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# A Stable Isotope Analysis to Quantify the Contribution of Basal Dietary Sources to Food Webs of Drinking Water Reservoirs

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**Abstract:** This study investigates the food web structure of the Xinlicheng Reservoir, a drinking water source of critical importance in Changchun, China, by employing stable isotope analysis (SIA) to quantify the contribution ratios of four basal dietary sources—phytoplankton, zooplankton, sediment organic matter, and particulate organic matter (POM)—to the diets of two key filter-feeding fish species, *Hypophthalm ichthys molitrix* and *Aristichthys nobilis*. The analysis reveals that phytoplankton is the dominant dietary source for both species, contributing 32.08% and 34.06%, respectively, whereas the POM contribution is discernably lower (13.25%). The average trophic level of the fish assemblage in Xinlicheng Reservoir is 3.03, while the trophic levels of the two filter-feeding species lie between 3.00 and 3.50. Furthermore, a random forest model was used to identify key environmental drivers of isotopic variations in these basal dietary sources, highlighting the significant role of pH, total nitrogen (TN), chloride ($Cl^-$), calcium ($Ca^{2+}$), phosphorus (TP), and silicate ($SiO_4^{4-}$) in influencing carbon and nitrogen isotopic ratios. These findings provide critical insights to optimize biomanipulation strategies aimed at improving water quality in drinking water reservoirs by enhancing our understanding of the environmental factors that govern trophic interactions and broader food web dynamics.

**Keywords:** drinking water reservoir; food web; dietary contribution proportions; stable isotope mixing models; random forest models; environmental factors

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**Citation:** Huang, F.; Zhao, W.; Qiao, X.; Xia, C.; Liu, Y.; Wei, J.; Yin, D.; Arhonditsis, G. A Stable Isotope Analysis to Quantify the Contribution of Basal Dietary Sources to Food Webs of Drinking Water Reservoirs. *Water* **2024**, *16*, 3338. https://doi.org/10.3390/w16223338

Received: 17 September 2024
Revised: 5 November 2024
Accepted: 12 November 2024
Published: 20 November 2024



## 1. Introduction

The protection of drinking water reservoirs has been a topical issue in the context of environmental management. These reservoirs, essential for human well-being and survival, face increasing threats from pollution due to overdevelopment and a multitude of other anthropogenic activities. Biomanipulation has emerged as a viable strategy for regulating aquatic ecosystems to improve water quality [1–6]. The fundamental concept of biomanipulation revolves around the modification of food web structures to enhance material cycling and energy flow and subsequently optimize ecosystem multifunctionality [1]. The success of biomanipulation strategies in drinking water reservoirs hinges upon a comprehensive understanding of food web structures and dynamics, especially the interactions between fish species and their dietary sources. However, the contribution of basal dietary sources is often surrounded by considerable uncertainty, which, in turn, leads to arbitrary decisions regarding the type and quantity of fish species introduced. To address this critical knowledge gap, the use of stable isotope analysis (SIA) offers an effective tool to delineate the relative strength of trophic relationships and elucidate the contributions of various dietary sources. By analyzing carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes, we can effectively characterize the interactions between fish species and their natural dietary sources. Stable carbon isotope ratios ($\delta^{13}C$) provide critical insights into the sources of primary production that directly reflect the nature of the base of food webs [7]. The $\delta^{13}C$ values of basal sources vary depending on their photosynthetic pathways, while the enrichment of $\delta^{13}C$ between successive trophic levels is minimal, which, in turn, makes $\delta^{13}C$ a useful tracer for mapping food chains [8,9]. Nitrogen isotope ratios ($\delta^{15}N$) typically increase by approximately 3.4‰ (±1‰) from prey to predators and can thus provide a reliable indicator of the trophic level positioning [9].

There is a growing body of literature that uses stable isotope data coupled with Stable Isotope Mixing Models (SIMMs) to quantify the contributions of different dietary sources to consumer nutrient intake. The application of SIMMs, such as SIAR, MixSIAR, and SIBER, provides researchers with powerful tools to unravel complex food web relationships along with the underlying dietary patterns [10–14]. In particular, SIMMs have been employed to investigate the degree of dependence of different fish species on basal dietary sources such as phytoplankton, zooplankton, and benthic organic matter [15–17]. The insights gained by these studies not only advance our understanding of aquatic food web structures, but also reveal how environmental changes can influence the availability and isotopic composition of basal dietary sources, thereby impacting the food selection strategies and nutritional status of higher-level consumers. The majority of these studies have focused on lakes, rivers, estuaries, and other aquatic environments, whereas drinking water reservoirs have received limited attention.

In drinking water reservoirs in China, artificial feeding and fertilization are prohibited, and thus their fish assemblages rely solely on natural food sources for survival. Variations in their physicochemical properties can profoundly shape the trophodynamics and the relative assimilation rates of basal dietary sources accordingly [18–20], and thus trigger shifts in their carbon and nitrogen isotope fractionation. Identifying the key environmental factors influencing the carbon and nitrogen isotopic variations of basal dietary sources can aid in comprehending their impact on the associated food web processes, and design biomanipulation treatments that can effectively bring about distinct water quality improvements.

In this broader context, the overarching goal of the present study is to shed light on critical aspects of the ecological systems typically characterizing drinking water reservoirs. We first use carbon and nitrogen stable isotope modeling to investigate the contribution of basal dietary sources to two key filter-feeding fish species, the silver carp (*Hypophthalmichthys molitrix*) and the bighead carp (*Aristichthys nobilis*). The first component of our work provides the foundation for designing management strategies in typical drinking water reservoirs. In such reservoirs, the introduction of the silver carp and the bighead carp is commonly used as a biological manipulation method to ameliorate the water quality conditions. The elucidation of the relative contribution of various dietary sources provides critical information about the optimal number and size of fish individuals to be introduced for effective water quality management. We then examine the temporal variations in the carbon and nitrogen isotope signatures of these basal dietary sources, which are then causally linked with the potential environmental drivers. The second part of our study will provide novel insights into the interplay among the physical, chemical, and biological constituents of the reservoir ecosystem and support more precise ecological management practices.

## 2. Materials and Methods

### *2.1. Sampling Time and Site Selection*

The study area is the Xinlicheng Reservoir, located at the southeastern part of Changchun City, Jilin Province, China, approximately 20 km from Changchun City (Figure 1). The Xinlicheng Reservoir is an important drinking water source for Changchun City. The

Xinlicheng Reservoir is located upstream of the Yitong River, with a river length of 114.8 km and a controlled watershed area of 1970 square kilometers. It has a total storage capacity of 551 million cubic meters and an average storage capacity of 322 million cubic meters over the past three years. With a normal water level of 219.63 m and corresponding capacity of 338 million cubic meters, the reservoir serves as the second-largest water source for Changchun city. It supplies water to the western urban area and provides 240,000 cubic meters of water daily, accounting for about one-third of the city's total water supply.

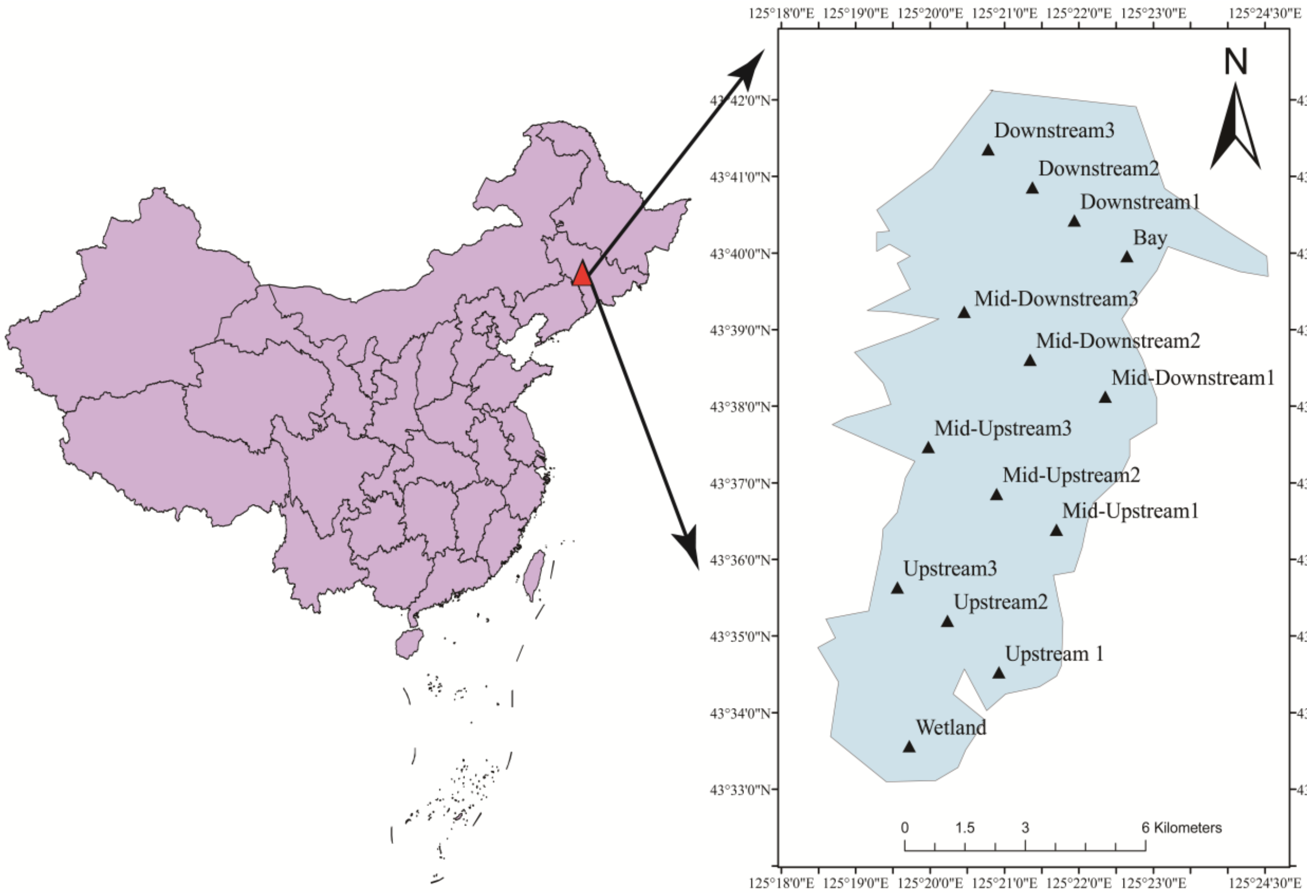


**Figure 1.** Geographic locations of study sites and sampling stations.

Sampling was conducted at representative stations in six sections of Xinlicheng Reservoir wetlands, upstream, mid-upper reaches, mid-lower reaches, downstream, and bay areas in September 2020 and April, June, September, and December 2021. Samples collected included water samples for physicochemical analysis, particulate organic matter (POM) in water, sediment organic particulate matter, phytoplankton, zooplankton, and fish catches. Fish samples were only collected in September 2020 and June 2021. Geographic coordinates and other location information of the sampling points are depicted in Figure 1.

2.1.1. Water Sample Collection and Water Quality Index Measurement Methods

In this study, the water quality parameters measured and their corresponding methods are presented in Table 1. All analytical methods employed for sample testing were conducted following internationally recognized standard procedures.

**Table 1.** Physicochemical water parameters and associated measurement methods.

| Main Water Quality Index | Method |
|---|---|
| Water temperature (WT) | Water thermometer |
| Water transparency (SD) | Secchi disk method |
| pH | Portable pH meter (Mettler Toledo) |
| Chemical oxygen demand (COD) | Acid digestion method |
| Dissolved oxygen (DO) | Portable oxygen dissolving meter (Milwaukee MW600) |
| Total nitrogen (TN) | Potassium persulfate ion oxidation—UV Spectrophotometry |
| Total phosphorus (TP) | Molybdenum antimony anti spectrophotometric method |
| Ammonia nitrogen ($NH_4$-N) | Spectrophotometric method with Knott's reagent |
| Nitrite nitrogen ($NO_2$-N) | Spectrophotometric method of N-(1-Nike)-ethylenediamine spectrophotometric method |
| Nitrate nitrogen ($NO_3$-N) | Ultraviolet spectrophotometry |
| Soluble reactive phosphorus (SRP) | Molybdenum antimony anti spectrophotometric method |
| Alkalinity (ALK) | Acid-base titration |
| Chloride ($Cl^-$) | Silver nitrate titration method |
| Silicate (${SiO_4}^{4-}$) | Silicomolybdenum yellow spectrophotometry |
| Potassium ($K^+$) | Sodium ion Tetraphenylborate Titration Method |
| Sodium ($Na^+$) | Difference method |
| Chlorophyll a | 90% Acetone extraction method |
| $Ca^{2+}$ | EDTA complexometric titration (two-step titration) |
| $Mg^{2+}$ | EDTA complexometric titration (two-step titration) |
| Total nitrogen in sediments (BTN) | Same as TN |
| Total phosphorus in sediments (BTP) | Same as TP |
| Sulfate (${SO_4}^{2-}$) | EDTA volumetric titration method |

### 2.1.2. Isotope Sample Processing Methods for Fish and Basal Dietary Sources

Fish samples (various fish species and other aquatic animals) were captured using fishing nets. The list of fish species captured in this study is provided in Table 2. After their collection, the fish samples were weighed for body weight, fresh weight, and dry weight. Muscle tissue from the dorsal quadrant was collected (for smaller fish species, three specimens were mixed and ground), dried using a freeze dryer, wrapped in aluminum foil, sealed in opaque bags, and stored in the dark. Particulate organic matter (POM) was collected and dried using a 100 μm plankton net. Zooplankton (ZOOP) and phytoplankton (PHYT) were collected by filtering water through No. 13 and No. 25 plankton nets, respectively. Sediment organic matter (BOTM) samples were collected using a Petersen grab sampler.

**Table 2.** List of Fish Species and Basal Dietary sources Collected in This Study.

| Latin Names | Dietary Habit |
|---|---|
| *Ctenopharyngodon idella* (CTEI) | Herbivorous |
| *Cyprinus carpio* (CYPC) | Omnivorous |
| *Protosalanx chinensis* (PROC) | Omnivorous/Carnivorous |
| *Hypophthalmichthys molitrix* (HYPM) | Filter-feeding |
| *Aristichthys nobilis* (ARIS) | Filter-feeding |
| *Megalobrama amblycephala* (MEGA) | Herbivorous |
| *Rhodeus ocellatus* (RHOO) | Omnivorous |
| *Pelteobagrus fulvidraco* (PELF) | Omnivorous |
| *Carassius auratus* (CARA) | Omnivorous |
| *Hemiculter leucisculus* (HEML) | Omnivorous |
| *Cultrichthys erythropterus* (CULE) | Omnivorous |
| Zooplankton (ZOOP) | --- |
| Phytoplankton (PHYT) | --- |
| Sediment organic matter (BOTM) | --- |
| Particulate organic matter (POM) | --- |

When required for measurement, a portion of the dried sample was removed and ground into powder using a high-throughput grinder in a 2 mL centrifuge tube. To analyze only organic carbon, we applied the following carbonate removal process to all POM, sediment, and plant samples: Samples were treated with 0.1 M hydrochloric acid (HCl) dropwise until visible bubbling stopped, indicating the complete removal of carbonate material. After acid treatment, the samples were thoroughly rinsed with distilled water to remove any residual acid. The treated samples were then dried at 60 °C for 24 h before further processing. The ground muscle samples were wrapped in tin cups and weighed, with approximately 300–500 µg of animal samples, around 1000 µg of water POM and sediment organic particulate matter samples, and about 700 µg of plant samples. Subsequently, stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) of the samples were measured using a stable isotope mass spectrometer (Delta V Advantage). For all isotope samples, measurements were taken in triplicate or more for each sample.

### *2.2. Data Processing*

#### 2.2.1. Stable Isotope Data Processing

Carbon and nitrogen stable isotope analyses were conducted using the international standards Pee Dee Belemnite (PDB) for carbon and atmospheric nitrogen for nitrogen. The results were expressed using $\delta^{13}C$ and $\delta^{15}N$ values, calculated according to the following formulas:

$$\delta X(‰) = \frac{\mathrm{Rsam} - \mathrm{Rsta}}{\mathrm{Rsta}} \times 10^3 \tag{1}$$

where X represents $^{13}C$ or $^{15}N$, and R represents $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Rsam represents the isotopic ratio of the sample, and Rsta represents the isotopic ratio of the standard reference sample. The trophic position (isotopic) for each species was calculated using the formula given by Post [9]:

$$TL = \left( \frac{\delta^{15}N_{\_fish} - \delta^{15}N_{\_baseline}}{\Delta^{15}N} \right) + TL_{\_baseline} \tag{2}$$

In the equation, $TL$ represent tropic level, $\delta^{15}N\_fish$ represents the nitrogen isotopic ratio of the studied fish species, while $\delta^{15}N\_baseline$ denotes the nitrogen isotopic ratio of baseline organisms (such as primary producers or organisms known to belong to specific trophic levels). $\delta^{15}N$ represents the average nitrogen isotopic fractionation between trophic levels, conventionally assumed to be 3.4‰, although this value may vary depending on the specific ecosystem and study organism. *TL_baseline* represents the trophic level of baseline organisms, typically assigned a value of 1, with particulate organic matter (POM) offering the benchmark level in this study.

#### 2.2.2. Isotope Mixing Model with SIMMR

Stable isotope mixing models are used to quantify the proportional contributions of various sources to a diet mixture [21–24]. Along with a series of digestion and metabolic processes accompanied by isotopic fractionation or fractionation adjustments, the isotopic signatures of consumers and their dietary sources are determined and exhibit a certain degree of similarity. Based on this similarity, an index of relative importance for each dietary source can be inferred. In this study, a Bayesian mixing model was developed using two isotopes ($\delta^{13}C$ and $\delta^{15}N$), which can estimate the contributions of four basal dietary sources (phytoplankton, zooplankton, sediment organic matter, and POM) to the assimilated diets of two filter-feeding consumers. The operation and calculation of this model were performed using the ffvb method built into the SIMMR-V: 0.5.1 package in the R programming language [25]. To ensure the repeatability of the model results, the seed number was set to 60 before running all ffvb models. This mixing model is based on the fundamental linear mass balance equations [23]:

$$X_{ij} = \frac{\sum_{k=1}^{K} p_k q_{jk} \left( s_{jk} + c_{jk} \right)}{\sum_{k=1}^{K} p_k q_{jk}} + \varepsilon_{ij} \tag{3}$$

$$s_{jk} \sim N\left(\mu_{jk}, \omega_{jk}^2\right) \tag{4}$$

$$c_{jk} \sim N\left(\lambda_{jk}, \tau_{jk}^2\right) \tag{5}$$

$$\varepsilon_{ij} \sim N\left(0, \sigma_j^2\right) \tag{6}$$

$X_{ij}$—observed isotope value j of the consumer *i*.
$s_{jk}$—source value *k* on isotope *j*; normally distributed with mean $\mu_{jk}$ and variance ${\omega_{jk}}^2$.
$c_{jk}$—Trophic Enrichment Factor (TEF) for isotope *j* on source *k*; normally distributed with mean $\lambda_{jk}$ and variance ${\tau_{jk}}^2$.
$p_k$—dietary proportion of source *k*; estimated by the model.
$q_{jk}$—concentration of isotope *j* in source *k*.
$\varepsilon_{ij}$—residual error, describing additional inter-observation variance not described by the model, drawn from a normal distribution with a mean equal to zero and variance ${\sigma_j}^2$ derived by the model.

In our study, the following steps were applied to quantify the dietary contributions with the isotopic data:

1. *Model Input:*

Collected isotopic data ($\delta^{13}$C and $\delta^{15}$N) from both consumers and their potential food sources. Applied fractionation values to adjust for changes during digestion and assimilation.

2. *Bayesian Inference:*

Used SIMMR's Bayesian framework to generate posterior distributions of each food source's proportion, providing both mean estimates and associated uncertainty.

3. *Result Interpretation:*

Reported the proportional contributions of food sources along with 95% credible intervals to ensure robust conclusions.

#### 2.2.3. Random Forest Algorithm

This study utilized random forest to analyze the relationship between twenty-two (22) environmental factors and isotope concentrations. Initially, we constructed a random forest model containing 500 decision trees using the random forest package in the R language. The importance of each environmental factor variable was assessed using the built-in feature importance evaluation function, aiming to identify the factors contributing most significantly to the variation in isotope concentrations. To ensure result reproducibility, the random seed number was set to 290. To elucidate the independent impacts of each environmental factor on isotope variation, we generated Partial Dependence Plots (PDPs) using the pdp package. These plots illustrate how individual environmental factors affect the isotopic concentrations while holding other factors constant. Based on the sorted importance results, the top six important environmental factors were identified, and individual Partial Dependence Plots were created.

## 3. Results

### *3.1. Carbon and Nitrogen Isotope Characteristics of the Food Web in Xinlicheng Reservoir*

Isotopic carbon signatures of fish varied between −28.28‰ in *C. erythropterus* (CULE) to −24.30‰ in *C. carpio* (CYPC), whereas in the four food sources, the values ranged between −29.63‰ in zooplankton (ZOOP) to −25.46‰ in benthic organic trophic matter (BOTM) (Table 3). Isotopic nitrogen signatures of fish varied between 8.43‰ in *C. idella*

(CTEI) or 8.65‰ in *C. carpio* (CYPC) to 22.25‰ in *P. fulvidraco* (PELF), whereas in the four basal dietary items, the values ranged between 9.74‰ in benthic organic trophic matter (BOTM) to 14.47‰ in zooplankton (ZOOP) (Table 3).

**Table 3.** Carbon and nitrogen isotope δ values of the food web in Xinlicheng Reservoir.

| Name (Abbr) | ($\delta^{15}N \pm SD$) ‰ | ($\delta^{13}C \pm SD$) ‰ |
|---|---|---|
| CTEI | 8.43 ± 0.18 | −(24.47 ± 0.08) |
| CYPC | 8.65 ± 0.21 | −(24.30 ± 0.06) |
| PROC | 19.24 ± 0.32 | −(26.84 ± 0.03) |
| HYPM | 17.10 ± 0.11 | −(25.10 ± 0.03) |
| ARIS | 18.09 ± 0.17 | −(25.36 ± 0.08) |
| MEGA | 16.37 ± 0.16 | −(24.85 ± 0.14) |
| RHOO | 14.01 ± 0.44 | −(25.69 ± 0.08) |
| PELF | 22.25 ± 0.44 | −(27.17 ± 0.07) |
| CARA | 17.03 ± 0.20 | −(23.53 ± 0.05) |
| HEML | 18.45 ± 0.11 | −(24.49 ± 0.02) |
| CULE | 18.63 ± 0.27 | −(28.28 ± 0.92) |
| POM | 11.67 ± 0.67 | −(28.76 ± 0.61) |
| PHYT | 13.07 ± 0.90 | −(28.22 ± 0.86) |
| ZOOP | 14.47 ± 0.49 | −(29.63 ± 0.71) |
| BOTM | 9.74 ± 0.55 | −(25.46 ± 0.39) |

The carbon and nitrogen isotopic signatures of the four major basal dietary sources across different locations can offer insights into their spatial variation (Figure 2). The iso-space plot is divided into four quadrants: Quadrant A, characterized by $\delta^{15}N$ enrichment and $\delta^{13}C$ depletion; Quadrant B, characterized by enrichment in both $^{15}N$ and $^{13}C$; Quadrant C, depleted in both $^{15}N$ and $^{13}C$, Quadrant D, enriched in $^{13}C$ and depleted in $^{15}N$ [26]. The locations within the reservoir, upstream, midstream-upper, midstream-lower, downstream, wetland, and bay are sequentially labeled with numbers 1–6; e.g., ZOOP1 represents upstream zooplankton. It can be observed that midstream-upper zooplankton (ZOOP2) and bay zooplankton (ZOOP6), as well as midstream-lower phytoplankton (PHYT3) and zooplankton (ZOOP3), predominantly fall within Quadrant A, indicating $\delta^{15}N$ enrichment and $\delta^{13}C$ depletion. The consumers, such as *H. molitrix* (HYPM) and *A. nobilis* (ARIS), which rely on the four major basal dietary sources, are distributed in Quadrant B, indicating enrichment in both $\delta^{15}N$ and $\delta^{13}C$. Additionally, midstream-lower and downstream zooplankton (ZOOP3 and ZOOP4) and midstream-lower particulate organic matter (POM3) are also located in this quadrant. Quadrant C primarily consists of upstream and wetland zooplankton (ZOOP1 and ZOOP5), as well as upstream and bay phytoplankton (PHYT1 and PHYT6), and upstream, downstream, and bay POM (POM1, POM4, POM6), indicating depletion for both $\delta^{13}C$ and in $\delta^{15}N$. All locations of bottom sediment organic matter (BOTM1-BOTM6) are situated in Quadrant D, along with midstream-upper, downstream, and wetland phytoplankton (PHYT2, PHYT4, PHYT5), and midstream-upper and wetland POM (POM2 and POM5), indicating depletion for $\delta^{15}N$ and enrichment for $\delta^{13}C$ (Figure 2).

Based on the $\delta^{15}N$ values, we can infer that the average trophic level of fish species in Xinlicheng Reservoir is 3.03, with a food chain length of 4.69. Surprisingly, two fish species, *C. idella* (CTEI) and *C. carpio* (CYPC), were assigned to very lower trophic levels around 1.5. Fish species with trophic levels ranging from two to three include *R. ocellatus* (RHOO) and *M. amblycephala* (MEGA). Likewise, fish species with trophic levels ranging from three to four include *C. auratus* (CARA), *H. molitrix* (HYPM), *A. nobilis* (ARIS), *H. leucisculus* (HEML), *C. erythropterus* (CULE), and *P. chinensis* (PROC). The only fish species occupying the highest trophic level (>4) is *P. fulvidraco* (PELF) (4.69) (Figure 3).

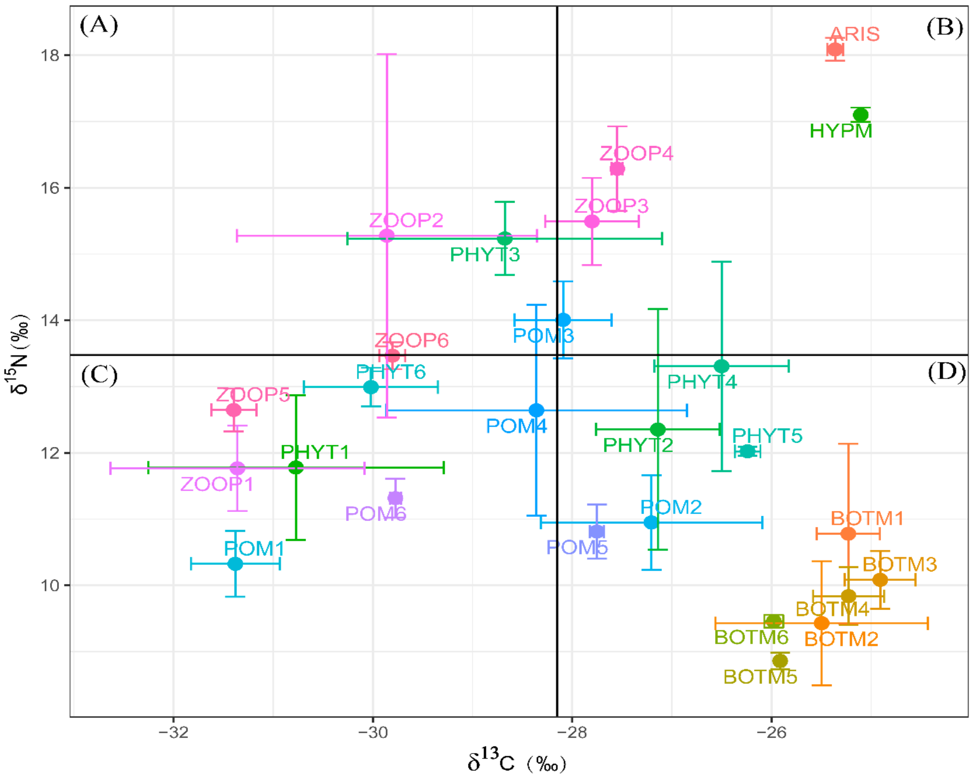


**Figure 2.** Spatial distribution of carbon and nitrogen stable isotope values of the four major basal dietary sources in the Xinlicheng Reservoir. Locations within the reservoir, upstream, midstream-upper, midstream-lower, downstream, wetland, and bay are sequentially labeled with numbers 1–6. The lines divide the niche map into four quadrants (**A**–**D**).

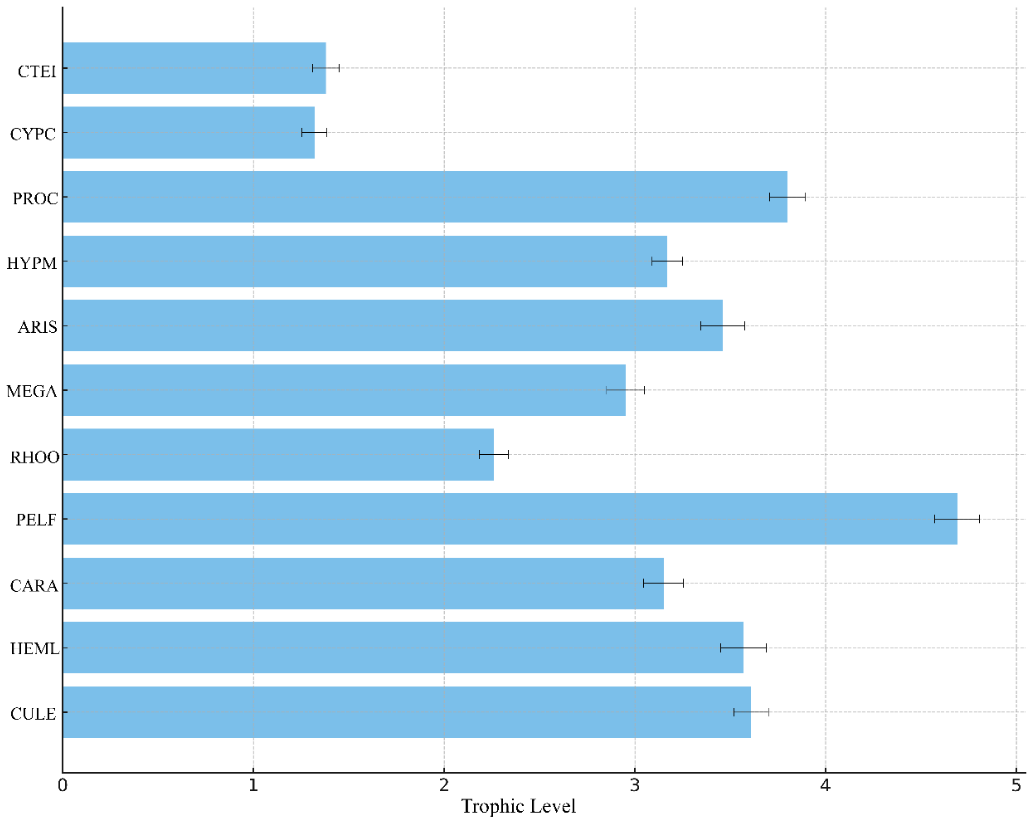


**Figure 3.** Trophic levels of fish in the Xinlicheng Reservoir.

*3.2. Contribution of the Four Major Basal Dietary Sources to the Filter-Feeding Fish Species, H. molitrix and A. nobilis*

According to the results of the stable isotope mixing model, phytoplankton (median 32.08%) displayed the highest contribution among the four major basal dietary sources to *H. molitrix*, followed by sediment organic matter (median 20.04%), and zooplankton (median 18.90%), with the lowest contribution from particulate organic matter (POM) (median 13.25%) (Figure 4). For *A. nobilis*, the highest contribution was similarly registered from phytoplankton (median 34.06%), followed by sediment organic matter (median 17.58%) and zooplankton (median 16.69%), with the lowest contribution from particulate organic matter (POM) (median 13.25%) (Figure 5).

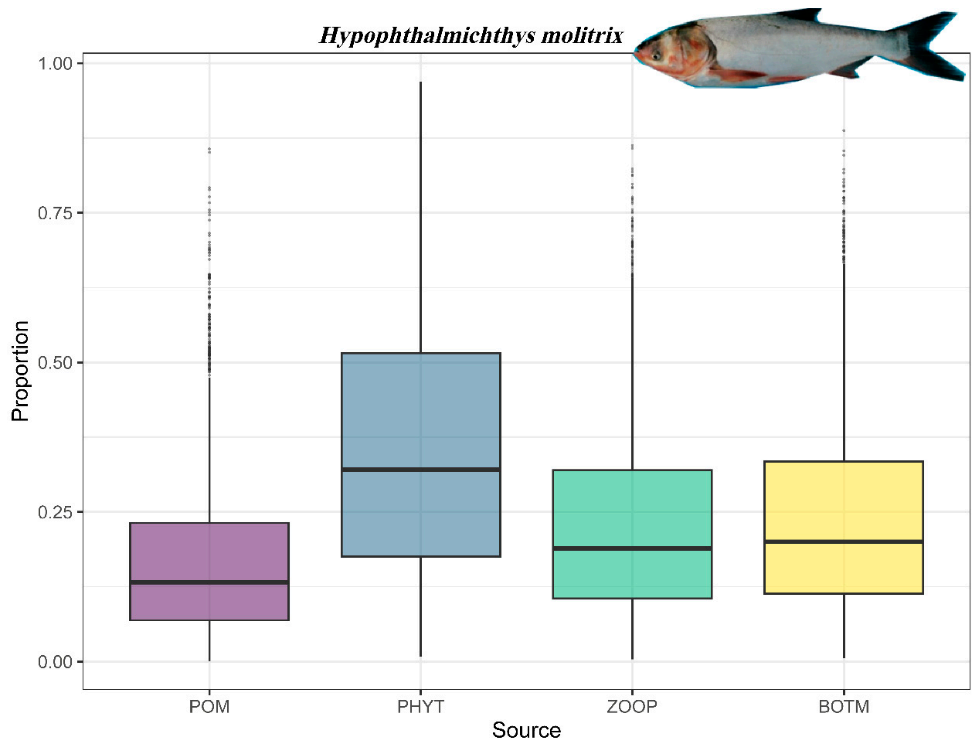


**Figure 4.** Box plots of the percentage contribution of four major basal dietary sources to the diet of *H. molitrix*.

During the feeding and growth of *H. molitrix* within the reservoir, the median contribution of zooplankton (ZOOP2) from the middle-upper reaches is the highest (9.10%), followed by organic particles (POM3), phytoplankton (PHYT3), and zooplankton (ZOOP3) from the middle-lower reaches, with the corresponding percentage contributions being 4.32%, 3.48%, and 3.30%, respectively. The lowest median contribution to *H. molitrix* stems from phytoplankton in the bay (PHYT6) (Figure 6). Similarly, for *A. nobilis*, the median contribution from zooplankton (ZOOP2) in the middle-upper reaches is the highest (7.91%), followed by zooplankton (ZOOP4) in the lower reaches, organic matter in the upper reaches (BOTM1), zooplankton (ZOOP3) and phytoplankton (PHYT3) in the middle-lower reaches, with percentage contribution of 4.66%, 4.18%, 3.99%, and 3.87%, respectively. The lowest median contribution to *A. nobilis* comes from POM in the bay (POM6), zooplankton, and phytoplankton in the bay (ZOOP6 and PHYT6) (Figure 7).

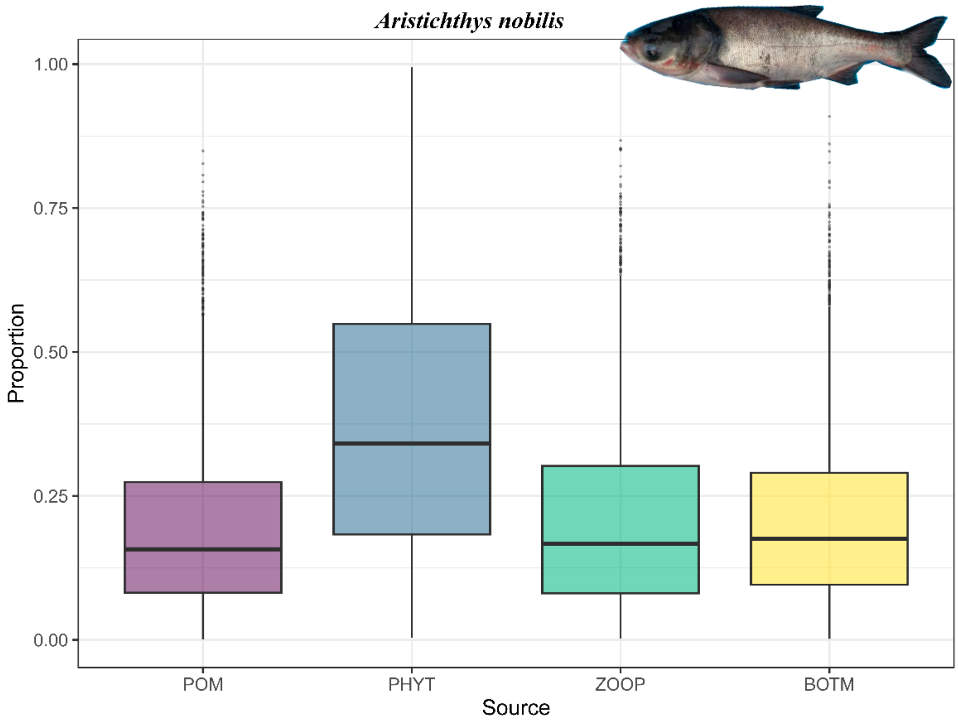


**Figure 5.** Box plots of the percentage contribution of four major basal dietary sources to the diet of *A. nobilis*.

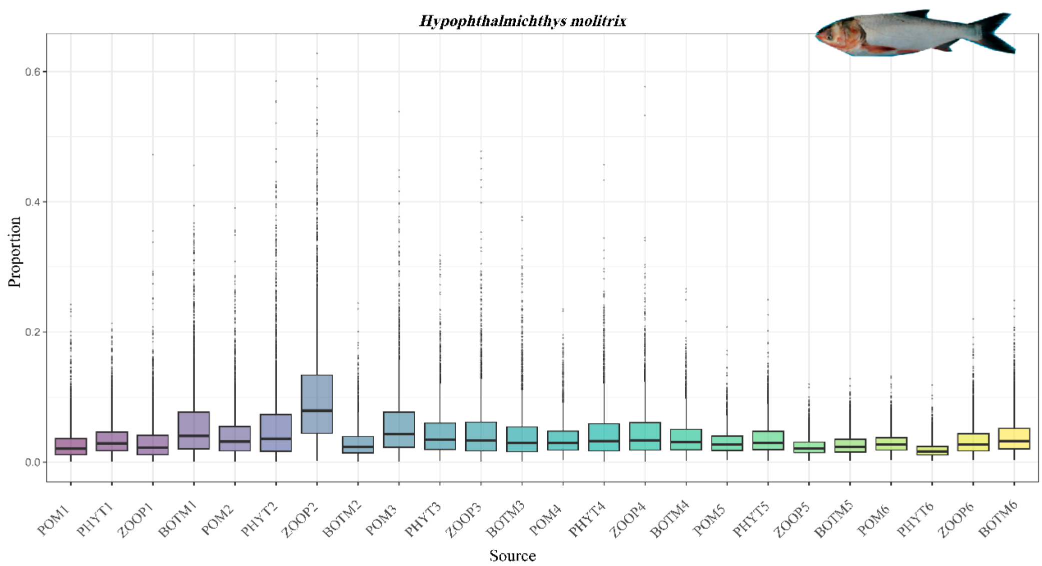


**Figure 6.** Box plots showing the percentage contribution of the four major basal dietary sources to the diet of *H. molitrix* across different locations within Xinlicheng Reservoir.

### *3.3. Relationship Between Isotopic Changes of Basic Dietary Sources and Environmental Factors*

#### 3.3.1. Relationship Between Carbon and Nitrogen Isotopic Signatures of Phytoplankton and Environmental Factors

The application of random forest allowed ranking twenty-two (22) environmental factors according to the importance in influencing the carbon and nitrogen isotope values of phytoplankton (Figure 8). According to the model, pH and water temperature (WT) had the greatest impact on $\delta^{13}C$ isotope values (Figure 8A), while total nitrogen (TN) and total phosphorus (TP) were the most influential factor for $\delta^{15}N$ isotope values (Figure 8B).

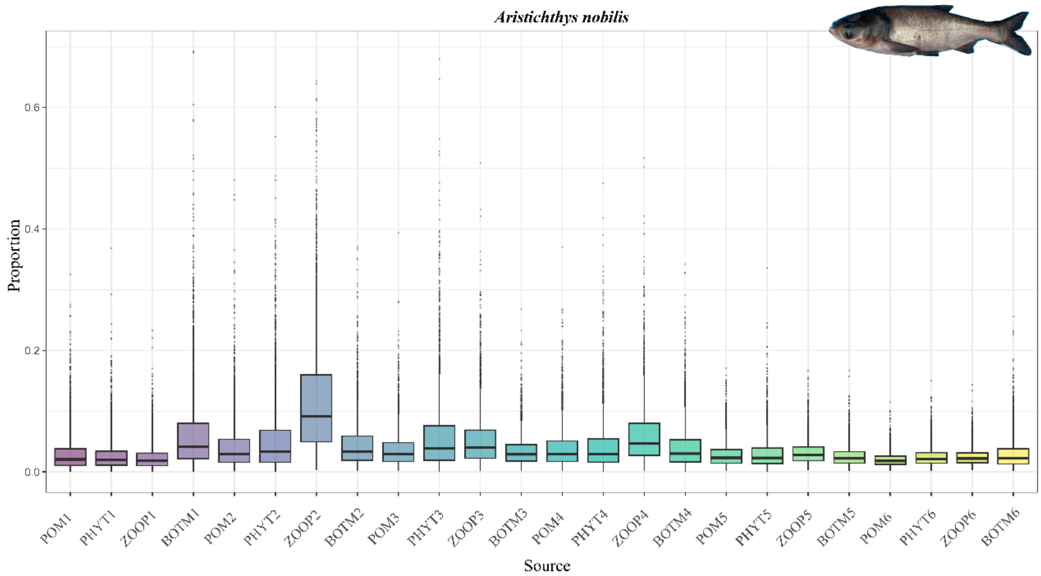


**Figure 7.** Box plots showing the percentage contribution of the four major basal dietary sources to the diet of *A. nobilis* across different locations within Xinlicheng Reservoir.

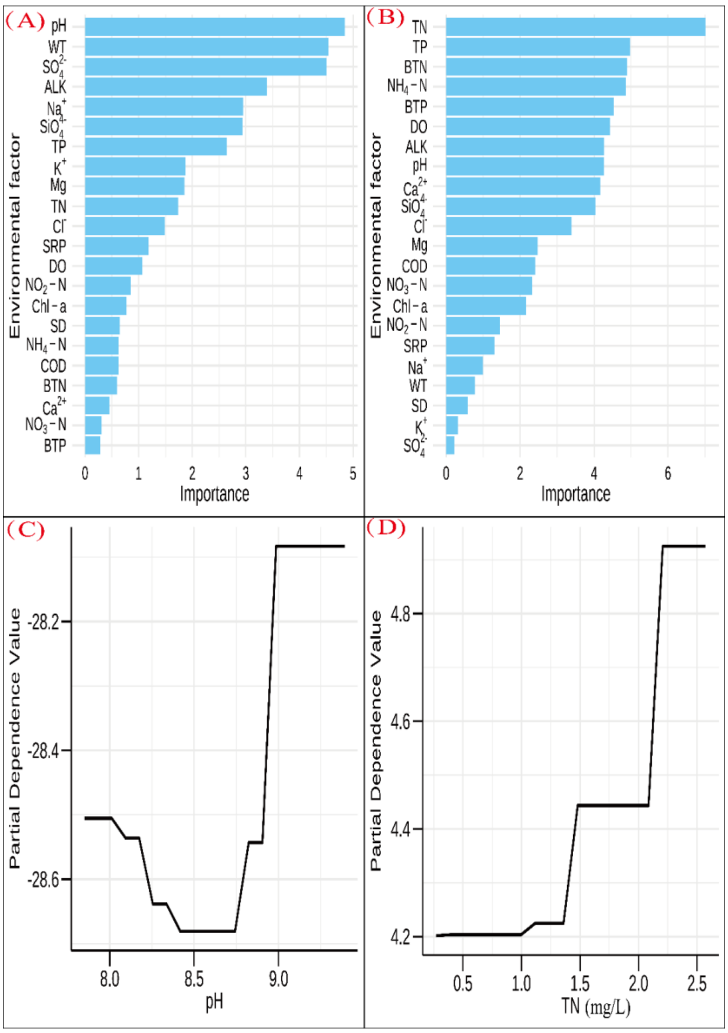


**Figure 8.** Order of importance of environmental factors related to carbon (**A**) and nitrogen (**B**) isotopic signatures of phytoplankton and partial dependence plots of key environmental factors (**C**: pH; **D**: TN).

Partial dependence plots further signified the relationships between phytoplankton carbon isotopes and environmental factors. Notably, a significant and non-monotonic relationship was registered between pH and $\delta^{13}C$ isotope values. In particular, when the pH ranged from 8.0 to 8.5, $\delta^{13}C$ isotope values decreased markedly, indicating a negative correlation within this range, but when the pH exceeded the level of 8.7, the relationship became positive (Figure 8C). Similarly, for $\delta^{15}N$ isotope values, a positive correlation with TN was observed when TN concentrations exceeded the level of 1.02 mg/L and became even more pronounced at TN levels greater than 2.08 mg/L (Figure 8D).

3.3.2. Relationship Between Carbon and Nitrogen Isotopic Signatures of Zooplankton and Environmental Factors

The environmental factor that had the greatest influence on $\delta^{13}C$ isotope values in zooplankton was the chloride ($Cl^-$) concentrations (Figure 9A), while the most critical factor for $\delta^{15}N$ was the calcium ($Ca^{2+}$) levels (Figure 9B). Partial dependence plots revealed a strongly positive relationship between chloride ion concentrations and $\delta^{13}C$ isotope values when they exceeded the level of approximately 30 mg/L (Figure 9C). Regarding the $\delta^{15}N$ isotope values, calcium ion ($Ca^{2+}$) exhibited an overall negative correlation, which was particularly pronounced after a concentration threshold of 32 mg/L (Figure 9D).

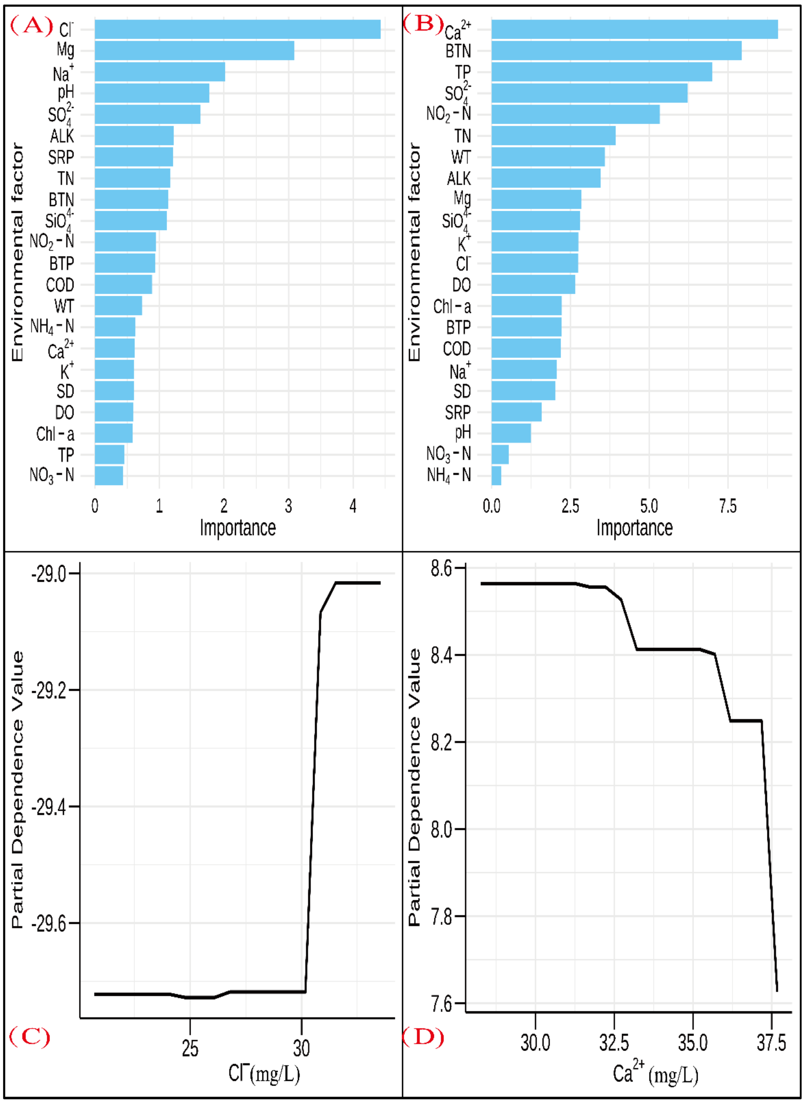


**Figure 9.** Order of importance of environmental factors related to carbon (**A**) and nitrogen (**B**) isotopes of zooplankton and partial dependence plots of key environmental factors (**C**: $Cl^-$; **D**: $Ca^{2+}$).

3.3.3. Relationship Between Carbon and Nitrogen Isotopic Signatures of Particle Organic Matter and Environmental Factors

Among the multitude of environmental factors examined, silicate ($SiO_4{}^{4-}$) had the greatest impact on $\delta^{13}C$ isotope values of particulate organic matter (POM) (Figure 10A), while the most critical factor influencing the POM $\delta^{15}N$ values was pH (Figure 10B). Partial dependence plots revealed that silicate ($SiO_4{}^{4-}$) displays a negative and nearly monotonic relationship with the carbon isotope POM values (Figure 10C). For the nitrogen isotope values, pH showed a negative correlation at levels higher than 8.00 (Figure 10D).

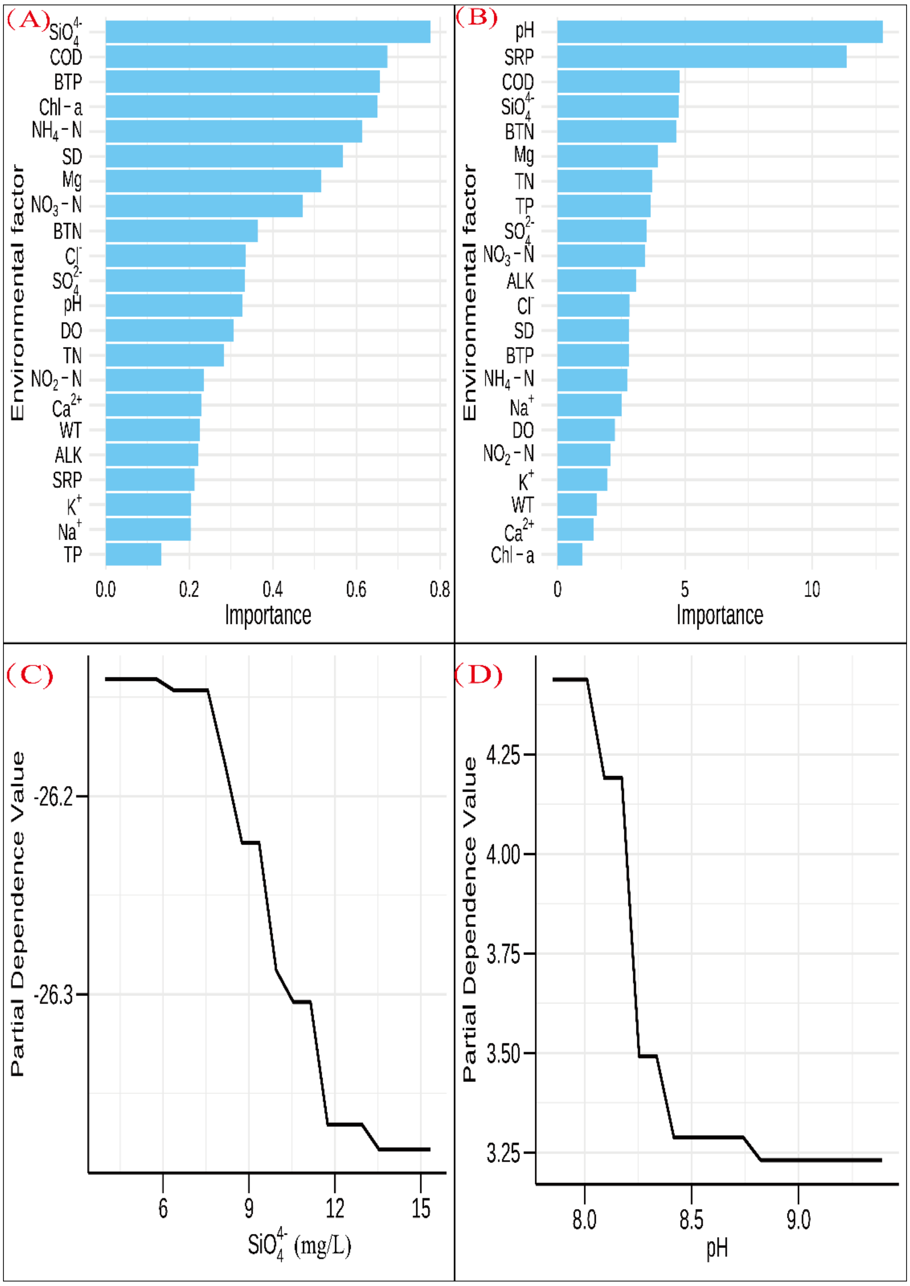


**Figure 10.** Order of importance of environmental factors related to carbon (**A**) and nitrogen (**B**) isotopes of particulate organic matter and partial dependence plots of key environmental factors (**C**: $SiO_4{}^{4-}$; **D**: pH).

3.3.4. Relationship Between Carbon and Nitrogen Isotopic Signatures of Bottom Sediment Organic Matter and Environmental Factors

The environmental factor with the greatest influence on $\delta^{13}C$ isotope values of bottom sediment organic matter (BOTM) was total phosphorus (TP) (Figure 11A). For $\delta^{15}N$ isotope values in BOTM, the most critical factor was silicate $\left(SiO_4^{4-}\right)$ (Figure 11B). Partial dependence plots further revealed that the total phosphorus (TP) concentrations were negatively correlated with $\delta^{13}C$ values when TP concentrations were below 0.12 mg/L (Figure 11C). Regarding the $\delta^{15}N$ values, sulfate ion ($SiO_4{}^{4-}$) exhibited a positive correlation when the corresponding concentrations were below 135 mg/L (Figure 11D).

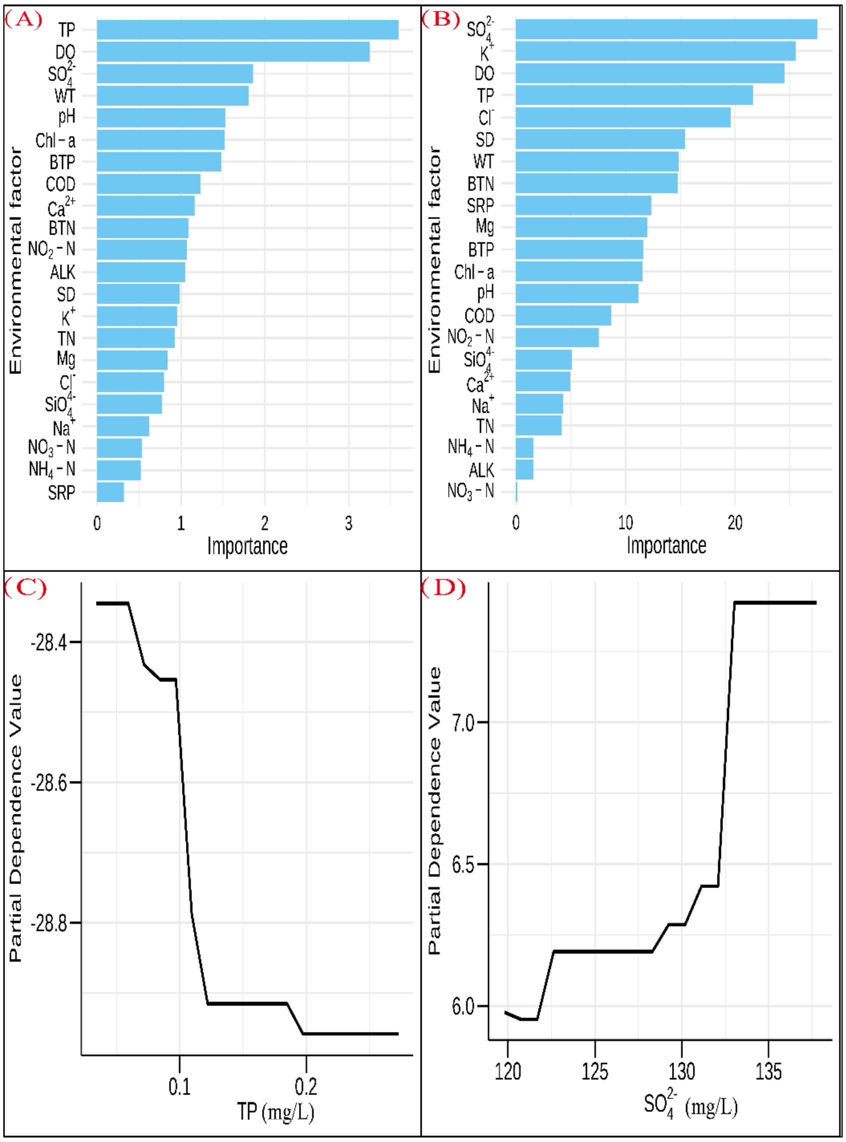


**Figure 11.** Order of importance of environmental factors related to carbon (**A**) and nitrogen (**B**) isotopes of bottom sediment organic matter and partial dependence plots of key environmental factors (**C**: TP; **D**: $SO_4{}^{2-}$).

## 4. Discussion

### *4.1. Bayesian Stable Isotope Mixing Models and Fish Dietary Sources*

Notwithstanding the importance of biomanipulation as a strategy for managing water quality, it is essential to optimize its design by characterizing the food web structures and determining the trophic levels of the various consumers. In this regard, the present study used stable isotopes to explore the trophodynamics of a typical drinking water reservoir, identify the trophic niche of various consumers through the differences of their carbon and nitrogen isotopic signatures, and quantify the contribution of basic dietary sources to two filter-feeding fish species. Even more so, we presented an attempt to link the spatiotemporal variations of carbon and nitrogen isotopes in basal dietary sources with key environmental factors. Considering also that the confounding effects of artificial feeding and fertilization are largely controlled in the drinking water reservoirs in China, the present assessment of the role of the basic dietary sources can directly guide the proportion and actual quantity of fish when stocking them. By accurately managing their introduction, biomanipulation efforts can be fortified to more effectively improve water quality.

The prevalent staples of the diet of *H. molitrix* and *A. nobilis* primarily comprise phytoplankton and zooplankton, respectively [27,28]. Fish assemblages dominated by *H. molitrix* and *A. nobilis* can efficiently consume prominent constituents of the planktonic community, thereby sequestering nutrients from the water column and transferring them to higher trophic levels through the multitude of food web interactions. Fish harvesting can then remove excessive nutrients from the system and to some extent control the proliferation of harmful algae [29,30]. The strong linkages between plankton and the two filter-feeding fish species have been established through methods such as stomach content analysis, laying the groundwork for the use of mixing stable isotope models [31]. Interestingly, the $\delta^{15}N$ values are suggestive of a trophic positioning for both fish species, *H. molitrix* and *A. nobilis*, within the mid-level of the food web, while several members (*C. idella*, *C. carpio*, *R. ocellatus*, *M. amblycephala*) of the fish assemblage appear to occupy lower trophic levels and could be affected, in terms of the food availability and feeding habits, by the changes induced from the introduction of additional silver and bighead carps in the Xinlicheng Reservoir.

Counter to prior evidence that *H. molitrix* mainly feeds on phytoplankton and *A. nobilis* on zooplankton, the present study suggests that both fish species predominantly rely on phytoplankton as their dietary source. The significant consumption of phytoplankton by *A. nobilis* may be attributed to the distinctly higher biomass of phytoplankton than zooplankton in the reservoir studied, signifying the importance of the availability of dietary sources and the spatiotemporal variations of their influence on fish feeding behavior [32]. Specifically, the spatial distribution of carbon and nitrogen isotopic signatures suggest significant adjustments in the diet composition, as food items from both upper-middle and lower sites appear to discernably contribute to fish feeding and growth in the studied reservoir. Likewise, our analysis identified considerable temporal variability in the isotopic signatures of four basal dietary sources (phytoplankton, zooplankton, sediment organic matter, and POM), likely related to differences in the utilization of carbon and nitrogen resources by algae under different environmental conditions [33]. Therefore, further investigation of the environmental drivers of carbon and nitrogen isotopic changes in the main staples of fish diets, such as temperature, pH, and nutrient concentrations, can advance our understanding of their ecological role during different seasons and trophic conditions [34,35].

### *4.2. Environmental Drivers of Isotopic Variations in the Primary Fish Dietary Sources*

The present study employed random forest models to conduct a detailed analysis of the carbon and nitrogen isotopic dynamics of the basal dietary sources in the Xinlicheng Reservoir. This method demonstrates significant advantages in handling complex interactions and high collinearity in ecological data. Compared to traditional multivariate statistical analysis methods, such as Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA), random forest models exhibit higher adaptability and accuracy in analyzing nonlinear data structures. While CCA and RDA are widely used in ecological studies to delineate the relationships between species and environmental covariates, they may be insufficient to capture nonlinear relationships when dealing with complex and high-dimensional data [36]. Random forest models address this potential challenge by constructing multiple decision trees, providing a more comprehensive examination of the complex interactions between environmental factors and biological responses.

Among the twenty-two (22) environmental variables analyzed in the Xinlicheng Reservoir, pH, total nitrogen (TN), chloride ($Cl^-$), calcium ($Ca^{2+}$), phosphorus (TP) and silicate ($SiO_4{}^{4-}$) were found to have a discernible impact on the carbon and nitrogen isotopic ratios of the basal fish dietary sources. These drivers play a critical role in shaping the chemical balance of water and directly (or indirectly) affect the availability of nutrients and metabolic activities of aquatic organisms. pH variations are intricately linked to the photosynthetic activity of phytoplankton as well as to the microbial processes involved in nitrogen cycling. The present study highlights the covariance of pH with both the $\delta^{13}C$ values of phytoplankton and $\delta^{15}N$ values of POM. Elevated phytoplankton photosynthetic activity directly reduces $CO_2$ availability from the water column, leading to an increased pH and altered $\delta^{13}C$ values of phytoplankton [37]. When a $CO_2$ drawdown is experienced in productive systems, rapid carbon uptake by phytoplankton may depend on carbon concentrating mechanisms, many of which rely on active uptake of bicarbonate which is less depleted in $^{13}C$ than $CO_2$, and thus uptake of different forms of inorganic carbon by phytoplankton can alter their organic $\delta^{13}C$ value [38]. Alternatively, phytoplankton may opt to concentrate $CO_2$ as a carbon source but with reduced isotopic fractionation associated with its fixation relative to the values expected by passive $CO_2$ diffusion.

The primary production of plankton also displays a positive relationship with the $\delta^{15}N$ values of suspended particulate organic matter as we shift from oligotrophic to eutrophic environments, but $\delta^{15}N$ tends to decrease when hypereutrophic conditions prevail [39]. The latter pattern is qualitatively similar to the negative correlation between pH and $\delta^{15}N$ of POM registered in our analysis, which, in turn, has been attributed to the dominance of cyanobacteria able to fix nitrogen from the atmosphere and, as a result, the $^{15}N$ concentration in POM is reduced [39]. In fact, during our investigation, we found that cyanobacteria often represent a significant fraction (>50%) of the total phytoplankton abundance in the Xinlicheng Reservoir, including several N-fixing species, which lends support to our explanation. The increase of total nitrogen (TN) in water is positively correlated with the rise in $\delta^{15}N$ values of phytoplankton, which presumably suggests that phytoplankton in nitrogen-rich environments tends to assimilate heavier nitrogen isotopes. However, the latter pattern should be viewed with caution for two basic reasons: (i) attempts to elucidate the relationship between nitrogen isotope fractionation and phytoplankton assimilation have not always been conclusive [39–41]; and (ii) while the Xinlicheng Reservoir may seem like a nitrogen-rich environments based on the TN concentrations (0.35–2.57 mg/L), the actual values for ammonia- and nitrate-nitrogen are distinctly lower (<0.120 mg/L) and thus the likelihood of nitrogen-limiting conditions to prevail for extended periods of the year cannot be ruled out.

Notwithstanding the directly negative impact on total zooplankton abundance, biomass, and richness, which declined with increasing chloride, the observed positive correlation between chloride ion ($Cl^-$) concentration and the $\delta^{13}C$ values of zooplankton can be attributed to composition shifts in the phytoplankton communities. Different species of phytoplankton exhibit distinct $\delta^{13}C$ signatures, and changes in the relative abundance of these species, driven by variations in chloride levels, can influence the $\delta^{13}C$ values of primary consumers, such as zooplankton [42]. This result highlights the zooplankton sensitivity to variations in environmental conditions, as their isotopic composition closely reflects the isotopic characteristics of their food sources. The present study also found a significant negative correlation between calcium ion ($Ca^{2+}$) concentration and $\delta^{15}N$ values of zooplankton. Generally, the ecological impacts of declining calcium on zooplankton community composition are widely recognized, as calcium is crucial for the fitness of zooplankton with calcified exoskeletons and is mostly taken up directly from water through respiratory surfaces [43]. The relationship found by the present study may arise from the regulatory role of calcium in zooplankton metabolism, influencing nitrogen uptake and utilization. Additionally, calcium ions may indirectly affect $\delta^{15}N$ values by modulating microbial nitrogen cycling processes, such as mineralization, nitrification and denitrification, in the aquatic environment [44].

Our analysis identified a negative correlation between total phosphorus (TP) concentration and the $\delta^{13}C$ values of bottom sediment organic matter (BOTM) when TP levels were below 0.12 mg/L. Although the latter TP level is discernably higher than any other threshold presented in the literature to distinguish between eutrophic and mesotrophic conditions, it may signify a quantitative and/or qualitative (stoichiometric) shift in the organic matter settling on the sediments, which could presumably modulate the sediment diagenesis rates along with the corresponding isotopic signals of the bottom sediment organic matter [45]. Silicate ($SiO_4{}^{4-}$) exerts a significant impact on the $\delta^{13}C$ values of particulate organic matter (POM). The results are suggestive of a negative correlation between silicate concentrations and the carbon isotopic composition of POM. This trend may arise from the direct linkages between silicates and the abundance/productivity of diatoms within the algal assemblage of the reservoir, which are a major component of POM in many freshwater systems and account for approximately 10% of the total algal abundance in the Xinlicheng Reservoir (Our unpublished data, 2021). Diatom species require silicate for their frustule formation, and thus variations in silicate levels can alter their ability to compete for resource procurement, leading to shifts in the POM isotopic composition [46].

## 5. Conclusions

Taking the Xinlicheng Reservoir as a representative case of drinking water source reservoirs, where biomanipulation is commonly employed to improve water quality, the present study was conducive to quantifying the contributions of basal dietary sources to key filter-feeding fish species (*H. molitrix* and *A. nobilis*) and guiding the optimal stocking accordingly. In determining the isotopic signatures of four primary dietary sources of the two filter-feeding fish species, we were able to pinpoint the role of phytoplankton as the primary staple of their diets, which deviates somewhat from earlier assertions that *A. nobilis* mainly feed on zooplankton. The strong linkages between the reservoir's plankton assemblage and the two fish species reinforces their potential to provide an effective strategy to design biomanipulation strategies and bring about distinct water quality improvements in the study reservoir. Our analysis also signified the importance of the relative availability of the main dietary sources together with their spatiotemporal variations as regulatory factors of fish feeding behavior and growth.

Employing random forest modeling, the study identified key environmental drivers underlying the variations of carbon and nitrogen isotopic ratios of the four primary dietary sources. Our results indicate that pH, total nitrogen (TN), chloride ion ($Cl^-$), calcium ion ($Ca^{2+}$), phosphorus (TP), and silicate $\left(SiO_4^{4-}\right)$ exert the most significant influence on the carbon and nitrogen isotopic values of dietary sources. Overall, the present characterization of the food web structure and identification of the environmental drivers of carbon and nitrogen isotopic variations of critical fish dietary sources offer critical benchmarks that could be used to more comprehensively connect the degree of water quality improvements with the functional and structural changes induced by biomanipulation efforts in the Xinlicheng Reservoir and elsewhere.

**Author Contributions:** F.H.: sample collection, data analysis, and original draft preparation. W.Z.: sample collection, conceptualization, and manuscript preparation. X.Q.: sample collection. C.X.: sample collection. Y.L.: methodology, laboratory supervision. J.W.: laboratory supervision. D.Y.: sample collection. G.A.: overall supervision and manuscript editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** The study was supported by the Survey of Fishery Resources in Xinlicheng Reservoir and Research Project on Optimal Water Fisheries Technology [2021–2023].

**Data Availability Statement:** All the data involved in the present study are provided in the manuscript.

**Acknowledgments:** The authors would like to express their gratitude to the staff of the Xinlicheng Reservoir Management Bureau for their invaluable assistance in providing both logistical and manpower support during the field sampling of this research.

**Conflicts of Interest:** The authors declare no conflicts of interests.

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