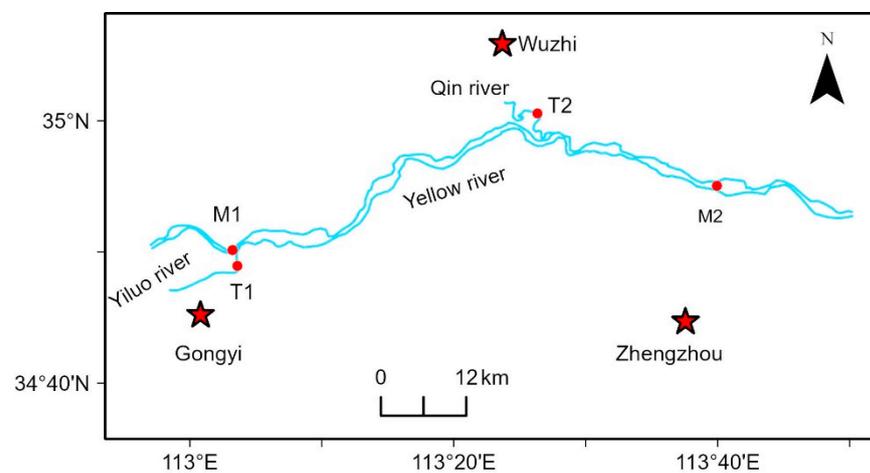


Figure 1. Sampling point layout of the middle reaches of the Yellow River basin.

During the dry season (2 January 2021, 31 March 2021, 5 December 2021, 23 February 2022, and 20 March 2022) and the wet season (4 July 2021 (due to extreme rainfall in the study area, no continuous sampling was conducted), 12 June 2022, 11 July 2022, 20 August 2022, and 17 September 2022), water samples were collected five times using a water sampler (TC-Y, Fushun, China) at a depth of 0.2 m below the water surface. In addition, surface sediment samples were collected using a grab-type sediment sampler (XY-TC1001, Qingdao, China) at the M1 and M2 sections of the Yellow River (2 January 2021, 20 March 2022, and 4 July 2021, 17 September 2022).

Dissolved oxygen (DO), temperature, and pH values were measured on-site using a portable water quality analyzer (YSI ProQuatro, Ohio, USA). The water samples were stored away from light at 4 °C and transported back to the laboratory within 12 h. Some of the samples were filtered using a 0.22 µm membrane for the measurement of $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$, and the rest were refrigerated at 4 °C for water quality analysis. $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ levels were determined using denitrifying bacteria methods [26]. Total nitrogen (TN) was determined via alkaline potassium persulfate digestion and UV spectrophotometry. NH_4^+-N content was determined using Nessler's reagent spectrophotometry, and $\text{NO}_3^- -\text{N}$ and $\text{NO}_2^- -\text{N}$ levels were determined using spectrophotometry [27]. Chloride ions were measured using an ion chromatograph (DIONEX ICS-1000, California, USA), while chemical oxygen demand (COD_{Cr}) and suspended substance (SS) levels were measured using the standard method [27].

2.3. SIAR Program Based on Bayesian Isotope Mixing Model

Recently, SIAR was successfully used to estimate the contribution of different nitrate sources [11]. SIAR can be expressed as follows in Equations (1)–(4) [11].

$$X_{ij} = \sum_{k=1}^K P_k (S_{jk} + C_{jk}) + \varepsilon_{jk} \quad (1)$$

$$S_{jk} \sim N(\mu_{jk}, \omega_{jk}^2) \quad (2)$$

$$C_{jk} \sim N(\lambda_{jk}, \tau_{jk}^2) \quad (3)$$

$$\varepsilon_{jk} \sim N(0, \sigma_{jk}^2) \quad (4)$$

where X_{ij} represents the isotope value of j (for ^{15}N and ^{18}O) in water sample i ($i = 1, 2, 3, \dots, N$); P_k is the proportion of source k calculated using the SIAR model; S_{jk} indicates the isotope values j of source k ($k = 1, 2, 3, \dots, K$), which is normally decided by the mean μ_{jk} and standard deviation ω_{jk} ; C_{jk} is the isotopic fractionation factor of source k of isotope j , generally expressed as mean λ_{jk} and standard deviation τ_{jk} ; and ε_{ij} is the residual error denoting the remaining unquantified variation between the mixtures, which is normally distributed with a mean of 0 and standard deviation σ_j [28]. In this study, four potential sources of nitrate were identified; the mean values of nitrate isotopes (μ_{jk}) and associated standard deviations (ω_{jk}) were acquired from other studies [14].

2.4. The Source of the Referenced Data

The data on the monthly average flow and the concentrations of TN and NH_4^+ -N in sections M2, T1, and T2 from 2020 to 2022 came from the bulletins of the Yellow River Conservancy Commission of the Ministry of Water Resources and the Ministry of Ecology and Environment of the People's Republic of China, respectively.

3. Results

3.1. Temporal and Spatial Variations of Hydrochemical Parameters

Temperature, DO, and pH are important physicochemical parameters governing the self-purification capacity of a river. As shown in Table 1, the temperature of each section in the study area ranged from 3.80 °C to 13.30 °C during the dry season, while the pH ranged from 7.60 to 8.60, and the DO content ranged from 7.60 mg L⁻¹ to 16.40 mg L⁻¹; during the wet season, the temperature ranged from 22.70 °C to 30.10 °C, the pH ranged from 7.70 to 8.70, and the DO content ranged from 6.70 mg L⁻¹ to 12.50 mg L⁻¹. The pH was weakly alkaline, with little variation over time. However, the DO content in the dry season was higher than that in wet season, mainly due to the lower temperature during the dry season [29].

Table 1. The statistical results regarding hydrochemical characteristics (Unit: mg L⁻¹, except for pH and temperature).

Location	Time	Statistic	pH	T	DO	COD _{Cr}	NH ₄ ⁺	NO ₃ ⁻	TN	Cl ⁻	SS
M1	Dry season	Max	8.40	12.10	13.20	16.12	0.22	3.87	4.57	116.13	1439.65
		Min	7.90	3.80	10.00	10.30	0.04	2.69	3.23	107.11	571.55
		Mean	8.26	8.22	11.36	12.49	0.15	3.21	3.87	111.61	1040.81
		SD	0.22	3.44	1.34	2.33	0.07	0.43	0.52	3.53	349.17
	Wet season	Max	8.10	29.90	8.40	15.10	0.22	2.96	3.62	118.78	2369.22
		Min	7.70	22.70	6.90	11.80	0.03	1.44	1.93	102.33	1372.26
		Mean	7.90	25.70	7.72	13.82	0.09	2.19	2.67	109.28	1861.44
		SD	0.15	2.88	0.57	1.27	0.08	0.66	0.73	8.34	422.26

Table 1. Cont.

Location	Time	Statistic	pH	T	DO	CODcr	NH ₄ ⁺	NO ₃ ⁻	TN	Cl ⁻	SS
M2	Dry season	Max	8.60	12.30	12.80	17.34	0.21	3.74	4.67	118.17	1198.87
		Min	7.80	4.20	9.60	10.50	0.09	2.65	3.17	106.34	632.09
		Mean	8.32	8.58	10.76	12.79	0.16	3.34	4.04	113.20	907.01
		SD	0.30	3.45	1.32	2.68	0.05	0.42	0.58	4.81	261.99
	Wet season	Max	8.10	30.10	8.20	14.90	0.34	3.06	3.80	116.87	1839.16
		Min	7.70	22.80	6.70	11.80	0.02	1.37	1.88	104.23	1089.23
		Mean	7.90	25.86	7.36	13.57	0.14	2.33	2.89	109.52	1462.57
		SD	0.15	3.01	0.67	1.31	0.12	0.71	0.79	5.98	314.45
T1	Dry season	Max	8.30	13.10	16.40	31.30	0.79	6.12	7.42	79.04	60.11
		Min	7.60	4.00	8.90	10.90	0.52	3.98	5.46	71.00	43.28
		Mean	8.04	9.14	11.80	15.77	0.67	4.78	6.16	73.24	51.23
		SD	0.26	4.01	2.94	8.78	0.10	0.83	0.82	3.27	6.31
	Wet season	Max	8.40	29.20	12.50	24.30	0.77	3.38	4.46	60.44	74.15
		Min	7.90	25.50	7.70	12.60	0.10	1.31	1.46	52.08	57.11
		Mean	8.18	28.18	9.46	18.60	0.57	2.02	2.93	56.37	65.20
		SD	0.21	1.52	2.13	5.35	0.27	0.82	1.08	3.10	6.83
T2	Dry season	Max	8.60	13.30	14.60	9.23	0.21	4.81	6.21	77.00	70.92
		Min	8.10	4.10	7.60	8.21	0.11	4.02	4.48	61.00	48.16
		Mean	8.35	9.16	10.66	8.65	0.16	4.41	5.48	66.64	56.64
		SD	0.18	3.98	2.53	0.46	0.05	0.28	0.67	6.55	8.84
	Wet season	Max	8.70	28.60	9.20	11.60	0.21	4.02	4.70	55.70	113.04
		Min	7.90	25.80	7.20	8.15	0.05	1.55	1.65	41.27	63.54
		Mean	8.51	27.30	8.24	10.39	0.16	3.13	3.97	49.81	92.95
		SD	0.31	0.99	0.73	1.41	0.07	0.94	1.30	5.32	19.48

Note: NO₂⁻ was not detected.

During the dry season, the $\rho(\text{NH}_4^+)$ values of M1, M2, T1, and T2 were $0.15 \pm 0.07 \text{ mg L}^{-1}$, $0.16 \pm 0.05 \text{ mg L}^{-1}$, $0.67 \pm 0.10 \text{ mg L}^{-1}$, and $0.16 \pm 0.05 \text{ mg L}^{-1}$, respectively, while the $\rho(\text{NO}_3^-)$ values were $3.21 \pm 0.43 \text{ mg L}^{-1}$, $3.34 \pm 0.42 \text{ mg L}^{-1}$, $4.78 \pm 0.83 \text{ mg L}^{-1}$, and $4.41 \pm 0.28 \text{ mg L}^{-1}$, respectively, and the $\rho(\text{TN})$ values were $3.87 \pm 0.52 \text{ mg L}^{-1}$, $4.04 \pm 0.58 \text{ mg L}^{-1}$, $6.16 \pm 0.82 \text{ mg L}^{-1}$, and $5.48 \pm 0.67 \text{ mg L}^{-1}$, respectively. The average values of TN and NO₃⁻ in each section were significantly higher than those in the wet season. During the wet season, the non-point source pollution load caused by surface runoff increased, but the concentration of nitrogen pollutants decreased significantly, and the concentrations of TN and NO₃⁻ decreased by 27.55–52.44% and 29.02–57.74%, respectively. A correlation analysis of river discharge and TN and NH₄⁺ concentrations from 2020 to 2022 was carried out. The results are shown in Table 2. The correlation coefficient between the TN and NH₄⁺ concentrations and discharge was very small, indicating that the dilution effect caused by the increase in river discharge in the wet season was not the main reason for the decrease in nitrogen pollutant concentrations, and the CODcr in the wet season was close to that in the dry season; however, the water temperature in the wet season was higher, and this higher temperature was conducive to the metabolic activities of microorganisms. Therefore, the decrease in TN concentration was mainly induced by biological transformation processes.

Table 2. Correlation coefficients of pollutant concentration and river flow.

Location	Correlation Coefficient	
	NH ₄ ⁺ -N	TN
M2	-0.06	-0.19
T1	-0.02	-0.15
T2	0.06	0.07

Spatial analysis showed that the concentrations of nitrogen pollutants in the two tributaries were higher than those in the main stream in both the dry and wet seasons, indicating that the sources of pollutants in the tributaries and the main stream were different, which may also be related to the fact that the high concentration of SS in the main stream was more conducive to the biological transformation of nitrogen pollutants [30,31]. Table 1 showed that the $\rho(\text{SS})$ in the main stream was 571.55–2369.22 mg L^{-1} , while the $\rho(\text{SS})$ in the two tributaries was 43.28–113.04 mg L^{-1} , and the $\rho(\text{SS})$ in the main stream was higher than that in the tributaries. Studies have shown that suspended sediments can provide greater adhesion area for microorganisms, strengthening the nitrification and denitrification of nitrogen in water, resulting in a 25–120% increase in nitrogen loss [30,31].

During the dry season, the $\rho(\text{Cl}^-)$ values of M1 and M2 were $111.61 \pm 3.53 \text{ mg L}^{-1}$ and $113.20 \pm 4.81 \text{ mg L}^{-1}$, respectively, while the $\rho(\text{Cl}^-)$ values of T1 and T2 were approximately $73.24 \pm 3.27 \text{ mg L}^{-1}$ and $66.64 \pm 6.55 \text{ mg L}^{-1}$, which were slightly higher than those during the wet season. Cl^- is widely distributed in the natural environment, with relatively high concentrations in feces and domestic sewage [32]. The $\rho(\text{Cl}^-)$ in rivers was relatively stable, and it was not affected by changes in physical, chemical, or biological factors; it was only affected by the mixing effect caused by flow changes [33,34]. As shown in Figure 2, the flow of the main stream and tributaries of the Yellow River increased in the wet season, and Cl^- was diluted, resulting in lower $\rho(\text{Cl}^-)$ than that in the dry season.

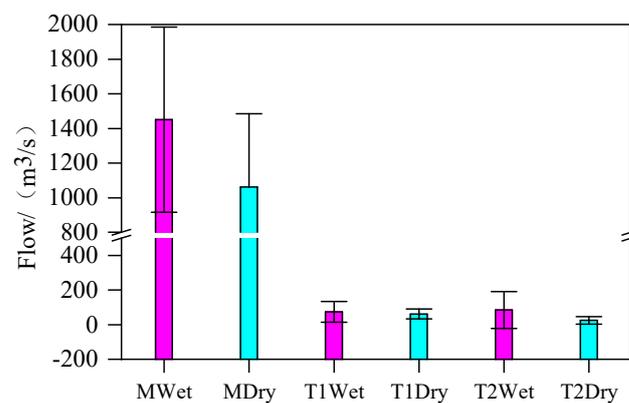


Figure 2. Comparison of river flow in different periods.

3.2. Spatial and Temporal Variations in $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$

As can be seen from Table 3, during the dry season, the $\delta^{15}\text{N}$ values of M1 and M2 were $8.78\text{‰} \pm 0.48\text{‰}$ and $9.39\text{‰} \pm 0.63\text{‰}$, respectively; the $\delta^{18}\text{O}$ values were $2.11\text{‰} \pm 0.32\text{‰}$ and $2.90\text{‰} \pm 0.77\text{‰}$; and the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of T1 were $13.77\text{‰} \pm 0.57\text{‰}$ and $5.91\text{‰} \pm 0.39\text{‰}$, respectively, and those of T2 were $13.14\text{‰} \pm 0.80\text{‰}$ and $4.92\text{‰} \pm 0.73\text{‰}$, respectively. During the wet season, the $\delta^{15}\text{N}$ values of M1 and M2 were $8.52\text{‰} \pm 0.08\text{‰}$ and $8.75\text{‰} \pm 0.20\text{‰}$, respectively; the $\delta^{18}\text{O}$ values were $1.42\text{‰} \pm 0.88\text{‰}$ and $2.13\text{‰} \pm 1.45\text{‰}$, respectively; and the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of T1 were $12.20\text{‰} \pm 1.48\text{‰}$ and $5.70\text{‰} \pm 0.16\text{‰}$, respectively, and those of T2 were $11.05\text{‰} \pm 0.76\text{‰}$ and $3.49\text{‰} \pm 0.84\text{‰}$, respectively. It can be seen that the $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values of the tributaries were higher than those of the main stream, and the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of both the main stream and tributaries were higher in the dry season than in the wet season. The $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values were not only influenced by the sources and contribution rate of nitrate but also by the transformation of nitrogen [17–19]. The $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values of the main stream and tributaries of the Yellow River showed different characteristics in different periods, indicating that there were different nitrate sources and transformation processes.

Table 3. $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ in overlying water (Unit: ‰).

Location	Statistic	$\delta^{15}\text{N-NO}_3^-$	$\delta^{18}\text{O-NO}_3^-$	$\delta^{15}\text{N-NO}_3^-$	$\delta^{18}\text{O-NO}_3^-$
		Dry Season		Wet Season	
M1	Max	9.45	2.67	8.60	1.95
	Min	8.22	1.86	8.43	−0.14
	Mean	8.78	2.11	8.52	1.42
	SD	0.48	0.32	0.08	0.88
M2	Max	10.10	3.55	9.05	4.25
	Min	8.67	1.86	8.58	0.21
	Mean	9.39	2.90	8.75	2.13
	SD	0.63	0.77	0.20	1.45
T1	Max	14.75	6.54	14.82	5.91
	Min	13.30	5.54	11.24	5.47
	Mean	13.77	5.91	12.20	5.70
	SD	0.57	0.39	1.48	0.16
T2	Max	13.83	6.16	11.96	4.98
	Min	11.98	4.25	9.91	2.91
	Mean	13.14	4.92	11.05	3.49
	SD	0.80	0.73	0.76	0.84

4. Discussion

4.1. Analysis of the Sources of Nitrate

The $\delta^{15}\text{N-NO}_3^-$ of the main stream in the study area ranged from 8.22‰ to 10.10‰, and the $\delta^{18}\text{O-NO}_3^-$ ranged from −0.14‰ to 4.25‰; these values are mainly located in the characteristic range of domestic sewage and manure and close to the characteristic range of soil nitrogen. The $\delta^{15}\text{N-NO}_3^-$ of the tributaries ranged from 9.91‰ to 14.82‰, and the $\delta^{18}\text{O-NO}_3^-$ ranged from 2.91‰ to 6.54‰, and these values are mainly located in the characteristic range of domestic sewage and manure (Figure 3). The higher temperature in the wet season was conducive to the nitrification and denitrification processes of microorganisms, and these biological transformation processes increase the $\delta^{18}\text{O-NO}_3^-$, which was confirmed by Yue's [3] study on the Yellow River. However, in this study, the $\delta^{18}\text{O-NO}_3^-$ in the wet season was lower than that in the dry season, and Li [35] also found the same result, which may have been precipitated by the increase in the loss of ammonia fertilizers and soil organic nitrogen during the wet season, and the influence of nitrate sources on $\delta^{18}\text{O-NO}_3^-$ exceeded the influence of nitrate transformations.

The SIAR model was used to estimate the contribution rate of nitrate from different sources in four sections, as shown in Figure 4. It can be seen that the main nitrate source of the main stream was soil nitrogen, accounting for 40.95–45.83%, followed by domestic sewage and manure, accounting for 30.15–32.60% and fertilizer, accounting for 21.88–24.46%; the contribution of atmospheric deposition was small, amounting to only 1.36–2.91%. Affected by the inflow of two tributaries, the contribution rate of domestic sewage and manure in the M2 section was slightly higher than that in the M1 section. However, due to the large discharge of the Yellow River, the pollution contribution of the two tributaries for the main stream was small, and the nitrate in the main stream came from the migration of pollution in the upper stream.

The main nitrate sources of the two tributaries during the dry season were domestic sewage and manure, accounting for 45.23–47.40%, followed by soil nitrogen, accounting for 31.35–34%, and fertilizer and atmospheric deposition, accounting for 17.98–18.89% and 1.88–3.27%, respectively. During the wet season, the primary nitrate source of T1 was still domestic sewage and manure, but for T2, it became soil nitrogen, accounting for 40.05%, and the proportion of domestic sewage and manure was reduced to 37.50%, mainly due to the fact that the discharge of T2 was significantly increased, resulting in increased soil erosion in the basin.

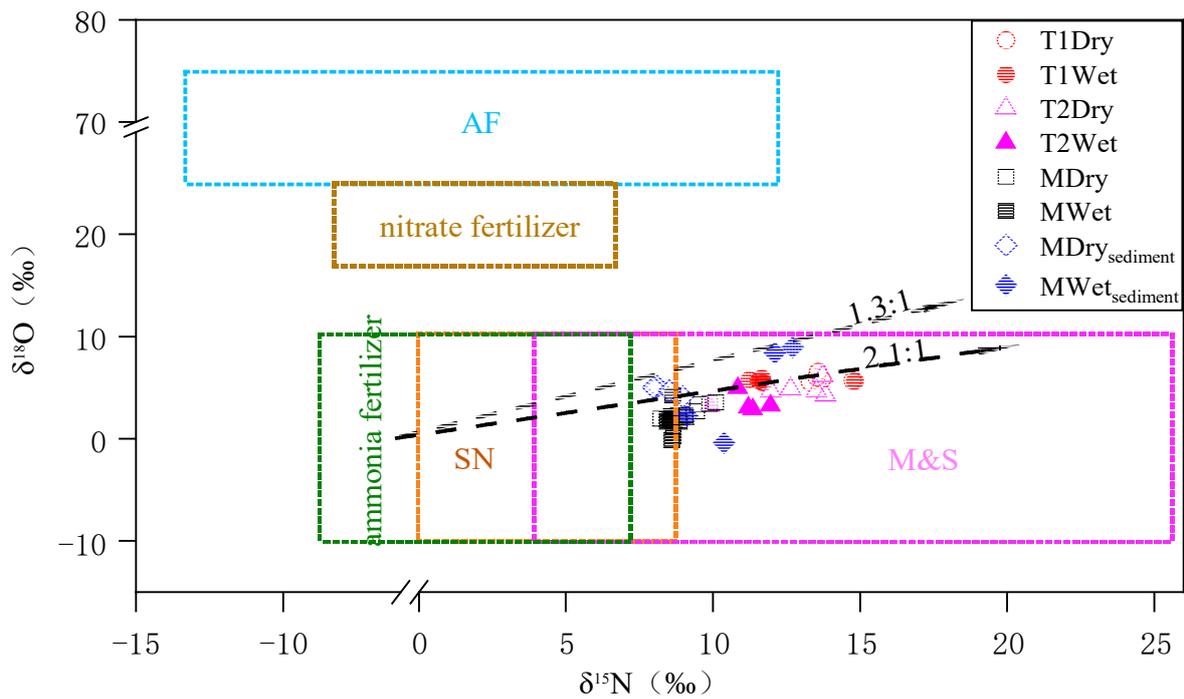


Figure 3. Relationship between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values during the different sampling periods. AF indicates atmospheric deposition of nitrogen; SN indicates soil nitrogen; M&S indicate manures and sewage.

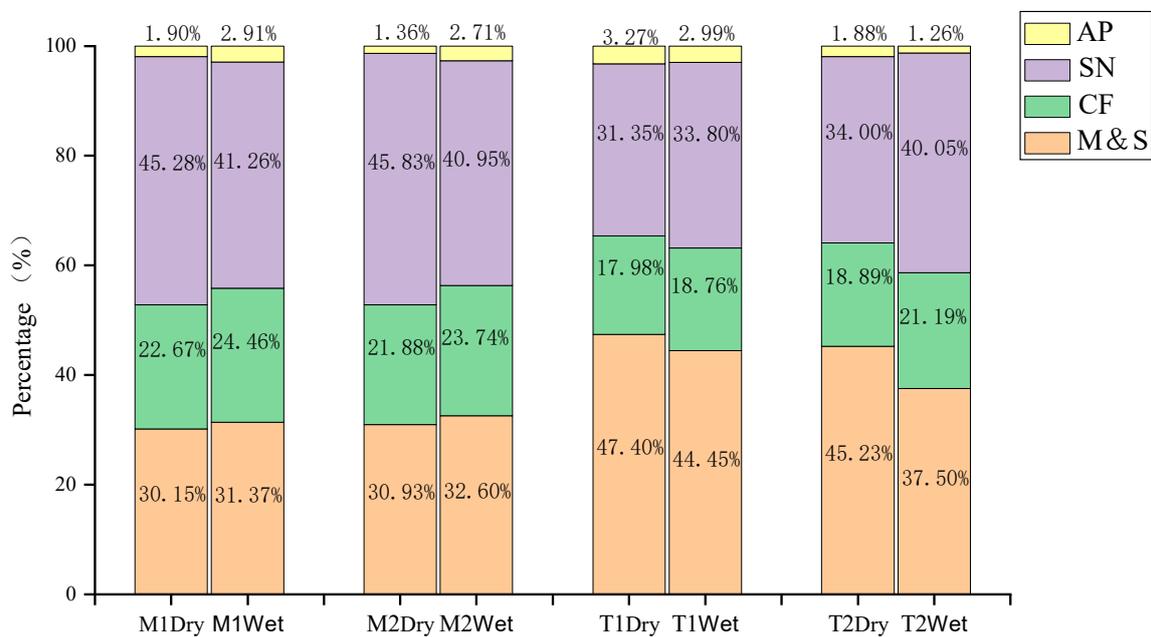


Figure 4. Seasonal and spatial contributions of nitrate sources estimated via SIAR. CF indicates chemical nitrogen fertilizer.

4.2. Analysis of the Transformation Pathways of Nitrogen Pollutants

The biological transformation processes of nitrogen, such as nitrification, assimilation, and denitrification, can all lead to isotopic enrichment and change. In the nitrification process, two-thirds of the O in the produced nitrate comes from H_2O , while one-third comes from O_2 in the atmosphere. Studies have shown that the $\delta^{18}\text{O}\text{-NO}_3^-$ value generated during the nitrification process is $-10\text{--}10\text{‰}$ [2]. In this study, the $\delta^{18}\text{O}$ of the main stream

ranged from 1.86‰ to 3.55‰, while that of the tributaries ranged from 4.25‰ to 6.54‰. Meanwhile, the higher temperature in the wet season resulted in lower concentrations of ammonia nitrogen compared to those in the dry season, indicating that the nitrification process was the main nitrogen conversion process in the river, which is consistent with the research results reported by Yue [3].

The denitrification process reduced the concentration of NO_3^- , while $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ enriched in NO_3^- , and there was an obvious linear relationship between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, with the $\delta^{15}\text{N}/\delta^{18}\text{O}$ ratio typically ranging from 1.3 to 2.1 [25,33,36]. During the wet season, the nitrate concentrations in the four sections were significantly lower than those in the dry season (Table 1), and the $\delta^{15}\text{N}/\delta^{18}\text{O}$ of some samples in T1 and T2 ranged from 1.3 to 2.1 (Figure 3), but the $\delta^{15}\text{N}/\delta^{18}\text{O}$ values in the main stream were not within the typical range of denitrification, indicating that denitrification occurred in the tributaries during the wet season. Qin's [37] research showed that the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the Qin River were mainly affected by the mixing and nitrification processes, and there was no obvious denitrification effect. Our research results are different, mainly because in the actual environment, affected by various factors, the isotope information on denitrification may be obscured [20–22], while the sampling time employed by Qin was only once in August, which could not fully reflect the isotope characteristics.

Due to the conservative chemical properties of Cl^- , the molar ratio of $\text{NO}_3^-/\text{Cl}^-$ can usually be used as an auxiliary indicator to judge the sources and transformations of nitrate. $\rho(\text{Cl}^-)$ did not change, while the $\text{NO}_3^-/\text{Cl}^-$ ratio changed significantly, indicating that denitrification may have occurred in the river. In contrast, if the $\text{NO}_3^-/\text{Cl}^-$ ratio remained constant but the $\rho(\text{Cl}^-)$ concentration changed, this would indicate that mixing processes occurred in the river [38].

According to Figure 5, compared with the dry season, the change in $\rho(\text{Cl}^-)$ in the main stream was not obvious during the wet season, but the $\rho(\text{Cl}^-)$ in tributaries was decreased, and the $\text{NO}_3^-/\text{Cl}^-$ ratios in both the main stream and tributary T1 were also decreased, possibly due to denitrification processes reducing the NO_3^- concentration. There was little change in the $\text{NO}_3^-/\text{Cl}^-$ ratio in tributary T2, indicating that T2 was more affected by mixing processes [36], which is also the reason why denitrification information in tributary T2 was easily obscured.

Further analysis of surface sediments of the main stream revealed that most of the $\delta^{15}\text{N}\text{-NO}_3^-/\delta^{18}\text{O}\text{-NO}_3^-$ values were between 1.3 and 2.1 (Figure 3), confirming denitrification occurred in the main stream, mainly in the surface sediment region. According to research, nitrate in the water of small rivers is more likely to interact with bed sediments compared with large rivers, so denitrification is more likely to occur in small rivers [39]. However, in large rivers, factors such as changes in discharge or small changes in isotope ratios would have a greater uncertain impact on the interpretation of isotope results [20]. The isotope information on the main stream in this study did not indicate a denitrification process, and this result was also confirmed. Therefore, increasing the sampling frequency and incorporating other methods for comprehensive analysis can accurately reflect the transformations of nitrogen in river, especially regarding the denitrification process.

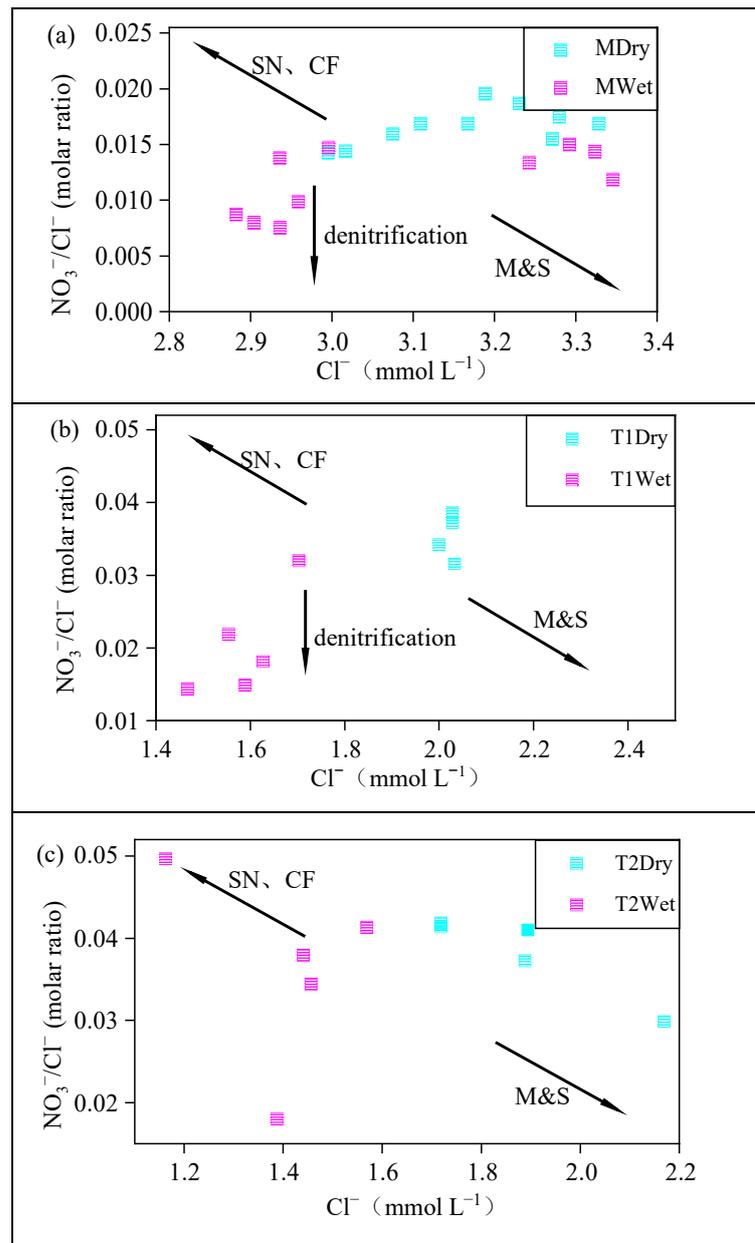


Figure 5. The relationship between Cl^- concentration and $\text{NO}_3^-/\text{Cl}^-$. (a) The mainstream of the Yellow River; (b) the Yiluo River (T1); (c) the Qin River (T2).

5. Conclusions

In this study, combined with water quality parameters and nitrogen and oxygen isotope values monitored several times, the sources and transformation processes of nitrate in the middle reaches of the Yellow River and its tributaries during the dry season and wet season were analyzed, and the proportions of each nitrate source were estimated using the SIAR model. Under the influence of pollution migration in the upper reaches, the main nitrate sources in the main stream of the Yellow River were soil nitrogen (40.95–45.83%) and domestic sewage and manure (30.93–32.60%), respectively, with little variation between the dry season and wet season. During the dry season, the nitrate sources of the two tributaries were mainly domestic sewage and manure (45.23–47.40%), followed by soil nitrogen (31.35–34.00%). However, the primary nitrate source of T2 (Qin River) became soil nitrogen (40.05%) during the wet season, mainly because of the significant increase in river discharge and the increase in soil erosion in the basin. Therefore, reducing domestic

sewage discharge and controlling soil nitrogen loss are the key measures for improving the water quality of the Yellow River.

During the wet season, the concentrations of TN and NO_3^- significantly decreased and nitrification and denitrification processes occurred in both the main stream and tributaries of the Yellow River. In addition, the T2 tributary (Qin River) was also significantly affected by mixed dilution. High-frequency sampling can reflect the isotopic information on nitrate in the river more comprehensively, contributing to a more accurate understanding of the conversion process of nitrate.

Author Contributions: Conceptualization, C.S. and R.Z.; methodology, X.X.; software, Y.S.; validation, C.S. and Y.S.; formal analysis, X.X.; investigation, C.S., Y.S., R.Z. and X.X.; resources, Y.S.; data curation, R.Z.; writing—original draft preparation, C.S. and Y.S.; writing—review and editing, C.S. and Y.S.; visualization, X.X. and J.L.; supervision, X.X. and J.L.; project administration, X.X. and J.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Opening Project of Key Laboratory of Lower Yellow River Channel and Estuary Regulation, MWR (HHNS202004).

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The funders had no role in the design of this study.

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