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Using $\delta^{15}N$ and $\delta^{18}O$ Signatures to Evaluate Nitrate Sources and Transformations in Four Inflowing Rivers, North of Taihu Lake

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Abstract: Taihu Lake is the third largest freshwater lake in China. Due to rapid economic development and excessive nutrient discharges, there is serious eutrophication in the northern part of the lake. Nitrogen (N) is one of the key factors for eutrophication in Taihu Lake, which mainly comes from the rivers around the lake. Samples from four inflowing rivers were analysed for $\delta^{15}N$ and $\delta^{18}O$ isotopes in December 2013 to identify the different sources of nitrogen in the northern part of Taihu Lake. The results indicated that the water quality in Taihu Lake was clearly influenced by the water quality of the inflowing rivers and nitrate (NO_3^--N) was the main component of the soluble inorganic nitrogen in water. The soil organic N represented more than 70% of the total NO_3^--N loads in the Zhihugang. Domestic sewage was the major NO_3^--N source in the Liangxi river, with a contribution of greater than 50%. Soil organic N and domestic sewage, with contributions of more than 30% and 35% respectively, were the major NO_3^--N sources in the Lihe river and Daxigang river. Denitrification might be responsible for the shifting $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values in the Daxigang river, and a mixing process may play a major role in N transformations in the Lihe river in winter. The results of this study will be useful as reference values for reducing NO_3^- pollution in the inflowing rivers in the north of Taihu Lake.

Keywords: nitrate; stable isotopes; nitrate sources; nitrogen transformations; denitrification

1. Introduction

Taihu Lake is the third largest freshwater lake in China, with an area of 2338 km^2 and an average depth of 2 m. The average growth rate of Gross Domestic Product (GDP) was 13.46% and the average growth rate of the population was 5.24‰ from 1991 to 2008 in Taihu Lake basin. This growth has resulted in large quantities of nutrients being discharged into Taihu Lake, which have caused eutrophication of the lake [1]. After years of environmental regulation, eutrophication problems are still serious. It has been reported that the main source of pollution into Taihu Lake was from the rivers around the lake, and nitrogen (N) was the main pollutant of these rivers [2–4]. Paired-sample T test analysis indicated that the input of total nitrogen (TN) to Taihu Lake was different ($p = 0.034 < 0.05$) between the 1990s and the early 21st century, and the inputs of TN to Taihu Lake were still rising (Figure 1) [5,6]. Some research illustrated that the inflowing rivers in the west or northwest of Taihu Lake were seriously polluted, and more than 70% of the TN entering Taihu Lake was from the northern rivers [7]. Therefore, it is important and necessary to study the sources of N in the northern rivers. With the rapid development of the economy and the population increases in recent decades, excess

nitrogenous substances have drained into the rivers [8] from synthetic N fertilizers [9], animal wastes and manure, sewage, and atmospheric deposition [10], which has caused adverse effects on the water's ecological environment, such as eutrophication and the degradation of ecosystems (National Research Council, 2000). According to the date of pollutants of Taihu Lake in 1998, the pollutants from domestic sewage and agriculture accounted for 25% and 28% of TN, respectively [11]. It is necessary to identify the sources and transformations of nitrate in water to control nitrate pollution.

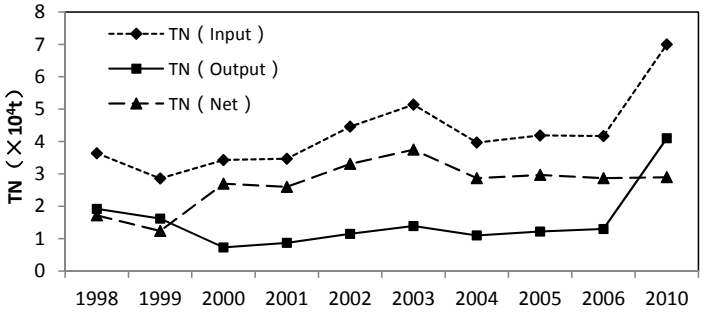

Figure 1. Total Nitrogen (TN) input and output throughout the years in Taihu Lake [2,4].

Surface water systems are very vulnerable to nitrate pollution. The sources of nitrate are mainly rain, chemical fertilizers, sewage and animal wastes, and nitrate derived from nitrification. Due to the distinct isotopic characteristics of nitrate, stable isotope techniques have been used to identify the sources of nitrate-nitrogen [12,13]. The range of $\delta^{15}N$ values of artificial fertilizers generally extends from −6‰ to +6‰. There are some natural factors and biological activities that cause small variations in the N isotopic ratios of soil organic N, such as soil depth, vegetation types, mineralization and nitrification. Therefore, $\delta^{15}N$ values for soil organic N generally range from 0‰ to +8‰ [14,15]. The N isotopic ratios in sewage and manure are more highly enriched than those of the other NO_3^--N sources. During the storage, treatment, and application of sewage and animal wastes, the ammonia in the sewage evaporates, causing ^{15}N enrichment in the residual NH_4^+-N. The remaining NH_4^+-N is subsequently oxidized into $\delta^{15}N$-enriched nitrate. As a result of this process, the $\delta^{15}N$ values of sewage generally range from +8‰ to +20‰ [16,17].

However, as a result of fractionation, the N isotope composition of NO_3^- could change during transformation [18]. For example, ammonification and denitrification results in N isotope fractionation between organic matter and NH_4^+ [19,20]. Therefore, the NO_3^--N source cannot be identified reliably by using the $\delta^{15}N$-NO_3^- technique on its own. In order to solve this problem, a dual isotope method with analysis of $\delta^{15}N$ and $\delta^{18}O$ values of nitrate (i.e., $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-) has been used to identify NO_3^--N sources [21,22]. Researchers have found that $\delta^{18}O$-NO_3^- is not only helpful for identifying sources of NO_3^--N, but it is also useful to help distinguish between NO_3^--N from atmosphere deposition, biological processes in soil and chemical fertilizers, and to study biological denitrification processes in water [23–28] . It has been suggested that $\delta^{18}O$ was more useful for separating atmospheric NO_3^--N deposition from biologically-produced soil NO_3^--N than $\delta^{15}N$ [28]. The $\delta^{18}O$ values in NO_3^--N fertilizers range from +17‰ to +25‰ and the $\delta^{18}O$ values of atmospheric NO_3^--N deposition are higher than 60‰ [29].

Many studies have used N and O stable isotope ratios to identify the sources of nitrate in Taihu Lake. In those studies of Taihu Lake, the researchers found that the value of ^{15}N-NO_3^- in winter was lowest of the four seasons, and human activities were the main source of nitrate of Taihu Lake in winter, but agriculture was the main source of nitrate of Taihu Lake in spring and summer [30,31]. In some studies, some researchers found that in winter, the biodegradation was weak and the N and O stable isotopes were similar from year to year in Taihu Lake basin [32]. Some research found that the main source of N pollution of water in Taihu basin was not the N fertilizer from farmland but the domestic sewage and human and animal excreta discharged into the water, and the atmospheric deposition

was also another source of the surface waters [33]. But, in some studies, the results indicated that the non-domestic sewage (not the domestic sewage) was the primary source of nitrate pollution in Taihu Lake [34]. The nitrate of groundwater was also a risk of lake eutrophication and water safety [35], but not the main nitrate source in Taihu Lake [36]. These studies have covered many fields, such as the nitrate source of Taihu Lake in different seasons, the reliability of the method to trace the nitrate source, and so on. However, the results of these studies have shown some inconsistencies with the main source of nitrate in Taihu Lake in the same season or the same region, and the contribution of the main sources has not been quantified either.

In order to obtain more reliable results, samples were collected in winter and N and O stable isotopes were used to identify and quantify the nitrate sources in four rivers (Zhihugang river, Liangxi river, Lihe river, and Daxigang river) flowing into the lake. We hope the results of this study will be useful as reference values for reducing NO_3^--N pollution in the inflowing rivers in the north of Taihu Lake, and assist the management of land-use and pollution-source control in the watershed.

2. Study Area

The Zhihugang river and the Liangxi river connect with Meiliang Bay, which is located in the northwest of Taihu Lake. Land in the watershed is mainly used for growing crops, industry and human settlements. According to data for the period from 2005 to 2007, the mean TN concentrations in the Zhihugang and Liangxi rivers were 5.38 mg/L and 6.90 mg/L respectively [37]. The Lihe and the Daxigang rivers are located to the northeast of Taihu Lake; they flow into Gonghu Bay. These catchments are dominated by forests and cropland, and land bordering the rivers which is used for construction [38]. Basic information about the Zhihugang, Liangxi, Lihe, and Daxigang rivers is shown in Table 1.

Table 1. Hydrologic data of the four studied rivers and annual amounts of contaminants imported.

River Name	Connected Lake Area	Length (km)	Bottom Width (m)	Annual Runoff ($\times 10^8$ m^3)	Annual Amounts of Contaminants Imported (ton)	
					TN	TP
Zhihugang river [a,b]	Meiliang Bay	20.51	20–40	3.15	930.15	33.33
Liangxi river [a,b]	Meiliang Bay	7.97	15–60	0.42	280.80	11.36
Lihe river [a]	GongHu Bay	6.87	10	1.07	910.60	37.61
Daxigang river [a,b]	GongHu Bay	5.04	8	1.31	105.40	29.10

Notes: [a] [39]; [b] [40].

3. Sample Collection and Measurement

Water samples were collected from the Zhihugang (ZH), Liangxi (LX), Lihe (LH), and Daxigang (DX) rivers from 5 to 15 December 2013. Sampling sites ZH1, LX1, LH1, and DX1 were located in the upper reaches of the rivers and were surrounded by many industries and houses. Sampling sites ZH4, LX4, LH4, and DX4 were located in the estuaries to Taihu Lake. Sampling sites ZH2, LX2, LX3, LH2, LH3, DX2, and DX3 were adjacent to settlements and cropland. Sampling sites ZH3 and LH3 were located in industrial areas (Figure 2).

pH and dissolved oxygen (DO) were determined directly in situ using a multi-parameter water quality monitoring instrument (METTLER TOLEDO, SevenGo Duo pro, SG68, America). Calibration of sensors was performed before the measurements. Ammonium (NH_4^+-N) was measured with Nessler' reagent; nitrate nitrogen (NO_3^--N) and total nitrogen (TN) were determined through ultraviolet spectrophotometry and the alkaline potassium persulfate oxidation-UV spectrophotometric method (GB3838-2002) respectively. Chloride (Cl^-) were measured using silver nitrate titration. Detection limits were 0.025, 0.02, 0.003 and 0.5 mg/L for NH_4^+-N, NO_3^--N, TN, and chloride (Cl^-) respectively. Total organic carbon (TOC) concentrations were determined by a TOC-V Analyser (SHIMADZU -TNM-1, Japan), with an estimated detection limit of 0.05 μg/L.

The concentrations of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were determined by the denitrifier method [41–43] using a stable isotope ratio mass spectrometer. The USGS-32 (180 ± 0.5‰, 25.7 ± 0.4‰), USGS-34 (−1.8 ± 0.2‰, −27.9 ± 0.4‰), USGS-35 (2.7 ± 0.3‰, 57.5 ± 0.4‰) and IAEA-N3 (4.7 ± 0.3‰, 25.6 ± 0.5‰) international reference materials for $\delta^{15}N$ and $\delta^{18}O$ were used to calibrate the sample data after correction for blanks. The sample detection error was 0.2‰.

The isotope results are expressed in δ units defined as:

$$\delta_{sample}(‰) = ((R_{sample} - R_{standard})/R_{standard}) \times 1000 \quad (1)$$

where R represents the $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratio, expressed as $\delta^{15}N$ and $\delta^{18}O$. $\delta^{15}N$ and $\delta^{18}O$ were expressed relative to air and the Vienna standard mean ocean water (V-SMOW), respectively.

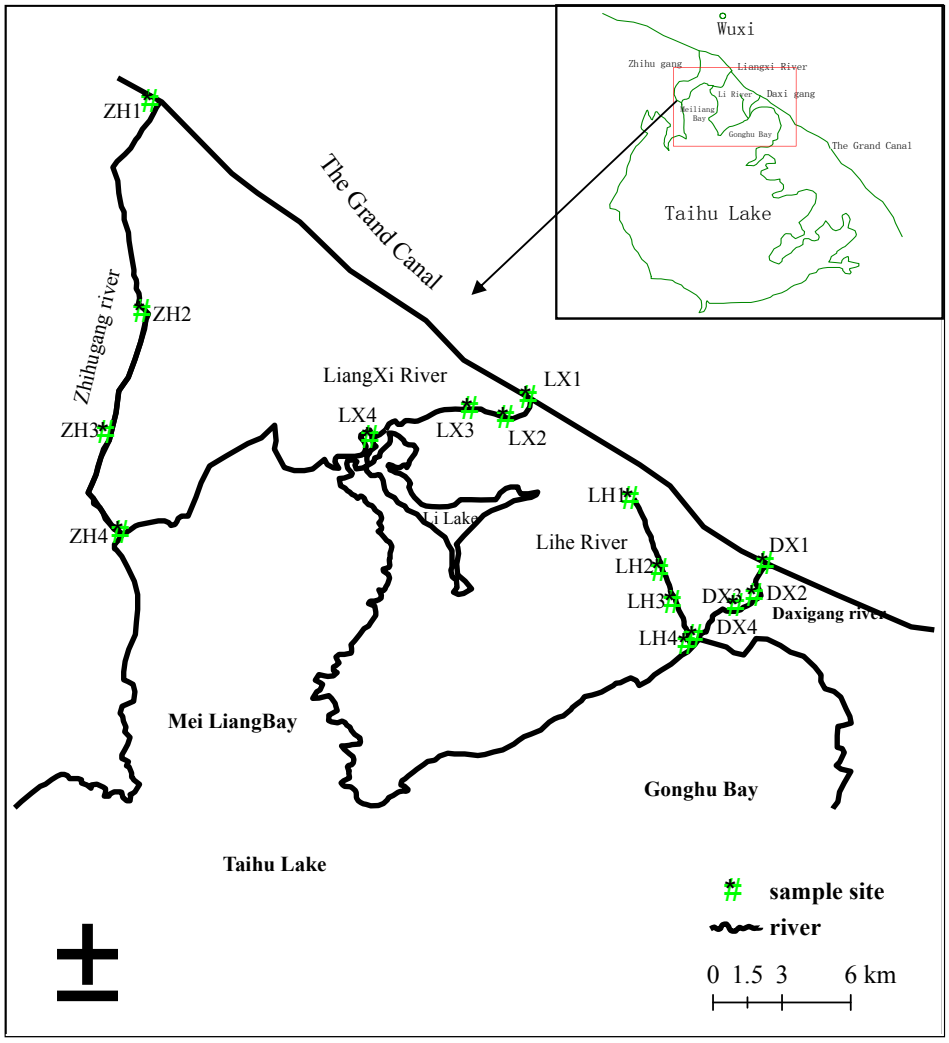

Figure 2. Location of the rivers studied (Zhihugang, Liangxi, Lihe, and Daxigang rivers) and their sampling sites.

4. Results and Discussion

4.1. Physicochemical Characteristic of Water Samples

Information on the water chemistry and concentrations of dissolution inorganic nitrogen (DIN) of the samples are presented in Table 2. The pH of the Zhihugang, Liangxi, Lihe, and Daxigang rivers ranged from 7.72 to 7.94 (mean = 7.79), 8.46 to 8.58 (mean = 8.51), 8.08 to 8.53 (mean = 8.30),

and from 8.09 to 8.84 (mean = 8.42), respectively. The four rivers were slightly alkaline. DO in the Zhihugang, Liangxi, Lihe, and Daxigang Rivers ranged from 5.90 to 9.24 mg/L (mean = 7.23 mg/L), 10.49 to 10.73 mg/L (mean = 10.60 mg/L), 8.37 to 10.07 mg/L (mean = 9.47 mg/L), and from 8.66 to 11.38 mg/L (mean = 9.84 mg/L), respectively. The method of Paired-sample T test indicated that DO in Zhihugang river was significantly different from Liangxi river and Li river (p = 0.022 and p = 0.042, respectively), and was not significantly different with Daxigang river (p = 0.082); and there was a significant difference between Liangxi river and Lihe river (p = 0.046), but not with Daxigang river (p = 0.314).

Table 2. Physicochemical characteristics and isotopic compositions of water in four studied rivers (mean values ± SD).

Sample	pH	DO	Cl^-	TOC	NH_4^+-N	NO_3^--N	TN	$\delta^{15}N$-NO_3^-	$\delta^{18}O$-NO_3^-
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(‰)	(‰)
ZH1	7.66 ± 0.23	6.24 ± 0.33	166.7 ± 2.4	28.9 ± 2.2	2.95 ± 0.43	4.45 ± 0.73	7.50 ± 1.03	7.75 ± 0.2	−0.12 ± 0.1
ZH2	7.72 ± 0.33	7.55 ± 0.44	90.7 ± 4.3	33.4 ± 2.1	1.09 ± 0.23	3.77 ± 0.43	5.12 ± 0.63	4.83 ± 0.1	−7.65 ± 0.2
ZH3	7.94 ± 0.23	5.9 ± 0.23	77.1 ± 3.2	48.4 ± 4.2	4.75 ± 0.37	2.40 ± 0.12	7.34 ± 1.31	8.35 ± 0.2	−6.28 ± 0.2
ZH4	7.84 ± 0.34	9.24 ± 0.46	81.9 ± 2.3	43.9 ± 2.2	1.25 ± 0.22	3.08 ± 0.27	4.55 ± 0.68	9.15 ± 0.1	−3.78 ± 0.2
LX1	8.46 ± 0.31	10.49 ± 0.13	59.9 ± 3.1	28.4 ± 2.7	0.46 ± 0.24	0.79 ± 0.22	1.35 ± 0.23	15.11 ± 0.2	4.35 ± 0.1
LX2	8.48 ± 0.13	10.68 ± 0.23	63.1 ± 2.7	29.2 ± 4.2	0.13 ± 0.04	0.83 ± 0.21	1.18 ± 0.33	10.81 ± 0.2	5.32 ± 0.1
LX3	8.53 ± 0.32	10.73 ± 0.51	59.1 ± 2.1	45.5 ± 1.7	0.32 ± 0.11	0.78 ± 0.14	1.18 ± 0.32	12.32 ± 0.1	0.11 ± 0.2
LX4	8.58 ± 0.31	10.57 ± 0.23	61.4 ± 3.3	31.1 ± 1.9	0.46 ± 0.13	0.76 ± 0.17	1.33 ± 0.28	11.80 ± 0.2	9.38 ± 0.1
LH1	8.37 ± 0.36	8.37 ± 0.38	63.7 ± 5.1	24.6 ± 2.3	0.51 ± 0.13	0.53 ± 0.31	1.14 ± 0.21	11.62 ± 0.2	12.35 ± 0.2
LH2	8.23 ± 0.43	9.57 ± 0.53	20.4 ± 4.2	31.8 ± 2.9	0.21 ± 0.03	0.29 ± 0.03	0.71 ± 0.53	10.22 ± 0.2	8.67 ± 0.1
LH3	8.08 ± 0.53	9.87 ± 0.37	61.8 ± 1.1	25.5 ± 3.3	0.10 ± 0.03	0.42 ± 0.11	1.14 ± 0.13	7.94 ± 0.2	−5.00 ± 0.1
LH4	8.53 ± 0.38	10.07 ± 0.49	68.2 ± 1.6	25.4 ± 2.5	0.24 ± 0.11	0.41 ± 0.15	1.35 ± 0.23	8.10 ± 0.1	2.52 ± 0.1
DX1	8.20 ± 0.31	8.79 ± 0.33	110.9 ± 2.1	30.2 ± 1.6	1.61 ± 0.55	3.31 ± 0.41	5.12 ± 0.53	8.84 ± 0.2	1.01 ± 0.2
DX2	8.09 ± 0.45	8.66 ± 0.53	129.5 ± 3.6	33.1 ± 1.7	0.57 ± 0.11	0.72 ± 0.23	1.30 ± 0.39	13.64 ± 0.2	−1.47 ± 0.1
DX3	8.84 ± 0.34	11.38 ± 0.56	47.7 ± 1.8	25.9 ± 2.2	0.46 ± 0.18	0.34 ± 0.02	1.68 ± 0.32	5.04 ± 0.2	−12.26 ± 0.1
DX4	8.56 ± 0.53	10.53 ± 0.43	54.8 ± 1.9	32.7 ± 2.2	0.24 ± 0.02	0.43 ± 0.11	1.14 ± 0.13	10.05 ± 0.2	−8.96 ± 0.1

Concentrations of NH_4^+-N in the Zhihugang, Liangxi, Lihe, and Daxigang rivers ranged from 1.09 to 4.75 mg/L (mean = 2.52 mg/L), 0.13 to 0.46 mg/L (mean = 0.35 mg/L), 0.10 to 0.51 mg/L (mean = 0.27 mg/L), and from 0.24 to 1.61 mg/L (mean = 0.72 mg/L), respectively. The highest concentration of NH_4^+-N in four rivers appeared at ZH3, LX1, LX4, LH1, DX1, and lowest concentration appeared at ZH2, LX2, LH3, DX4, respectively. NO_3^--N concentrations in the Zhihugang, Liangxi, Lihe, and Daxigang rivers ranged from 2.40 to 4.45 mg/L (mean = 3.43 mg/L), 0.76 to 0.83 mg/L (mean = 0.79 mg/L), 0.29 to 0.53 mg/L (mean = 0.42 mg/L), and from 0.43 to 3.31 mg/L (mean = 1.20 mg/L), respectively. The highest concentrations of NO_3^--N in four rivers appeared at ZH1, LX2, LH1 and DX1, and the lowest concentrations appeared at ZH3, LX4, LH2 and DX3. TN concentrations in the Zhihugang, Liangxi, Lihe, and Daxigang rivers ranged from 4.45 to 7.50 mg/L (mean = 6.12 mg/L), 1.18 to 1.35 mg/L (mean = 1.26 mg/L), 0.71 to 1.35 mg/L (mean = 1.08 mg/L), and from 1.14 to 5.12 mg/L (mean = 2.31 mg/L) respectively.

By one-way analysis of Variance (ANOVA) analysis of NH_4^+, NO_3^- and TN of four rivers (p = 0.015 < 0.05, 0.001 < 0.05 and 0.00 < 0.05, respective), the concentration of NH_4^+, NO_3^- and TN in four rivers had significant differences, and it might indicate that the sources in the four rivers were different.

Variations of the NH_4^+-N, NO_3^--N, and TN concentrations in the Zhihugang river, Liangxi river, Lihe river, and Daxigang rivers are shown in Figure A1 (shown in Appendix A). Because of the extensive area the Grand Canal basin covers and the large number of industries and residential areas within it, NH_4^+-N and NO_3^--N concentrations are high in the Grand Canal. The NH_4^+-N and NO_3^--N concentrations at site ZH1 in the Zhihugang river and at site DX1 in the Daxigang river were higher than at the other sampling sites because of inputs from the Grand Canal and high density population and factories around the sample sites. The NH_4^+-N and NO_3^--N concentrations at LX1 were lower than those at ZH1 and DX1, because the NH_4^+-N and NO_3^--N concentrations in the Grand Canal were lower in the section that fed the Liangxi river than in the section that fed the Zhihugang and Daxigang rivers [44] and there is lower density of population and factories. NH_4^+-N and NO_3^--N

concentrations were higher at the source of the Lihe river (LH1) than at the other sampling sites in this river; this is because LH1 is located at the junction of two rivers, one of which (the Liangtang river) received inputs from the Grand Canal. Out of all the rivers, there was more variation in the NH_4^+-N and NO_3^--N concentrations in the Zhihugang river, mainly because it is the longest of the four rivers, and there are more pollution sources with a wide range of NH_4^+-N and NO_3^--N concentrations along the river [45–47]. The NH_4^+-N and NO_3^--N concentrations were higher in Meiliang Bay than in Gonghu Bay, and the NH_4^+-N and NO_3^--N concentrations were higher at ZH4 and LX4 than at LH4 and DX4, because of mixing processes.

NO_3^--N concentrations were higher than NH_4^+-N concentrations in the four rivers, and NO_3^--N in the four rivers was not from the nitrification of NH_4^+-N in water, but from the external pollution sources. Pearson correlation analysis (shown in Appendix A, Table A1) showed that there were no strong positive relationships between DO and NO_3^--N or negative relationships between DO and NH_4^+-N, but there was a strong positive relationship between NO_3^--N and NH_4^+-N ($p < 0.01$). The low temperatures ($\approx 5 \pm 3$ °C) were not beneficial for nitrification (Brookshire, et al., 2011), indicating that nitrification in surface water might be not intense in our study. As reported, water quality and level of eutrophication in Meiliang Bay were more severe than in Gonghu Bay [48,49]. It indicated that the water quality of rivers had significant effect to the lake, and it is necessary to study the nitrate source and N transformation of the rivers.

4.2. Nitrate Sources and Nitrogen Transformation

The values of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in the Zhihugang river ranged from +4.83‰ to +9.15‰ and from −0.12‰ to −7.65‰, with mean values of +7.52‰ to −4.46‰, respectively (Table 2). The values of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in the Liangxi river ranged from +10.81‰ to +15.11‰ and from +0.11‰ to +9.38‰, with mean values of +12.50‰ and +4.49‰ respectively. The values of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in the Lihe river ranged from +7.94‰ to +11.62‰ and from −5.00‰ to +12.35‰ (Table 2), with mean values of +9.47‰ and +4.63‰ respectively. The δ^{15}N-NO_3^- values in the Lihe river gradually declined in clear steps, such that the δ^{15}N-NO_3^- value at L3 was negative, and was different from the values at the other Lihe river sites. The δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values in the Daxigang river ranged from +5.04‰ to +13.64‰ and from −12.26‰ to +1.01‰, with mean values of +9.39‰ and −5.42‰ respectively. The δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values in the Daxigang river fluctuated significantly, indicating that N transformations may be influenced by a range of factors, such as microbial nitrification, denitrification, mixing or assimilation by algae [25,26,50,51].

The δ^{15}N values in water in this study ranged from +4‰ to +15‰ (Table 2), which indicates that the NO_3^--N source of these four rivers is very complicated. Because of the wide variability of NO_3^--N from different sources, in this study we used the δ^{18}O-NO_3^- ratio to help identify NO_3^--N sources [15,52]. The δ^{18}O-NO_3^- values in our samples were lower than 15‰, further suggesting that neither atmospheric precipitation [29] (δ^{18}O-NO_3^- > 60‰,) nor nitrate-fertilizer is major sources of riverine nitrate in our study.

After one-way ANOVA analysis of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in the four rivers, it was found there were no significant differences of δ^{15}N-NO_3^- ($p = 0.071 > 0.05$), but significant differences of δ^{18}O-NO_3^- ($p = 0.032 < 0.05$) in the four rivers. Thus, we inferred that the sources of nitrate in the four rivers might be different.

Figure 3 shows that there may be differences between the NO_3^--N sources in the four rivers. The NO_3^--N that primarily originates from sewage has δ^{15}N-NO_3^- values of nearly 10‰, such as were reported for sites ZH1, ZH3, ZH4, and DX1. These sites could be assigned to one source. Water samples with a low NO_3^--N content, such as those at sites LH3, LH4, and DX3, have δ^{15}N-NO_3^- values between +3‰ and +8‰, suggesting that the NO_3^--N might be originally derived from nitrification of ammonium in precipitation, fertilizer, sewage and manure, as well as soil organic N. But the value of δ^{18}O-NO_3^- at these sites was from −12.26‰ to +2.56‰, that was much less than the value of δ^{18}O-NO_3^- coming from the nitrification of ammonium (+25‰–+75‰) in precipitation and fertilizer

(+17‰–+25‰) [53–55]. Thus we inferred that the source of nitrate of these sites was from soil organic N (−10‰–+15‰) [56]. The low water temperature (≈5 ± 3 °C) and the high dissolved oxygen (DO > 6 mg/L) was not beneficial for denitrification, and there was no significant negative correlation between NO_3^- and $\delta^{15}N\text{-}NO_3^-$ by Pearson correlation analysis, so we inferred that the denitrification at these points was not the main reason for the low concentration of nitrate nitrogen and high $\delta^{15}N\text{-}NO_3^-$ values in the water [57,58]. So the samples with low NO_3^--N concentrations have $\delta^{15}N\text{-}NO_3^-$ values greater than 10‰, which indicates that the NO_3^--N was the result of a mixture of sewage and soil organic N; this was the case at sampling sites ZX2, LX1, LX2, LX3, LX4, LH1, LH2, DX2, and DX4.

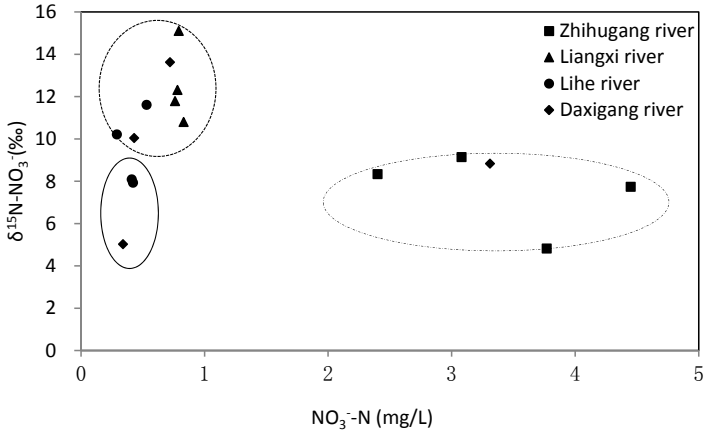

Figure 3. Relationships between NO_3^--N and $\delta^{15}N\text{-}NO_3^-$ in the Zhihugang, Liangxi, Lihe, and Daxigang rivers.

Several influence factors were found, which influence isotopic compositions of NO_3^--N sources, such as mixing, ammonification, nitrification, denitrification and so on. Although each NO_3^--N source has its own distinctive isotopic composition, the mixture may lead the intermediate values. The ammonification and nitrification results in some fractionation of $\delta^{15}N\text{-}NO_3^-$, the enrichment factors are between −1‰ to +1‰ and 12‰ to 29‰ [59]. Microbial denitrification may result in some fractionation of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, however the enrichment factors are within −40‰ to −5‰ and −18‰ to −8‰ respectively [60,61]. Therefore, Cl^- may be a useful indicator of sewage and manure, because of its stability in water and its resistance to physical, chemical and biological processes. Therefore, the NO_3^--N/Cl^- ratio can be used to obtain further information about the effects of N transformation processes, such as dilution or denitrification [21,53]. The Cl^- concentrations in the Zhihugang river gradually declined along the river (Table 2), and there was generally a positive correlation (Figure 4, R^2 = 0.7223) between Cl^- and NO_3^--N, which indicates that mixing and dilution processes had a major effect on NO_3^--N transport. A positive relationship (Figure 5) was found between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, with a linear regression slope of 0.8628 in the Zhihugang river, which indicated that denitrification occurred in the Zhihugang river but less significantly. There was little variation in Cl^- concentrations in the Liangxi river (Table 2), and Cl^- and NO_3^--N in the Liangxi river were not correlated (Figure 4, R^2 = 0.4555), which indicates that mixing and dilution processes had limited effects on NO_3^--N transport. There was a negative correlation between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ in the Liangxi river (Figure 5, k = −0.4337), which suggests that denitrification may not be the reason for the shift in the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values, and $\delta^{18}O\text{-}NO_3^-$ values indicates a mixing of different nitrate sources. Algae would easily grow in the Liangxi river because of its slow flow, and assimilation by algae would lead to enrichment of residual NO_3^--N with heavy isotopes [62,63]; this helps explain the low NH_4^+-N and NO_3^--N concentrations, and the high $\delta^{15}N\text{-}NO_3^-$ values, in the Liangxi river.

The ranges of Cl^- concentrations in Lihe river exhibited no large variation, but a positive correlation (Figure 4, R^2 = 0.6497) between Cl^- and NO_3^--N had been observed, indicating that the mixing process had an effect on nitrate transportation in Lihe river. No negative relationship between NO_3^--N and $\delta^{15}N$/ $\delta^{18}O$-NO_3^- was observed (not shown) in the Lihe river. But a positive relationship was found between $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- with the slope of the linear regression of 3.95 (Figure 5, R^2 = 0.8526) in Lihe river, which indicated that denitrification might be responsible for the shifting $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values, although the slope was bigger than that reported for denitrification (1.3–2.1) [64–66]. No clear positive correlation (Figure 4, R^2 = 0.2651) between Cl^- and NO_3^--N was observed, but a gradual decline of Cl^- concentrations was observed in the Daxigang river, which may indicate that dilution plays a major role in the variation of Cl^- and NO_3^-. A strong positive (Figure 5) between $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-, with a linear regression slope of 1.0797 of the Daxigang river (Figure 5, R^2 = 0.378), which indicated that denitrification might be mainly responsible for the shifting $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values.

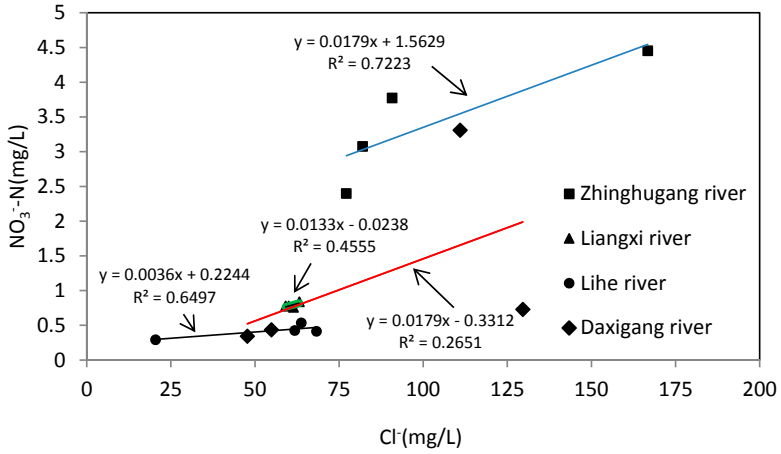

Figure 4. Relationships between Cl^- and NO_3^--N concentrations in the Zhihugang, Liangxi, Lihe, and Daxigang rivers.

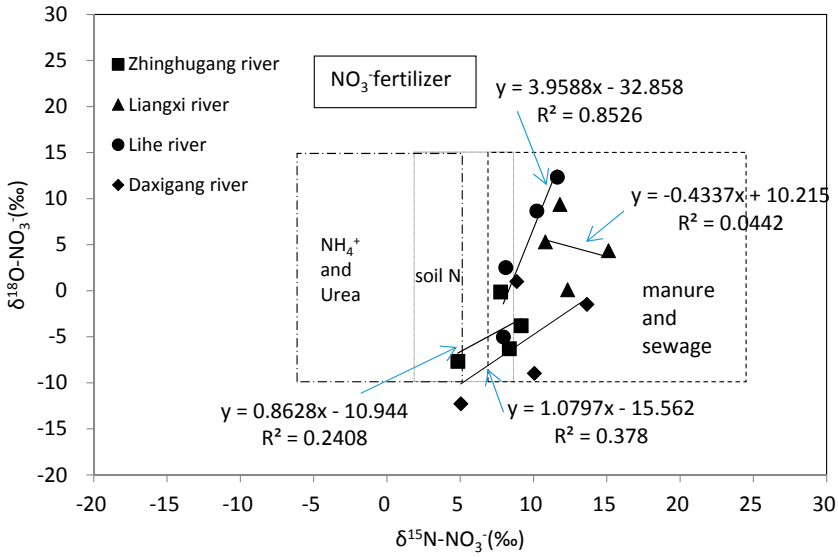

Figure 5. Relationships between $\delta^{15}N$ and $\delta^{18}O$ of NO_3^--N in the Zhihugang, Liangxi, Lihe, and Daxigang rivers. The isotopic compositions of various sources in the diagram were modified after [15,16,53,67].

4.3. Quantification of Nitrate Sources in Rivers

The sampling sites were close to areas of human habitation, agricultural land and forest land [68]. In order to quantify the NO_3^--N sources in the rivers, it was assumed that the nitrate of the rivers mainly comes from three sources (chemical fertilizer, soil organic N, and sewage) because of the type of land use around the rivers. When NO_3^--N from these three sources was mixed together, an isotope mixing model could be used to determine the relative importance of these sources. The mixing model is described by the following formulae:

$$\delta^{15}N_M = f_1\delta^{15}N_c + f_2\delta^{15}N_{son} + f_3\delta^{15}N_s \quad (2)$$

$$\delta^{18}O_M = f_1\delta^{18}O_c + f_2\delta^{18}O_{son} + f_3\delta^{18}O_s \quad (3)$$

$$1 = f_c + f_{son} + f_s, \quad (4)$$

where $\delta^{15}N_M$ and $\delta^{18}O_M$ are the NO_3^--N isotope values from the mixture. The subscripts c, son and s represent the NO_3^--N sources of chemical fertilizer, soil organic N and sewage. f is defined as the proportional contribution of the respective source. Results of the calculations are shown in Table 3.

Table 3. Contribution of nitrate sources in rivers.

River Name	N_M (‰)	O_M (‰)	Contributions of the Respective Sources		
			Chemical Fertilizer (%)	Soil Organic N (%)	Sewage (%)
Zhihugang river	7.51	−4.46	5.43–19.45	75.06–79.47	1.44–17.04
Liangxi river	12.51	4.49	7.68–9.85	27.89–35.63	56.70–71.13
Lihe river	9.47	4.63	15.50–26.97	33.24–41.20	35.43–48.51
Daxigang river	9.39	5.42	17.79–29.01	30.09–38.48	35.06–49.36
Value of different NO_3^--N sources					
chemical fertilizer [a]	0–0.8	17 [c]–19			
soil organic N [a]	6.6–9	−10–−9			
Sewage [b]	15–16.3	10–11			

Notes: [a] Date from [64]; [b] Data from [16];[c] [69].

As shown in Table 3, nitrification of soil organic N and sewage were the dominant NO_3^--N source in Liangxi, Lihe, and Daxigang rivers, which is consistent with results of Ding et al. [36]. In this paper, nitrification of soil organic N, but not the sewage, was the dominant NO_3^--N source in the Zhihugang river, which was inconsistent with the results of Chen et al. [70]. The reason for this difference might be that there are a lot of tributaries and farmland in the Zhihugang river basin, and also the fact that winter is the wheat season in the Taihu basin. During the wheat season, NO_3^--N accounted for 62–91% of the TN [71] that entered the main channel of the Zhihugang river through surface runoff or tributaries. This result indicated that the main source of nitrate in the Zhihugang river was soil organic N in winter. Domestic sewage was the major NO_3^--N source in the Liangxi river, which is consistent with the results of Lin et al. [30]. Domestic sewage and soil organic N were the major NO_3^--N sources in the Lihe river and the Daxigang river, which is consistent with the results of Yang et al. [72].

In order to assist pollution source control in the watershed, the annual amount of NO_3^--N imported to the four rivers has been quantified. The estimating formula is described by the following formulae:

$$T\ (NO_3^-\text{-}N^-)_I\ (ton/a) = C_i\ (mg/L) \times Q_i\ (m^3/a) \times f_i\ (\%) \times 10^{-6} \quad (5)$$

where T $(NO_3^-\text{-}N)_I$ are the annual total amounts of the three NO_3^--N sources (chemical fertilizer, soil organic N, and sewage). C_i is the average concentration of NO_3^--N of four rivers. According the Table 2, the average concentration of NO_3^--N in the Zhihugang, Liangxi, Lihe and Daxigang rivers

was 3.42 (mg/L), 0.79 (mg/L), 0.41 (mg/L) and 1.20 (mg/L) respectively. f_i are the contributions of the respective sources of NO_3^--N. Q_i was the annual runoff of four rivers (Table 1). Results of the calculations are shown in Table 4.

Table 4. Import of nitrate in the four rivers.

River Name	Annual Amounts of NO_3^--N (ton)	Respective Sources Imported (ton)		
		Chemical Fertilizer	Soil Organic N	Sewage
Zhihugang river	1078.8	58.5–209.8	809.8–857.3	15.5–183.8
Liangxi river	33.5	2.5–3.3	9.3–11.9	19..3–23.8
Lihe river	44.13	6.8–11.9	14.6–15.7	15.6–21.4
Daxigang river	157.2	27.9–45.6	47.3–60.4	55.1–77.5

Table 4 shows that the annual amounts of NO_3^--N in the Zhihugang river and Daxigang river were higher than the annual amounts of TN in the two rivers (in Table 1).This could be because the annual amount of TN in the four rivers in Table 1 was the net amount of TN, but the result of the Table 4 was the gross amount. But this result indicates that nitrate pollution was the main nitrogen pollution in the Zhihugang and Daxigang rivers, and the N transformations in the two rivers were complicated, such as the denitrification. So, pollution control and harnessing in the Zhihugang and Daxigang river basins should be strengthened. Annual amounts of NO_3^--N from sewage in the four rivers discharging into Meiliang Bay and Gonghu Bay were 34.8 to 207.6 ton and 70.7 to 98.9 ton respectively. Some reports have shown that the annual average proportions of the domestic sewage source in Meiliang Bay and Gonghu Bay were 17.2% and 15.3% respectively [34]. Based on the results of Zhen et al. [34] and Wang et al. [40], annual total amounts of NO_3^--N from sewage discharging into Meiliang Bay and Gonghu Bay were about 357 and 253 ton respectively. The sewage was not the main NO_3^--N source in Zhihugang river, but the maximum annual amount of NO_3^--N from sewage accounted for about 51.5% of annual total amounts NO_3^--N imported into Meiliang Bay. Therefore, it is very necessary to pay attention to the control of sewage pollution in the watershed of the Zhihugang river.

5. Conclusions

Results from this study of four rivers to the north of Taihu Lake show that the water quality of the rivers to the northwest of Taihu Lake was worse than that of rivers in the northeast (according to the national quality standards for surface waters, China (GB3838-2002)). Also, NO_3^--N was the dominant inorganic N species in the four rivers during the sampling season. It means that we need more strict protection measures, emission standards and wastewater treatment with the abilities of denitrification to protect the rivers to the northwest of Taihu Lake. Because it was winter, we ignored the contribution of atmospheric NO_3^--N to rivers. Soil organic N, with a contribution from 75.06% to 79.47%, was the major NO_3^--N source in the Zhihugang. Domestic sewage, with a contribution from 56.70% to 71.13%, was the major NO_3^--N source in the Liangxi river. Dilution and mixing processes may play a major role in N transformations in the Zhihugang river, but might contribute to assimilation by algae in the Liangxi river. Soil organic N and domestic sewage were the main NO_3^--N sources in the Lihe and Daxigang rivers, with contributions greater than 30% and 35% respectively. Denitrification might result in the enrichment of heavy isotopes of NO_3^--N in the Daxigang river, but mixing process may play a major role in N transformations in the Lihe river. The maximum annual amount of NO_3^--N from sewage in the Zhhugang river was highest of the four rivers. Therefore, while sewage was not the main NO_3^--N source in the Zhihugang river, it is very necessary that we pay attention to the control of sewage pollution in the watershed of the Zhihugang river.

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Author Contributions: Da Li, Xia Jiang and Binghui Zheng conceived, designed, conducted and analyzed experimental data of this research; Da Li wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Ethical Statement: In this paper, I guarantee that information I provide above is correct, and any part of the paper has not been published or being reviewed elsewhere, and that I did not violate ethical standards and there is no plagiarism in this study.

Appendix A

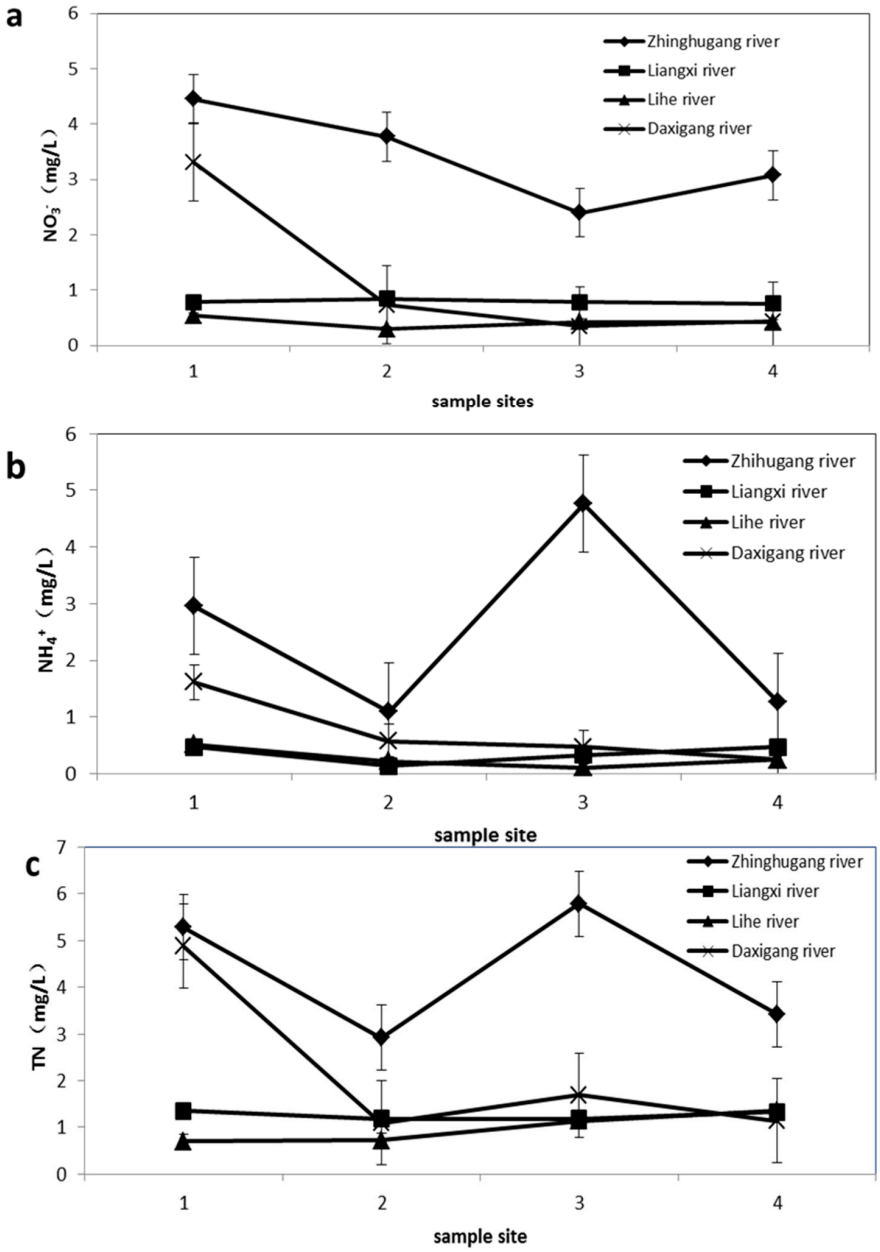

Figure A1. Spatial variations of (**a**) $NH4^+$-N; (**b**) NO_3—N; and (**c**) TN in the Zhihugang, Liangxi, Lihe and Daxigang rivers.

Table A1. Correlation coefficients between nitrogen and physicochemical variables in the Zhihugang, Liangxi, Lihe and Daxigang rivers.

Zhihugang River							
	pH	DO	TOC	Cl^-	NH_4^+-N	NO_3^--N	TN
pH	1	0.013	0.992 **	−0.783	−0.993 **	−0.993 **	−0.023
DO		1	0.136	−0.391	−0.060	−0.060	−0.933 *
TOC			1	−0.806	−0.986 **	−0.986 **	−0.124
Cl^-				1	0.850	0.850	0.550
NH_4^+					1	1.000 **	0.106
NO_3^-						1	0.106
TN							1
Liangxi River							
	pH	DO	TOC	Cl^-	NH_4^+-N	NO_3^--N	TN
pH	1	0.196	0.349	−0.073	−0.591	−0.591	0.075
DO		1	0.704	0.057	0.418	0.418	−0.959 *
TOC			1	−0.652	−0.256	−0.256	−0.553
Cl^-				1	0.675	0.675	−0.172
NH_4^+					1	1.000 **	−0.642
NO_3^-						1	−0.642
TN							1
Lihe River							
	pH	DO	TOC	Cl^-	NH_4^+-N	NO_3^--N	TN
pH	1	−0.077	−0.275	0.358	0.257	0.257	0.479
DO		1	0.212	−0.047	−0.625	−0.625	0.297
TOC			1	−0.982 *	−0.895	−0.895	−0.832
Cl^-				1	0.806	0.806	0.922
NH_4^+					1	1.000 **	0.530
NO_3^-						1	0.530
TN							1
Daxigang River							
	pH	DO	TOC	Cl^-	NH_4^+-N	NO_3^--N	TN
pH	1	0.993 **	−0.963 *	−0.713	−0.533	−0.533	−0.362
DO		1	−0.632	−0.971 *	−0.620	−0.620	−0.463
TOC			1	0.532	0.035	0.035	−0.169
Cl^-				1	0.515	0.515	0.373
NH_4^+					1	1.000 **	0.978 *
NO_3^-						1	0.978 *
TN							1

Notes: * Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

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