

Article

Ecological Stoichiometry Characteristic of Phytoplankton in Mountain Stream

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Abstract: This research investigated the phytoplankton ecological stoichiometry characteristics and the balance of the relationship between elements in a mountain river in a cold region. The samples of phytoplankton of four seasons were collected in May 2020, August 2020, November 2020, and February 2021 from the Taizicheng River in Chongli, Zhangjiakou City, China. We determined the contents of carbon (C), nitrogen (N), phosphorus (P), sulfur (S), hydrogen (H), and iron (Fe), and analyzed their ecological stoichiometric characteristics and correlation. Our results showed that the contents of C, N, P, S, H, and Fe in phytoplankton were 82.14 ± 32.12 g/kg, 9.22 ± 3.5 g/kg, 1.46 ± 0.55 g/kg, 1.96 ± 0.86 g/kg, 2.36 ± 1.36 g/kg, and 12.64 ± 10.57 g/kg, respectively. Generally, the contents of C, N, and P were relatively stable, while the contents of S, H, and Fe fluctuated greatly, and the coefficient of variation of Fe content was as high as 83.62%. The elemental molar composition of phytoplankton in the Taizicheng River is $C_{156.00}N_{15.41}S_{1.54}H_{51.17}Fe_{5.10}P$, which showed a significant difference compared with the classical Redfield ratio $C_{106}N_{16}P$. The high proportion of element C indicated that phytoplankton in the Taizicheng River have a high demand for C and a strong ability to consolidate C. The ratio of N:P was consistent with previous research results. The N:P ratio of phytoplankton in the Taizicheng River was 15.41, suggesting that the growth of phytoplankton in the Taizicheng River was restricted by both N and P. The contents of C, N, and P were positively correlated, while there was no significant correlation among S, H, and Fe. C:P was significantly positively correlated with C:N and N:P, while there were no strong correlations between C:N and C:P, as well as H:S, Fe:S, and H:Fe, indicating that the coupling correlation between phytoplankton elements was different and C, N, and P were highly correlated as important phytoplankton nutrient elements. This study contributes to our understanding of the phytoplankton ecological stoichiometry characteristics and the limiting factors of nutrients in a mountain river and provides a scientific basis for further ecological conservation and management efforts.

Keywords: cold region; mountain river; phytoplankton; Taizicheng River; elements; ecological stoichiometry



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1. Introduction

Phytoplankton plays a vital role in the biogeochemical cycles of aquatic ecosystems, such as the elemental cycles of carbon (C), nitrogen (N), phosphorus (P), and iron (Fe) [1]. Phytoplankton cells absorb these resources and use them in their activities [2]. Then, the elements are introduced into the environment in the form of organic matter. Some of this organic matter (particles or dissolved) is taken up, absorbed, and discharged by the next trophic level. And others precipitate to the bottom of the river and enter nature through microbial action and water exchange [3]. Therefore, phytoplankton absorb these nutrients and allocate them to their key activities. It will produce changes in its own element composition, food networks, and surrounding environmental elements. In the interaction

process between the exchange of substances and life activities in this ecosystem, the balance of various chemical elements in phytoplankton will form a unique stoichiometric ratio [4]. Ecological stoichiometry is an important means to study the balance of life elements and characteristics [5].

Redfield proved that the elemental composition of surface plankton was generally consistent with the proportion of dissolved nutrients in the ocean, and determined the Redfield mole ratio of 106C:16N:1P at the beginning of the 20th century [6]. This led to a hypothesis that the marine phytoplankton had evolved to have the ability to change their elemental levels to resemble those of the water environment in which they grew [7]. However, the C:N:P ratios of different heterotrophic and autotrophic aquatic organisms are now known to not necessarily conform to the ratio of Redfield. Because the unique C:N:P stoichiometric ratio of biology was generated by the interaction of nutrient elements and physiological control, the N:P ratios of the same species of phytoplankton in different environments and different phytoplankton in the same environment are different. Mountainous rivers differ significantly from marine environments in factors like water flow, temperature, light, and nutrient sources. These differences may cause riverine phytoplankton to deviate from the Redfield ratio. Additionally, the diverse species and seasonal variations in rivers further necessitate adjustments to the Redfield model. The optimal N:P ratio of phytoplankton was between 8.2:1 and 45.0:1 according to statistics, and the conclusion proposed by Redfield was more like the average N:P ratio [8]. Furthermore, phytoplankton C:N:P was higher than the Redfield ratio in Xiamen Bay and it may be limited by P due to imbalanced C:N:P input to seawater [9]. Although carbon, nitrogen, and phosphorus were traditionally emphasized for their roles in primary production and nutrient cycling, sulfur, hydrogen, and iron also played crucial roles in the physiology of phytoplankton and the functioning of ecosystems. Sulfur is vital for synthesizing amino acids and proteins, and its availability can influence the overall metabolic processes in phytoplankton. Hydrogen is a key element in energy transfer, participating in the formation of organic molecules through photosynthesis and respiration. Iron is essential for photosynthesis and serves as a cofactor in various enzymatic reactions, particularly in cold regions where its availability may be limited due to low solubility in cold water. In cold areas, the availability and cycling of these elements are especially critical because of extreme environmental conditions, which can alter the biochemical processes in phytoplankton. Therefore, understanding the stoichiometric ratios of these elements in phytoplankton is crucial for a comprehensive understanding of nutrient dynamics and ecosystem functioning in cold region aquatic environments. With the development of analytical testing techniques, new results have been produced on the ecological stoichiometric characteristics of not only aquatic ecosystems but also various ecosystems under the global pattern [5,10].

While much research has been conducted on phytoplankton stoichiometry in marine and temperate freshwater systems, there was a relative lack of studies focusing on cold mountain rivers. Unique environmental conditions such as extreme temperatures, seasonal ice closures, and limited growing seasons in cold regions create a challenging environment for primary producers such as phytoplankton. Environmental changes significantly affect the ecological dynamics of phytoplankton, leading to adaptive changes in phytoplankton stoichiometric ratios. Mountain rivers are dynamic ecosystems characterized by rapid water flow, fluctuating nutrient levels, and changing light conditions. These factors affect nutrient availability and phytoplankton growth capacity. Understanding how phytoplankton in these rivers respond to environmental variables can provide insights into broader biogeochemical cycles and potential downstream impacts on coastal ecosystems.

Mountain river ecosystems exhibit distinct characteristics, including a high altitude gradient, fast flow velocity, a scarcity of fish, and low temperature. These unique features make it an ideal object of study. In the current study, we aimed to investigate the spatial and temporal distribution and stoichiometric characteristics of carbon (C), nitrogen (N), and phosphorus (P) elements, as well as sulfur (S), hydrogen (H), and iron (Fe) of phytoplankton in the Taizicheng River ecosystem. Our objective was to better understand the characteris-

tics of the ecological stoichiometric and element balance relationship of river organisms in cold mountain areas. Specifically, we focused on the following aspects: (1) to explore the effects of various elements on the nutrient cycle mechanism; (2) to reveal the stoichiometric characteristics of multi-element balance of phytoplankton in mountain rivers.

2. Materials and Methods

2.1. Study Area

Taizicheng, situated at the heart of the Chongli District in Zhangjiakou, China, is a mountain river that holds great significance as part of the core area for the 2022 Winter Olympics (Figure 1). The river originates from densely forested mountains. It stretches across a length of 30.5 km, with an average width of about 2.0 m. Spanning an altitude range of 1180 m and 1910 m, the river boasts a fast and forceful water flow. The region surrounding the river experiences a typical continental monsoon climate, featuring cool and humid summers along with cold and long winters. The average temperature is around 19 °C in summer and −12 °C in winter. Its altitude and seasonal temperature variations might influence the ecological stoichiometry of the phytoplankton studied. Clastic sediment represents the prevailing sediment type within the river, which holds abundant nutrients, including nitrogen and phosphorus, with their composition exhibiting significant seasonal variations.

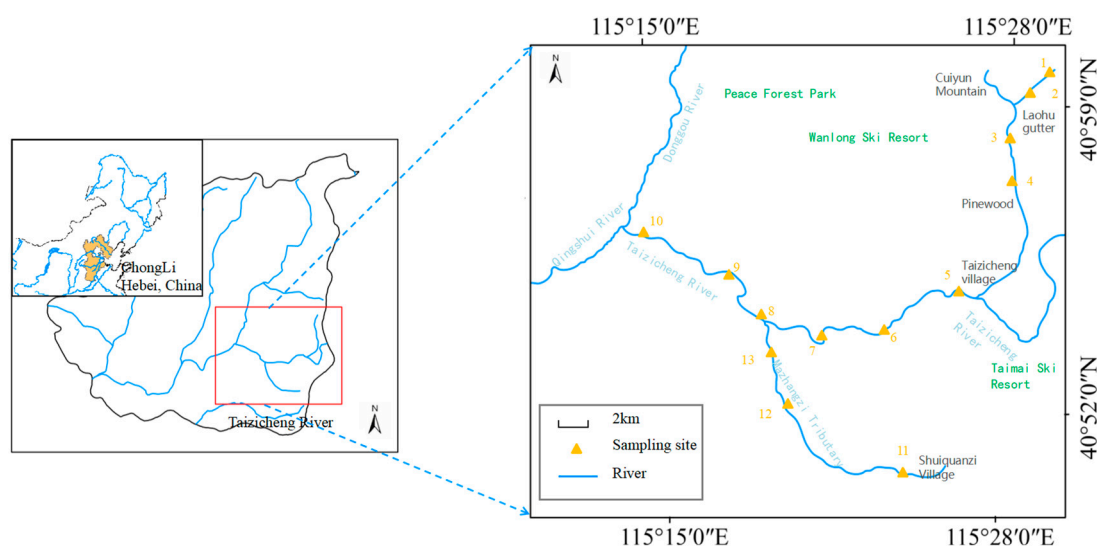


Figure 1. Location of the Taizicheng River and sampling sites.

2.2. Field Sample Collection

In order to study the ecological stoichiometric characteristics of phytoplankton in the Taizicheng River, 13 sampling sites were set up along the river in May 2020, August 2020, November 2020, and February 2021. Phytoplankton samples measuring 500 mL were collected (collection using plankton nets 13 and 25 nested towing) (Figure 1). At the upstream of the Taizicheng River, we set Sites 1–4, and Site 1 is the eye point of the Tiger Ditch spring as the source of the whole river. The river is slow, and the speed is not affected by the construction of the Winter Olympics. At the mainstream of the Taizicheng River, we set Sites 4–6, and there are construction sites along the riverbank that affect the water quality of the river. At the downstream of the Taizicheng River, we set Sites 7–9, and the construction site affects the water quality of the river. At the tributary of the Taizicheng River, we set Sites 10–12. The geographical location of each sampling point is shown in Figure 1.

2.3. Laboratory Experimental Analysis

There are two methods for ecological stoichiometry to study the elemental composition of phytoplankton. One is to directly determine the elemental composition of plankton organisms and organic detritus, and the other is to perform reverse deduction according to the concentration changes of biogenic elements in the water. The first one was adopted in this study. The collected phytoplankton samples were picked up under the stereo microscope and separated from the suspended particles and other debris in the river. The selected phytoplankton were, respectively, put into transparent vials and dried in a freeze-dryer to obtain powdery samples for element testing. Carbon (C), nitrogen (N), hydrogen (H), and sulfur (S) were determined by an element analyzer (Vario EL cube, Elementar Corporation, Langenselbold, Germany); phosphorus (P) and iron (Fe) were determined by inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7800, Heng Sheng Mass Spectrometer Company, Shanghai, China).

2.4. Statistical Analysis

We analyzed the mean value, standard deviation, coefficient of variation, and one-way ANOVA of the experimental data using SPSS25.0. Bilateral Pearson correlation test was used to conduct the correlation among C, N, P, S, H, Fe elements and their stoichiometric ratios (1). The results of data processing were made into a table in Excel 2019 and a box plot was drawn using R 4.2.2 and visualized using the “ggplot2” package [11].

$$\begin{aligned} n_i &= m_i / M_i \\ S_t &= n_c : n_N : n_s : n_H : n_{Fe} : n_P \end{aligned} \quad (1)$$

In the equation, n_i is the number of moles of elements, m_i is element concentration, M_i is molar mass of elements. S_t is the stoichiometric ratio of mountain river phytoplankton.

3. Results

3.1. Characteristics of Phytoplankton Element Content

The elemental composition of phytoplankton in the Taizicheng River is shown in Figure 2, revealing distinct trends for the six elements analyzed. Element C was the highest in February, which was cold and slow-growing. And C was the lowest in the fast growth period in August. Nitrogen content was lower in May and November 2020, and higher in August 2020 and February 2021. The content of element P was the lowest in May and increased gradually in November. However, the content of element P gradually decreased due to the decreased growth rate when the weather turned cold. The content of the S element decreased gradually after reaching the highest value in August, and the two protein-related elements N and S reached the peak value in August, indicating that the protein content in phytoplankton was high at this time. The content of the H element was high in May and November, which was opposite to the N element. The content of Fe was slightly lower in May, and gradually increased with the strengthening of photosynthetic respiration in August and November, while it dropped sharply in February, and the overall content was less than one-tenth of that in other months.

On the whole, the stability of C, N, and P elements was slightly higher than that of S, H, and Fe elements. Due to the demand of plant life activities and protein synthesis during growth and development, the overall changes of C, N, and P were in a relatively small range. The content of elements of S, H, and Fe were not as stable as C, N, and P, even though they were essential elements for plant growth. It was worth noting that a large number of Fe elements were lost with the weakening of plant life activities in February, and the stoichiometric proportion showed a significant decrease.

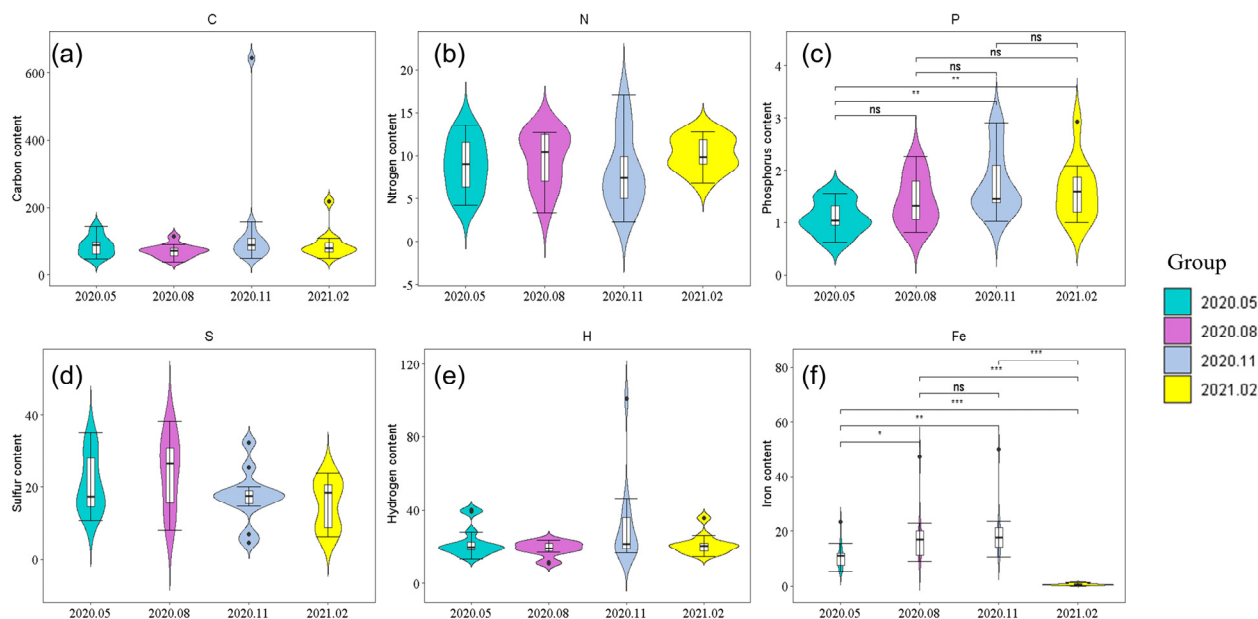


Figure 2. Content characteristics of phytoplankton C, N, P, S, H, and Fe in Taizicheng River. (a) The distribution of C content in four groups; (b) the distribution of N content in four groups; (c) the distribution of P content in four groups; (d) the distribution of S content in four groups; (e) the distribution of H content in four groups; (f) the distribution of Fe content in four groups. Different colors represent four different groups. Significance: * < 0.05; ** < 0.01; *** < 0.001 and ns indicates not significant.

3.2. Stoichiometric Characteristics of C, N, and P in Phytoplankton

The ecological stoichiometric ratios of elements C, N, and P of phytoplankton in the Taizicheng River were determined by the molar ratio (Figure 3). The average value of the C:P ratio in spring was 190.77 with a fluctuation range of 106–360. The mean value in summer was 136.49 with a range of 86–246. The mean value in autumn was 133.16 with a fluctuation range of 70–191. And the average value in winter is 161.09 with a fluctuation range of 73 to 308. The variation coefficients of the C:P ratio in the four seasons were 0.37, 0.33, 0.31, and 0.47, respectively. The average value of the N:P ratio was 19.23 in spring, and the variation range was 7–35. The mean value of summer was 15.83 with the fluctuation range of 6–32. In autumn, the mean value was 10.22, and the fluctuation range was 3–16. The winter mean was 15.04 with a fluctuation range of 6–28. The variation coefficients of the N:P ratio in the four seasons were 0.46, 0.49, 0.33, and 0.36, respectively. Additionally, the average value of the C:N ratio was 10.85 in spring, and the variation range was 4–15. In summer, the mean value was 9.80 with a range of 5–21. In autumn, the mean value was 14.29 and the fluctuation range was 8–28. The winter mean of the C:N ratio was 10.50 with a range of 6 to 23. The four seasonal variation coefficients were 0.29, 0.40, 0.39, and 0.41, respectively. The overall stoichiometric ratio of phytoplankton C:N:P in the Taizicheng River was 156.00:15.41:1. The C:P and N:P ratios are considered direct indicators of the growth rate, while the C:N ratio tends to be more stable. The C:P and N:P ratios began to decrease from May, and gradually increased in February of the following year due to plankton needing to enrich a large amount of P element to transcribe RNA during the exponential growth period. The C:P ratio of phytoplankton in the Taizicheng River was higher than the default value of 106 in marine plankton, while the N:P ratio was similar to that of marine plankton.

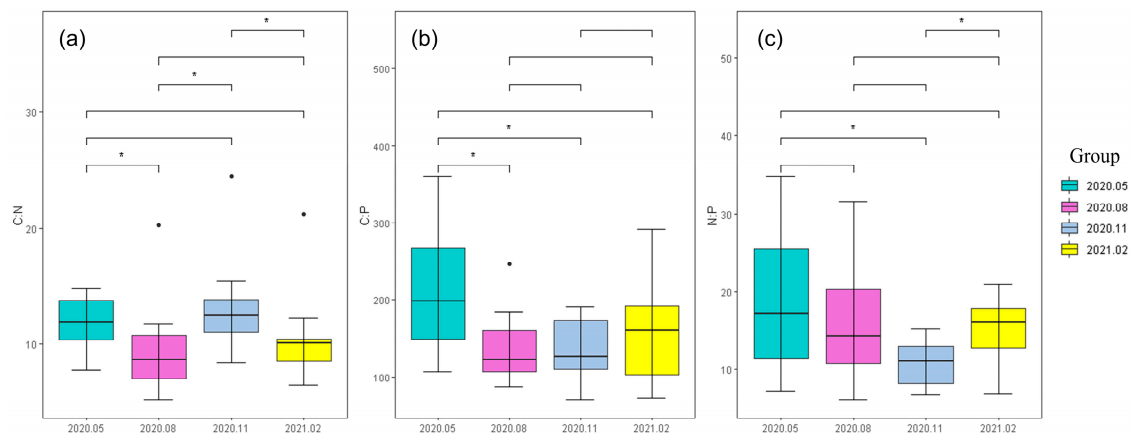


Figure 3. The box plots were used to represent mole ratio characteristics of phytoplankton C, N, and P in Taizicheng River. (a) The mole ratio characteristics of C:N; (b) the mole ratio characteristics of C:P; (c) the mole ratio characteristics of N:P. Different colors represent four different groups. Significance: * $p < 0.05$.

The contents of C, N, and P elements and the ratios of C:P, N:P, and C:N in phytoplankton samples from the Taizicheng River were analyzed by Spearman correlation analysis (Figure 4). The content of C was positively correlated with the content of N ($r = 0.57$, $p < 0.001$) and P ($r = 0.33$, $p < 0.05$), and the N content was also positively correlated with P content ($r = 0.24$, $p < 0.05$). The C:P ratio was significantly positively correlated with the content of C ($r = 0.53$, $p < 0.001$), while it was negatively correlated with the content of P ($r = -0.56$, $p < 0.001$). Similarly, the ratio of N:P was positively correlated with the content of N ($r = 0.65$, $p < 0.001$), but was negatively correlated with the content of P ($r = -0.52$, $p < 0.01$). C:N ratio had little correlation with the content of C but was correlated with the content of N ($r = 0.58$, $p < 0.001$). There was a significant negative correlation between the content of C:N and N ($r = -0.52$, $p < 0.001$), which showed that the change in C:N ratio had little relationship with the change in C element content, and mainly depends on the change in N element content. The ratio of C:P had a significant positive correlation with the ratio of N:P ($r = 0.59$, $p < 0.001$) as well as the ratio of C:N ($r = 0.23$, $p < 0.05$), while there was a weak negative correlation between C:P and C:N ($r = 0.23$, $p > 0.05$).

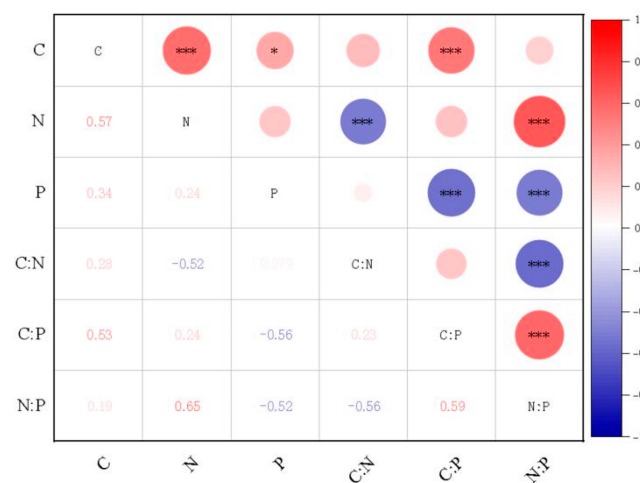


Figure 4. Spearman correlation coefficients among phytoplankton C, N, P, C:P, C:N, N:P in Taizicheng River. Significance: * $p \leq 0.05$; *** $p \leq 0.001$.

3.3. Stoichiometric Characteristics of S, H, and Fe in Phytoplankton

The ecological stoichiometric ratios of elements S, H, and Fe of phytoplankton in the Taizicheng River were also determined by molar ratio (Figure 5). The mean value of H:S in spring was 18.02 with a fluctuation range of 8.56–27.79, and the average value in summer was 14.12, with a fluctuation range of 9.09–21.83. The mean value in autumn was 49.99 and the fluctuation range was 13.19–339.64. The average value of 28.22 in winter ranged from 9.75 to 60.91, and the coefficients of variation in the four seasons were 0.26, 0.34, 0.42, and 0.60, respectively. The mean value of the Fe:S ratio was 0.36 in spring, with a range of 0.08–1.25, and 0.51 in summer, with a range of 0.17–1.18. The mean value in autumn was 0.78, and the fluctuation range was 0.24–1.97. The average value in winter was 0.03, the fluctuation range was 0.01–0.08. The coefficients of variation in the four seasons were 0.79, 0.61, 0.68, and 0.89, respectively. The mean value of the H:Fe ratio was 67.80 in spring with a range of 22.23–204.88, and 34.32 in summer. The fluctuation range was 12.60–58.06. The autumn average value was 50.44 with a fluctuation range of 24.12–172.25, and 1327.52 in winter with a range of fluctuation of 355.9–2803.88. The coefficients of variation were 0.38, 0.45, 0.44, and 0.66, respectively.

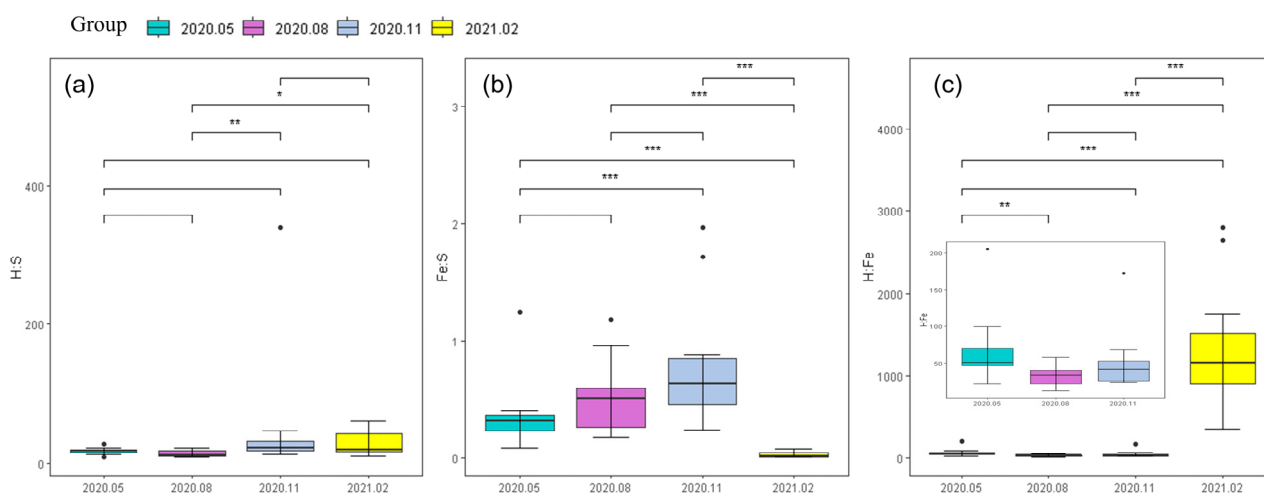


Figure 5. Mole ratio characteristics of phytoplankton H, S, and Fe in Taizicheng River. (a) The mole ratio characteristics of H:S in four groups; (b) the mole ratio characteristics of Fe:S in four groups; (c) the mole ratio characteristics of H:Fe in four groups. The subgraph shows the distribution characteristics of H:Fe in the first three groups. Different colors represent four different groups. Significance: * < 0.05 ; ** < 0.01 ; *** < 0.001 .

The overall stoichiometric ratio of phytoplankton H:Fe:S in the Taizicheng River was 41.47:40.28:1. The H:S ratio was relatively stable in May and August 2020 and the proportion of S element tended to increase, while the element ratio was unstable and gradually increased in November and February due to the decrease in protein content in phytoplankton. However, the ratio of Fe:S and H:Fe had changed significantly due to the significant decrease in Fe content in February, which led to a large abnormal condition in February 2021. Fe element was related to photosynthetic respiration of phytoplankton, and its content decreased by 95% in February with the weakening of photosynthetic respiration. Before this, the Fe:S ratio rose steadily and H:Fe remained stable. In February, the H:Fe ratio increased significantly and the Fe:S ratio was significantly reduced.

Spearman correlation was carried out on the contents of S, H, Fe, and the ratios of H:S, Fe:S, and H:Fe in all samples of phytoplankton in the Taizicheng River (Figure 6). The results are shown in Figure 6. There was no significant correlation between Fe and S, H, and there was a correlation between S and H ($r = 0.29$, $p < 0.05$). H:S was not significantly correlated with H:Fe, but was positively correlated with Fe:S ($r = 0.25$, $p < 0.05$). Additionally, there was a negative correlation between the Fe:S and H:Fe ratios ($r = -0.85$, $p < 0.001$).

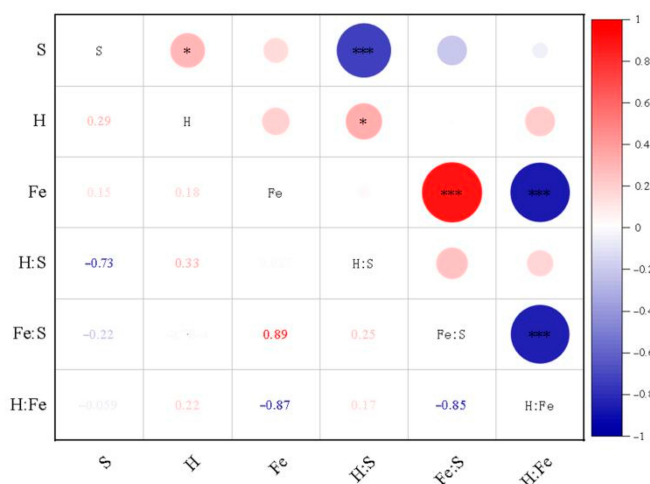


Figure 6. Spearman correlation coefficients among phytoplankton H, S, Fe, H:S, Fe:S, H:Fe in Taizicheng River. Significance: * $p \leq 0.05$; *** $p \leq 0.001$.

4. Discussion

Although the application of ecological stoichiometry began with plankton, the research of ecological stoichiometry in China has been more concentrated on soil, wetland, forest, grasslands, meadow, etc., and there are few studies on aquatic ecosystems, especially on river plankton [12]. Based on the classical Redfield ratio and the previous research results, our study analyzed the distribution pattern of elements C, N, P, S, H, and Fe of phytoplankton in the Taizicheng River, which is a mountain river in a cold area. Element C is a structural element of phytoplankton, and mainly participates in the synthesis of carbohydrates, with the highest content and the highest proportion, but its coefficient of variation was higher than that of N and P [13]. The coefficient of variation of N and P was about 37%, while that of C was 40.31%. The low content of N and P was relatively stable, which may be due to the fact that the input of nutrient elements N and P in the Taizicheng River was small and stable, and phytoplankton was limited by the supply of elements, absorbing only N and P elements for its use, resulting in its stable content. Phytoplankton can utilize dissolved CO_2 and HCO_3^- from the river water to meet their carbon demands, particularly given the global increase in atmospheric CO_2 concentrations [14]. According to its own growth stage, the absorption of element C was also different, resulting in a large fluctuation in its element C content and a large coefficient of variation. Although the content of element C in phytoplankton changed greatly, it was rarely limited by C, and more nutrient elements N and P limit the growth of phytoplankton. The coefficient of variation of S, H, and Fe elements was slightly larger than that of C, N, and P. As important nutrient elements of plankton, S and Fe were mainly involved in protein synthesis, while the latter were indispensable elements in plant photosynthesis, respiration, and other processes. The differences in plant life activities in different seasons form the temporal distribution characteristics of S and Fe. The absorption and action of hydrogen elements were related to water, with less content and relatively large fluctuations.

Redfield studied the elemental composition of marine plankton for the first time and proposed the elemental ratio of $\text{C}_{106}\text{H}_{263}\text{N}_{16}\text{P}$. According to the changes in hydrological environment and geological factors in the water ecosystem, there are differences in plankton stoichiometric ratio and biological composition [12]. Anderson proposed a new element composition of plankton, $\text{C}_{106}\text{H}_{263}\text{N}_{16}\text{P}$, through actual measurement. Wollast added the study of the S element and obtained the molecular formula of $\text{C}_{106}\text{H}_{263}\text{N}_{16}\text{S}_{1.7}\text{P}$ [13]. Xu Delin measured the elements of $\text{C}_{113.14}\text{N}_{20.06}\text{P}$ and $\text{C}_{105.86}\text{N}_{14.75}\text{P}$ in the grass and algal lakes of Taihu Lake [14]. Klausmeier calculated the N:P ratio between the phytoplankton growth explosion period and the relative plateau period, and found that the N:P ratio was higher in the competitive equilibrium period rather than the growth explosion period. The optimal N:P ratio of phytoplankton was between 8.2 and 45.0. It was suggested that the

classical ratio N:P = 16, which was the average ratio of each species in different periods and environments. Walker and Asams showed that organic C, N, P, and S were strongly related, both among different soils and with soil depth, and that the organic N:P ratio falls with depth in New Zealand grassland soils [15]. Kirkby et al. concluded that SOM of mineral soil has an approximately constant stoichiometry, and calculated a rounded stoichiometry of 52:5:1:1 (C:N:P:S) according to the data they collated for soils from 22 countries [16]. Our results showed that the phytoplankton element ratio was $C_{156.00} N_{15.41} S_{1.54} H_{51.17} Fe_{5.10} P$ in the Taizicheng River, and there were higher C, N, P contents than the classical Redfield ratio, and the proportion of H share fell dramatically.

The shallow water and strong illumination of the Taizicheng River may be the reason for the high proportion of phytoplankton element C. Berger studied the stoichiometry of plankton at different depths in 65 lakes in central Europe, and proved the positive correlation between light and C:P ratio [17]. The succession of phytoplankton community structure can also lead to a change in stoichiometry. For example, diatom was the main contributor to C element composition in river plankton, and the higher the dominance of diatom in phytoplankton community structure, the greater the stoichiometric ratio of phytoplankton C element. Diatom was one of the main species of phytoplankton community in the Taizicheng River, and it was also the reason for the relatively high proportion of C element. The N:P ratio of phytoplankton in the Taizicheng River was 15.41, which basically accorded with the classical Redfield ratio. The content of P increased in November and February, and the content of N increased in February. Low temperatures would induce the plant to store nutrients to resist the adverse effects of winter on growth. In stoichiometric studies, the ratio of N:P was usually used to determine the limiting element. When N:P was less than 14, N was a restrictive nutrient element, and when N:P was greater than 16, P was the limiting nutrient element. Between 14 and 16, it was subject to the comprehensive limitation of N and P. Combined with the actual data, it can be concluded that the growth of phytoplankton in the Taizicheng River was restricted by N and P. Additionally, phosphorus was a limiting factor in river ecosystems, which may have important implications for predicting the effects of environmental changes, such as climate change or land use change, on ecosystem functioning.

Phytoplankton elements were coupled with each other through their life activities. N was mainly used for plant protein production and P was mainly involved in the synthesis of nucleic acid. The storage of other elements relies on ion balance to adjust. The growth of plants needs to synthesize protein, which would inevitably increase the consumption of P element with the transcription of DNA to generate RNA, and this process significantly enhances the correlation between N and P. As a “skeleton element”, element C played a supporting role in the cell wall, and its content increased with the growth and development of plants. The contents of C, N, and P in plants were significantly correlated, while the correlation between S, H, and Fe was not significant. Only the elements S and N were significantly correlated at the level of 0.01, and both of them participated in protein synthesis. However, this did not prove that there was no interaction between elements. Some studies have shown that Fe in diatoms can affect the ratio of C:N:P by affecting their own growth rate and body size [18,19]. Boyd and Geider proved that iron in algae can promote some enzyme activities during the synthesis of proteins and nucleic acids and the absorption of CO₂ in the atmosphere, thus affecting the stoichiometric characteristics of their own C, N, and P [20].

5. Conclusions

In this paper, the ecological stoichiometric characteristics of C, N, P, S, H, and Fe elements in river phytoplankton in cold mountain areas were studied, and the results were compared with the Redfield ratio and previous research results. We know that the phytoplankton elements of the Taizicheng River were $C_{156.00} N_{15.41} S_{1.54} H_{51.17} Fe_{5.10} P$, fluctuating seasonally. Compared with the value of Redfield and previous studies, element C accounted for a higher proportion, element H accounted for a lower proportion, element

Fe content changed greatly, and the remaining elements fluctuated within a small range. Further, we know that the growth of phytoplankton in the Taizicheng River was limited by both N and P elements according to the theory of chemometrics restrictive elements. In addition, the contents and ratios of elements C, N, and P of phytoplankton in the Taizicheng River were coupled through life activities, which had a strong correlation and had a great influence on the growth of phytoplankton. However, the S, H, and Fe elements were relatively independent and had no significant correlation. The elements and their proportions in river bodies and zooplankton need to be further explored in future studies. Overall, our results represent a considerable advancement in understanding the distribution patterns of phytoplankton stoichiometry and the impact of seasonal changes on phytoplankton stoichiometry in cold temperate area. It paves the way to better understand how these ecosystems work and for further guidance and a better methodology of maintaining the balance and stability of the freshwater ecological environment.

Author Contributions: H.Z. designed experiments, determined the article framework and research methods, and wrote the paper. L.J. completed experiment sampling, performed data analysis, and wrote the paper. Z.W., Y.T., W.T. and Z.L. contributed to the research and writing of the paper. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: If anyone would like data from this study, please contact the first author at geelyz@163.com for a link to the data.

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