# Distribution Characteristics of Nitrogen and Its Stable Isotopes in Water Bodies of a Subtropical Agricultural Small Watershed (Postprint)

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## Abstract

To control nitrogen nutrient loss from watersheds and improve aquatic environment, continuous experimental observations were conducted on ammonium nitrogen (NH4+-N) and nitrate nitrogen (NO3−-N) concentrations in surface water, as well as \$ $15Nofnitratenitrogen($ \$15N-NO3−) in water and \$ $15Noforganicmatter($ \$15N-Org) in sediments, using the typical subtropical agricultural small watershed of Tuojia River as the study area. The spatiotemporal characteristics of nitrogen concentrations and their stable isotope values were analyzed, and the environmental factors affecting nitrogen distribution as well as the possible sources of water NO3− and sediment organic nitrogen were discussed. The results showed that: water NO3−-N concentration was significantly higher than NH4+-N, with mean values of 1.62 mg · L−1 and 0.90 mg · L−1, respectively, being higher in June, August, and winter; NH4+-N concentrations in urban and farmland areas showed significant differences from other types of areas (P<0.05), and were significantly higher than other water bodies, while NO3−-N concentrations were higher in urban, farmland, and forested mountain areas, and lower in reservoir areas. Tributary NH4+-N concentrations were higher than those in the main stream, both exhibiting winter > spring > summer > autumn; main stream and tributary NO3−-N concentrations exhibited winter > summer > autumn > spring and autumn > winter > summer > spring, respectively. Both source and outlet waters showed NO3−-N concentrations higher than NH4+-N, with nitrogen concentrations at the source lower than at the outlet. The distribution ranges of water \$ \$15N-NO3− and sediment \$ \$15N-Org values were −19.87‰~8.11‰ and −0.69‰~6.51‰, respectively; the highest water \$ \$15N-NO3− value occurred in Grade river sections, and the lowest value appeared in Grade river sections, with small differences in water \$ \$15N-NO3− among different river sections in November, but obvious differences in January and February; the highest riverbed sediment

$ $15N-Org value was also located in Grade river sections, while the lowest value was in Grade river sections, with $ $15N-Org values in Grade and river sections showing relatively consistent temporal variation trends, and the minimum $ $15N-Org values in Grade and river sections appearing in January. In conclusion, nitrogen pollution exists in Tuojia River water, dominated by external input; the main sources of water nitrogen are soil organic matter, synthetic fertilizers, and terrestrial organic matter; conducting research on nitrogen distribution and sources in watersheds has scientific significance for understanding source apportionment of nitrogen pollutants at the watershed scale.

## Full Text

### Distribution of Nitrogen and Its Stable Isotope from a Small Agricultural Catchment in the Subtropics

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**Abstract:** To control nitrogen nutrient loss and improve water quality in the Tuojia River basin, a typical small agricultural catchment in the subtropics, we conducted continuous monitoring of ammonium nitrogen ($NH_4^+ - N$), nitrate nitrogen ($NO_3^- - N$), $\delta^{15}N - NO_3^-$ in water, and $\delta^{15}N - Org$ in sediment. The spatial and temporal characteristics of nitrogen concentrations and stable isotope values were analyzed, and environmental factors influencing nitrogen distribution were examined to identify potential sources of $NO_3^- - N$ and sediment organic nitrogen. Results showed that $NO_3^- - N$ concentrations were significantly higher than $NH_4^+ - N$ concentrations, with mean values of 1.62 mg · L $^{1}$ and 0.90 mg · L $^{1}$, respectively. Elevated concentrations occurred in June, August, and winter. $NH_4^+ - N$ concentrations in urban and farmland areas differed significantly from other regions (P<0.05) and were notably higher than in other water bodies. $NO_3^- - N$ concentrations were elevated in urban, farmland, and forested mountain areas, but lower in reservoir zones. Tributary $NH_4^+ - N$ concentrations exceeded those in the mainstream, following the pattern winter > spring > summer > autumn. Mainstream and tributary $NO_3^- - N$ concentrations showed patterns of winter > summer > autumn > spring and autumn > winter > summer > spring, respectively. Both source and outlet locations exhibited higher $NO_3^- - N$ than $NH_4^+ - N$, with lower nitrogen concentrations at the source than at the outlet. $\delta^{15}N - NO_3^-$ values ranged from -19.87‰ to 8.11‰, while sediment $\delta^{15}N - Org$ ranged from -0.69‰ to 6.51‰. The highest $\delta^{15}N - NO_3^-$ occurred in Reach III, the lowest in Reach IV. Inter-reach differences in $\delta^{15}N-NO_3^-$ were minor in November but pronounced in January and February. Sediment $\delta^{15}N-Org$ showed similar patterns, with the highest value in Reach III and lowest in Reach I. Reaches III and IV exhibited consistent temporal trends, while Reaches I and II showed minimum values in January. Overall, the Tuojia River exhibits nitrogen pollution dominated by external inputs, with primary sources including soil organic matter, synthetic fertilizers, and terrestrial organic matter. This study provides scientific insight into nitrogen pollutant source apportionment at the catchment scale.

**Keywords:** Tuojia River; subtropical agricultural catchment; nitrogen pollution; nitrogen isotope; nitrogen source

## Introduction

The nitrogen cycle is one of the most critical processes in nature, with nitrogen being an essential element for all organisms and widely used in industrial and agricultural production [1-2]. Human activities have had the most pronounced impact on the nitrogen cycle over the past 2.5 billion years [3], with contributions increasing dramatically since the 20th century [4]. While nitrogen use is vital for societal development, excess nitrogen is a major cause of eutrophication in freshwater ecosystems, estuaries, and coastal regions [5]. Nitrogen pollution has attracted widespread research attention. Previous studies indicate that nitrogen loss in runoff primarily occurs as $NH_4^+-N$ and $NO_3^--N$ [6], with influencing factors including climate (precipitation), soil properties, topography, vegetation, tillage practices, cropping structure, and livestock management [7]. For example, Zhu et al. [8] examined nitrogen pollution in the Danjiangkou Reservoir watershed and identified dissolved organic nitrogen and nitrate as key control targets. Liao et al. [9] analyzed nitrogen distribution in the Dongjiang River system, finding severe nitrogen pollution primarily from agricultural non-point sources and direct domestic/industrial point sources. Zhao et al. [10] studied nitrogen loading from rivers, wet/dry deposition, and sediment sources in Erhai Lake, identifying the early rainy season as critical for controlling external nitrogen loads. While these studies analyzed spatial-temporal distribution characteristics and environmental factors, traditional methods relying on land use surveys combined with physicochemical properties yield indirect conclusions that cannot definitively identify nitrogen sources [11].

Stable isotope tracing technology can reveal food sources and trophic relationships, assess aquatic ecosystem nutrient status, and reflect human impacts on water environments [12]. Nitrogen isotope composition serves as an effective tracer of anthropogenic nitrogen sources, with variations monitoring changes in shallow aquatic ecosystems affected by human activities and wastewater [13]. This method has become crucial for identifying nitrogen sources in aquatic ecosystems, with domestic applications reported for groundwater [14], large rivers [15-16], lakes [17-19], and reservoirs [20]. However, research on subtropical small agricultural catchments remains limited. Such catchments, heavily influenced by local agricultural and industrial activities, exhibit nitrogen cycling with distinct anthropogenic signatures. Complex water system types in small catchments receive substantial terrestrial nitrogen inputs [21-22]. The Tuojia River basin, a typical subtropical agricultural catchment with intensive farming activities and evident nitrogen pollution, was selected to analyze spatial-temporal nitrogen distribution and use stable isotopes to examine $NO_3^- - N$ and sediment organic matter $\delta^{15}N$ composition. This research identifies environmental factors influencing nitrogen distribution and potential sources, providing a basis for controlling nutrient loss and nitrogen pollution.

### 1.1 Study Area Description

The Tuojia River basin is a typical small agricultural catchment in the mid-subtropics, located in Jinjing Town, Changsha County, Hunan Province (27°55 - 28°40 N, 112°56 -113°30 E). As a second-order tributary of the Xiang River system, it covers 52.10 km$^2$ with an average elevation of 98.3 m and a general north-high-south-low topography characteristic of red soil hilly landscapes. The region has a subtropical humid monsoon climate with an average annual temperature of 17.2°C and annual precipitation of 1,422 mm [23], featuring distinct seasons. Land use is diverse, including forest, paddy fields, tea plantations, orchards, and vegetable plots, dominated by forest, paddy fields, and tea plantations. Rice cultivation occupies 32% of the catchment area [23]. Red soil predominates, with intensive agricultural activity centered on double-cropping rice systems in valley and floodplain areas. Heavy fertilizer and pesticide application, particularly unreasonable nitrogen fertilizer use, has resulted in significant non-point source pollution.

### 1.2 Sampling Site Selection

Based on literature review and field investigation of the Tuojia River basin, 20 sampling sites were established according to water system structure [Figure 1: see original paper]. Sites A1-A13 represent mainstream locations, while B1-B7 represent tributaries.

Based on land use characteristics, the water system was divided into five subtype zones: urban area (A1, A2, A3, B1), farmland area (A4, A5, A6, A8, A9, A10, B2, B3, B4, B5, B6), residential area (A11), forested mountain area (A7), and reservoir area (A12, A13, B7). For analyzing nitrogen distribution in different river components, the system was further classified as source area (A7, A12, A13), river outlet (A1), mainstream (A2-A11), and tributaries (B1-B7). Based on stream order characteristics, the river was divided into Reaches I-IV from source (A7) to estuary (A1). Stable nitrogen isotope samples were collected at A7, B3, A5, and A1, representing Reaches I-IV, respectively.

### 1.3 Sample Collection and Analysis

#### 1.3.1 Water Sample Collection and $NH_4^+-N$, $NO_3^--N$ Determination

At each site, approximately 200 mL of surface water (0-30 cm) was collected in polyethylene bottles with three replicates. Samples were transported to the laboratory within 3 hours and stored refrigerated (1-4°C) or frozen (-20°C) until monthly batch analysis for $NH_4^+-N$ and $NO_3^--N$ concentrations. Refrigerated samples were analyzed directly, while frozen samples were thawed at 4°C for 24 hours before analysis [23]. Concentrations were determined using a flow injection analyzer (FIA-500 star, FOSS) at the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

#### 1.3.2 $\delta^{15}N$ Determination in Water and Sediment Organic Matter

Water and sediment samples for $\delta^{15}N$ analysis were collected as described above. Sediment samples were dried at 60-70°C for 24-48 hours, then ground with a mortar, ball mill, or grinder and sieved through a 60-mesh screen to ensure homogeneity. Sieved samples were weighed and introduced via a solid autosampler into an elemental analyzer, where nitrogenous compounds were converted to $N_2$ through oxidation and reduction furnaces. The $N_2$ was then transferred through a ConfloIV interface to an isotope ratio mass spectrometer (IRMS-MAT253) to obtain $^{15}N/^{14}N$ ratios. Liquid samples were acidified, concentrated, dried to powder, and analyzed directly by EA-IRMS [24]. All procedures were performed at the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Isotopic composition was calculated as:

$$\delta^{15}\mathrm{N}(‰) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (1)$$

where $R = {}^{15}\mathrm{N}/{}^{14}\mathrm{N}$ (2), with $R_{\text{standard}}$ representing the atmospheric nitrogen standard. Analytical error was <1‰.

### 1.4 Data Processing

SPSS 17.0 was used for statistical analysis. $NH_4^+ - N$ and $NO_3^- - N$ concentration data were tested for normality (Shapiro-Wilk test) and found to be non-normally distributed (P<0.05), prompting non-parametric Kruskal-Wallis tests. One-way ANOVA examined spatial-temporal differences in nitrogen concentrations. Microsoft Excel 2007 was used for calculating means and standard errors, with error bars representing standard errors in all figures.

## 2.1 Nitrogen Concentration Characteristics in Surface Water

During the study period, $NH_4^+ - N$ and $NO_3^- - N$ concentrations in Tuojia River surface water ranged from 0.30-1.35 mg · L$^{1}$ (mean 0.90±0.10 mg · L$^{1}$) and 0.82-2.45 mg · L$^{1}$ (mean 1.62±0.16 mg · L$^{1}$), respectively [Figure 2: see original paper]. $NH_4^+ - N$ concentrations exceeded the mean in March, June, December, and February, while $NO_3^- - N$ concentrations were above average in August, November, and December-February, with both peaking in winter. $NO_3^- - N$ concentrations were significantly higher than $NH_4^+ - N$.

## 2.2 Nitrogen Concentration Variations in Different Land Use Zones

As shown in [Figure 3a: see original paper], $NO_3^- - N$ concentrations exceeded $NH_4^+ - N$ in all zone types throughout the study period, with the most pronounced differences in forested mountain areas. $NH_4^+ - N$ concentrations in urban and farmland zones differed significantly from other areas ($P<0.05$) and were substantially higher than in other water bodies. $NO_3^- - N$ concentrations were elevated in urban, farmland, and forested mountain zones but lower in reservoir areas.

Seasonally, urban and farmland zones showed higher $NH_4^+ - N$ concentrations in summer compared to other seasons [Figure 3b: see original paper], while $NO_3^- - N$ concentrations were higher in winter and lower in spring, with urban and farmland zones exceeding other water bodies and forested mountain and reservoir zones showing lower values. Forested mountain zones also exhibited higher $NO_3^- - N$ in summer [Figure 3c: see original paper].

## 2.3 Nitrogen Variations in Mainstream, Tributaries, Source, and Estuary

All river components (mainstream, tributaries, source, and estuary) showed higher $NO_3^- - N$ than $NH_4^+ - N$ concentrations . Seasonally [Figure 4: see original paper], mainstream $NH_4^+ - N$ concentrations differed significantly between winter and autumn ($P<0.05$), while tributary $NH_4^+ - N$ showed significant differences between winter and the other three seasons. Spring, summer, and autumn differences were not significant. $NH_4^+ - N$ concentrations were consistently higher in tributaries than mainstream. $NO_3^- - N$ concentrations showed significant differences between winter and spring ($P<0.05$), being slightly higher in mainstream during spring, summer, and winter, but higher in tributaries during autumn.

Both source and estuary exhibited higher $NO_3^- - N$ than $NH_4^+ - N$, with source concentrations lower than estuary . Source $NH_4^+ - N$ concentrations differed significantly among spring, autumn, and winter compared to summer ($P<0.05$), while estuary $NH_4^+ - N$ showed significant differences between winter and autumn ($P<0.05$), with summer concentrations exceeding other seasons. Source $NO_3^- - N$ showed no significant seasonal differences, being higher in summer and winter and lower in spring and autumn, while estuary $NO_3^- - N$ differed significantly between winter and other seasons. $NO_3^- - N$ concentrations were consistently lower at the source than at the estuary, with both showing winter peaks [Figure 5: see original paper].

## 2.4 $\delta^{15}N$ Distribution Characteristics in Water and Sediment Organic Matter

During the study period, $\delta^{15}N - NO_3^-$ values ranged from -19.87‰ to 8.11‰ [Figure 6a: see original paper], with mean values of (-11.42±7.02)‰, (3.61±0.78)‰, (2.55±0.55)‰, and (3.41±2.37)‰ for Reaches IV (A1), III (A5), II (B3), and I (A7), respectively. The maximum occurred in Reach III and the minimum in Reach IV. Inter-reach differences were minor in November but pronounced in January and February, with Reach IV showing decreasing and more negative values and Reach III showing increasing values. Reach I showed elevated values in January.

Sediment $\delta^{15}N - Org$ values ranged from -0.69‰ to 6.51‰ [Figure 6b: see original paper], with means of 1.39‰, 2.83‰, 5.51‰, and 3.46‰ for Reaches I-IV, respectively. The maximum also occurred in Reach III, while the minimum was in Reach I. Reaches I and II showed consistent temporal trends with low, negative values in January, while Reaches III and IV showed similar patterns but with higher January values.

Sediment nitrogen isotope signatures reflect relative contributions from terrestrial and aquatic sources. Increased external nitrogen loading can elevate $\delta^{15}N - Org$, while $\delta^{15}N - NO_3^-$ variations indicate agricultural fertilizer and urban pollutant inputs [16]. Literature indicates terrestrial organic matter $\delta^{15}N$ ranges from -10‰ to 10‰, with aquatic organic matter averaging ~6.5‰ [25]. Soil nitrogen loss shows $\delta^{15}N$ of 3‰-8‰, synthetic fertilizers -4‰-4‰ [26], human/livestock waste nitrate 10‰-20‰ [27], and atmospheric $NO_3^- - N$ deposition 0.2‰-0.8‰ [28]. Using these characteristic $\delta^{15}N$ ranges with measured nitrogen concentrations allows identification of pollution sources. [Figure 7: see original paper] indicates that Tuojia River nitrogen is dominated by external inputs, with domestic sewage, agricultural non-point source pollution, and livestock waste as primary environmental factors.

## Discussion

Mean annual $NH_4^+ - N$ and $NO_3^- - N$ concentrations of 0.90 mg · L $^{1}$ and 1.62 mg · L $^{1}$ indicate nitrogen pollution. These values are similar to subtropical rivers [9,29-30] and lakes such as Poyanghu and Taihu [31-32] but differ from studies in the Ziya and Haihe Rivers [33-34], which have extensive urban-industrial discharges, large agricultural areas with severe nitrogen loss, and major livestock operations [35]. In contrast, the Tuojia catchment is a small agricultural watershed with extensive forest, farmland, tea plantations, and construction land, generating relatively small pollutant loads. Compared with the Danjiangkou Reservoir area, Tuojia shows higher $NH_4^+ - N$ due to significant agricultural impacts versus urbanization and water transfer project effects in Danjiangkou [33].

Elevated $NH_4^+ - N$ in June may relate to rice fertilization and drainage activities, while increased rainfall and runoff transport more livestock manure to rivers. The conversion of $NH_4^+ - N$ to $NO_3^- - N$ requires approximately one month [38], and July's early rice harvest and late rice planting may explain peak $NO_3^- - N$ concentrations in August from residual and basal fertilizers, consistent with Song et al. [37]. Winter low flow reduces self-purification capacity while external inputs from urban sewage and wastewater continue, and low temperatures limit microbial activity, resulting in nitrogen accumulation [8,23].

According to national surface water quality standards [39], urban and farmland zones meet Class III ($NH_4^+ - N \leq 1.5$ mg • L $^{1}$) and Class IV ($NH_4^+ - N \leq 1.0$ mg • L $^{1}$) criteria, respectively, with both zones exceeding other areas seasonally. Rice cultivation covers 32% of the catchment with nitrogen application rates of 374 kg • ha $^{1}$, with approximately 212.2 kg • ha $^{1}$ accumulating in soil or entering water bodies [41]. The plow pan layer in paddy soils restricts nitrogen migration to deeper layers, promoting runoff losses. Small factories and livestock farms distributed along riverbanks, combined with dense residential areas and inadequate wastewater treatment facilities [36], facilitate nitrogen entry into rivers, consistent with Wang et al. [41] and Ongley et al. [42]. Wang et al. [41] noted that nitrogen fertilizer use makes crop soils important contributors to river nitrogen loads. Lin et al. [43] found that excessive nitrogen fertilizer application and improper manure management created agricultural surplus nitrogen (72% of catchment load) and domestic discharge nitrogen (28%). Thus, agricultural fertilizer and domestic sewage are important pollution factors in urban and farmland zones.

Tributaries, classified as Class IV water, showed higher $NH_4^+ - N$ than the mainstream (Class III), while source water quality approached Class II standards. Tributaries extending through paddy fields with vegetable plots receive substantial nitrogen from fertilizer application. The permanently flooded, anaerobic conditions in paddy soils limit nitrification [44], while hospital wastewater discharge year-round contributes to higher tributary $NH_4^+ - N$. Liao et al. [9] found that besides mainstream nitrogen inputs, tributary transport is a major nitrogen source, a pattern consistent with Tuojia River. Additionally, nitrate's negative charge prevents soil adsorption, while positively charged $NH_4^+$ is readily adsorbed, contributing to higher $NO_3^- - N$ in mainstream and tributaries [45]. Elevated $NO_3^- - N$ in forested mountain (source) areas may result from aerobic conditions and low pH in subtropical red soils that inhibit denitrification [46]. Source area $NH_4^+ - N$ was relatively low due to minimal human impact and high vegetation cover that absorbs and degrades pollutants. Although plant uptake, sediment adsorption, and microbial decomposition affect nitrogen concentrations, the mainstream still receives substantial nitrogen inputs that converge at the outlet.

Based on characteristic $\delta^{15}N$ ranges, Tuojia River nitrogen sources are complex, similar to Erhai inflow rivers [18] but different from the Shiwuli and Nanfei Rivers [19], which flow through urban functional zones and industrial areas. As a typical agricultural catchment, Tuojia River is significantly impacted by agricultural activities, small factory wastewater, and rural domestic sewage. Reach III contains extensive farmland and livestock farms, likely causing its highest $\delta^{15}N$ values from fertilizer and waste, consistent with Xing et al. [47]. Elevated $\delta^{15}N-NO_3^-$ and $\delta^{15}N-Org$ values in January-February may reflect dominant denitrification at low temperatures [48]. In summary, nitrogen isotope distribution shows spatial-temporal variation with sources primarily from soil nitrogen loss, synthetic fertilizers, and terrestrial organic matter, consistent with identified pollution factors.

## Conclusions

1) The Tuojia River exhibits evident nitrogen pollution, most severe in urban (Class III) and farmland (Class IV) zones. Tributaries (Class IV) are more polluted than the mainstream (Class III), while the source area, minimally impacted by human activities, maintains better water quality approaching Class II standards.

2) Nitrogen distribution shows significant spatial-temporal variation, with catchment water environments heavily influenced by domestic and agricultural activities. External nitrogen inputs dominate, with domestic sewage, agricultural non-point source pollution, and livestock waste representing primary environmental factors.

3) Although $\delta^{15}N-NO_3^-$ and $\delta^{15}N-Org$ show spatial-temporal differences across reaches, nitrogen sources are similar throughout—characterized by combined pollution from soil nitrogen loss, synthetic fertilizers, and terrestrial organic matter, consistent with identified pollution factors.

4) Given limitations of single nitrogen isotope tracing, future research should combine nitrogen, hydrogen, and oxygen stable isotopes to comprehensively identify direct nitrogen pollution sources, providing scientific support for controlling nutrient loss and improving water quality.

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