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A High Accumulation of Dissolved Organic Matter in the Water Resulting from Terrestrial Input into a Large, Shallow Steppe Lake

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Abstract: Dissolved organic matter (DOM) is generally the dominant carbon pool in freshwater lakes and plays a vital role in the regional or even global carbon cycle. In recent years, steppe lakes have been subject to two stressors: eutrophication and abnormally high concentrations of organic matter. However, little is known about the sources and composition of the organic matter. In this study, carbon/nitrogen (C/N) ratios and three-dimensional excitation–emission matrix (3DEEM) fluorescence spectroscopy were adopted to identify the sources and composition of DOM in Hulun Lake, a large shallow steppe lake. The physicochemical and water quality parameters of Hulun Lake and three inflow rivers, Crulen River, Orshen River and Hailar River, were also investigated. The results showed that visible fulvic-like fluorescence (peak C) and tryptophan-like substances (peak T) were the main components of the DOM. The C/N atomic ratios and spectral characteristics of the DOM suggested that approximately 82% of the DOM in Lake Hulun is derived from terrigenous material. The results underscore that it is necessary to devote more research attention to terrestrial inputs into steppe lakes.

Keywords: Hulun Lake; steppe; organic matter; eutrophication; source identification

Citation: Zhang, C.; Li, W.; Du, W.; Cao, B.; Wang, W.; Pang, B.; Dou, H.; Ao, W.; Liu, B.; Yao, S. A High Accumulation of Dissolved Organic Matter in the Water Resulting from Terrestrial Input into a Large, Shallow Steppe Lake. *Water* **2023**, *15*, 1646. https://doi.org/10.3390/w15091646

Academic Editor: Roohollah Noori

Received: 20 February 2023
Revised: 8 March 2023
Accepted: 21 April 2023
Published: 23 April 2023

1. Introduction

With increasing eutrophication in lakes across the world, the number of cyanobacteria outbreaks has increased significantly, and the biodiversity of aquatic systems has decreased [1,2]. Dissolved organic matter (DOM) is the largest organic carbon source, and it plays an important role in the physicochemical and microbial degradation processes of aquatic systems [3–5]. Chromophoric dissolved organic matter (CDOM) can absorb energy and re-emit it as fluorescence through chemical processes, thus constituting the colored component of DOM [6]. DOM has both autochthonous and allochthonous sources. Autochthonous production results from organisms that grow in situ, such as hydrophytes and other photoautotrophs, and release of substances from sediment. Allochthonous inputs are discharged into a lake by rivers, atmospheric deposition or runoff, and can contain anthropogenic products [7–9].

The shallow steppe lake, considered to be a crucial ecosystem, provides a unique habitat for local fish, birds and other aquatic life [10]. As one of typical steppe lakes in the cold and arid regions of China, Hulun Lake is located on the Hulun Buir Steppe, where the surrounding population density is low and human influence is relatively limited [11]. However, it was found that the DOM content in Hulun Lake was unusually high and reached approximately 59 mg C/L [11]. It is several times that of freshwater lakes and salt lakes in China, which have DOM concentrations of 6.68 mg C/L and 27.4 mg C/L, respectively [12]. Therefore, it is necessary to carry out in-depth research on the phenomenon of high DOM concentration in these steppe lakes in order to explore whether the DOM derives from internal production or terrestrial input.

In recent years, several techniques have been widely used to analyze the sources of DOM in lakes. Atomic C/N ratios are commonly used to distinguish between terrestrial (soils, vascular plant debris) and autochthonous microbial (phytoplankton) sources of DOM [13]. Higher C/N values indicate DOM derived from pure soil organic matter (C/N > 10) and vascular plant debris (C/N > 20), while lower C/N values indicate organic matter from phytoplankton (C/N ≈ 4–10) [12,14]. Recently, the combined technique of excitation–emission matrix (EEM) fluorescence with parallel factor analysis (PARAFAC) has been developed and applied to evaluate the sources and composition of DOM in aquatic ecosystems [15,16]. Furthermore, the composition and optical properties of DOM are affected by the nutrient level. The relative molecular size and the slope ratio of DOM have been found to be negatively correlated with nutrient level [17]. The nutrient level could lead to an increase in the endogenous ratio of DOM by accelerating phytoplankton growth, but the spectral characteristics of DOM were not directly affected by the phytoplankton community [4]. Therefore, the effect of the nutrient level on the composition and spectral characteristics of DOM is an important and cutting-edge scientific topic in the field of DOM research.

Hulun Lake is a lake characteristic of grassland areas and is in a high-altitude region. Until now, comprehensive analysis of concentrations and characteristics of DOM in Hulun Lake and of the origins of the DOM has not been conducted. Furthermore, there have been few studies on similar lakes. In this study, we analyzed the fluorescence and sources of CDOM in Hulun Lake and its principal inflow rivers. The objectives of the study are the following: (1) characterize the variation in spectral characteristics of DOM; (2) determine the correlations between CDOM fluorescence and water-quality parameters; and (3) reveal the origin of DOM and the factors influencing it.

2. Materials and Methods

2.1. Study Sites

Hulun Lake, located in northeast Inner Mongolia, is the fifth largest lake in China (48°31′–49°20′ N; 116°58′–117°48′ E). Its average water depth is around 5.7 m, with a maximum water depth of 8 m recorded in 1968, and a minimum water depth of 3 m observed in 2009 [18]. The water storage of the lake ranges from 14.0 billion m^3 to 3.3 billion m^3, and its area ranges from 1609.6 km^2 to 2406.0 km^2 [19]. In 1992, the Hulun Lake National Nature Reserve was established, covering an area of 7400 km^2 [19].

The whole area is sparsely populated, and anthropogenic influence has been limited to inflow discharge with regard to three inlet rivers, Crulen River, Orshen River and Hailar River, and one outlet river, Xinkai River (Figure 1). The average annual runoff of Crulen River and Orshen River were 1.91 million m^3 and 4.07 million m^3, respectively, between 2001 and 2019 (data acquired from Hulun Buir Bureau of Water). Hailar River, located in southeastern Hulunbeir in China, is the mother river of the Hulun Buir Steppe. The river runs out of the Da Xingan mountains, and the length of its main channel is 708.5 km [20].

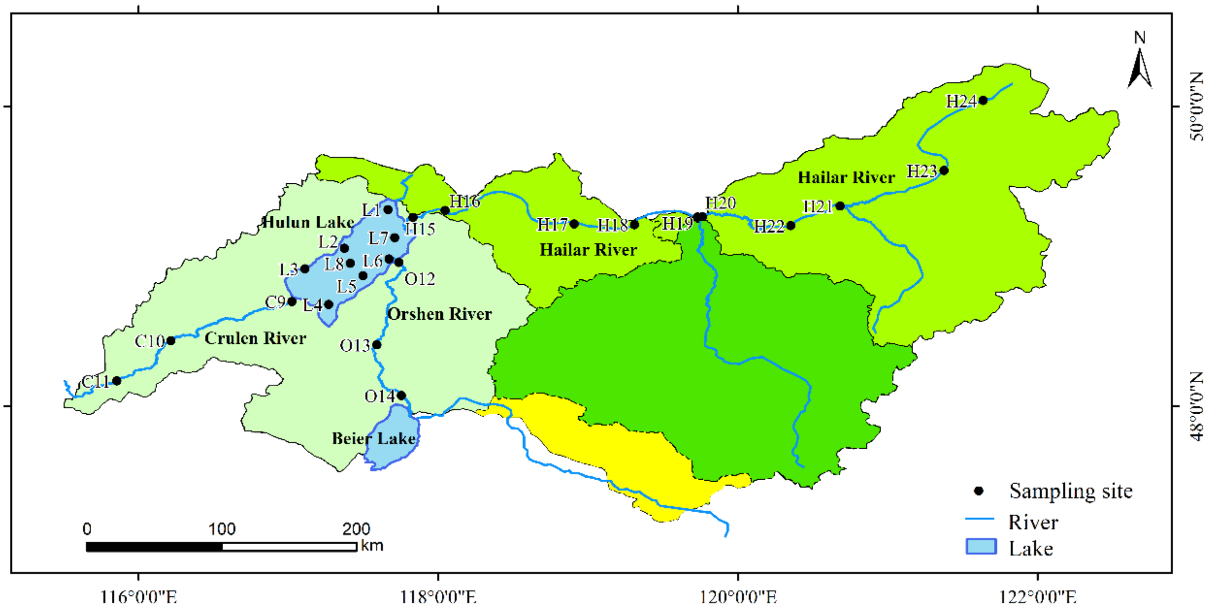

Figure 1. Allocation of sampling sites in Hulun Lake and the three headwater streams of Crulen River, Orshen River and Hailar River.

2.2. Sampling

The investigation was conducted in August 2019. In total, 24 water samples were collected from Hulun Lake (L1 to L8), Crulen River (C9 to C11), Orshen River (O12 and O14) and Hailar River (H15 to H24) (Figure 1). Monitoring stations were set up in the upper, middle and lower reaches of each river to monitor water quality along its length. The stations were set up on bridges or in places that were relatively easy to monitor. Eight points were set up in Hulun Lake to allow the study to fully characterize the differences between different regions. The samples were carefully collected from 50 cm beneath the air–water interface, with three collected from each site. They were stored in amber glass bottles at a cool temperature and immediately transported to the laboratory for quantification.

2.3. Physical and Chemical Analytical Methods

Water temperature (T), the pH and dissolved oxygen (DO) levels were measured in situ using a multiparameter water quality monitor at each sampling site (YSI 6600, Washington, DC, USA). In the laboratory, total nitrogen (TN) was analyzed using a UV-6100 spectrophotometer (Mapada, Shanghai, China) and the method of Raveh and Avnimelech [21]. Total phosphorus (TP) was tested using colorimetry after digestion with $K_2S_2O_8$ and NaOH [22]. Samples for dissolved total nitrogen (DTN) and phosphorus (DTP) were filtered first and then measured with the same methods used for TN and TP. Total organic carbon (TOC) was analyzed using a total organic carbon analyzer (TOC-L CPN, Shimadzu, Japan). The C/N ratio was calculated based on TOC and TN values. Chemical oxygen consumption (COD_{Mn}) was measured using the acid-potassium permanganate method. Measurements of chlorophyll a (Chl.a) were obtained via acetone extraction [23]. The 3DEEM spectra of samples were measured with an RF-6000 fluorescence spectrometer.

2.4. Analysis of Trophic Level Index and Contribution of Terrigenous DOM

The trophic level index (TLI) method based on Chl.a was used to evaluate the eutrophication state [24]. In accordance with the empirical recommendations for lake water quality in China, the relative weights of water quality parameters were selected (Table 1). In summary, different trophic state indices, including Chl.a, TP, TN and COD_{Mn}, were calculated, followed by the normalized correlation weight of the parameters. Then, the weighted average of the comprehensive trophic state index of the water body was calculated as part of the study [24].

Table 1. The correlation between chlorophyll a and the parameters of lake water (reservoir) in China [24].

	Chl.a	TP	TN	COD_{Mn}
r_{ij}	1	0.84	0.82	0.83
r_{ij}^2	1	0.7056	0.6724	0.6889

Note: Chl.a: chlorophyll a; TP: total phosphorus; TN: total nitrogen; COD_{Mn}: chemical oxygen demand.

The contribution of terrigenous DOM was calculated by substituting the FI values in the model equation [25]. The modeled relationship between FI and the proportional contribution from terrestrial sources is as follows:

$$y = 3.94(0.18x + 10)^{-0.316} \quad (1)$$

where y represents FI, and x represents the contribution of allochthonous DOM.

2.5. Statistical Analysis

Statistical Package for the Social Sciences 24.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. Pearson's correlation analyses were used for exploring relationships between water quality variables and odorants ($p < 0.05$). A dendrogram was produced by CA using Ward's method with squared Euclidean distances. The graphs were plotted using Origin 9.0 software.

3. Results

3.1. The Physicochemical Characteristics of Water Samples

The physical and chemical characteristics of sampling sites from August 2019 are listed in Figure 2. The concentration of DO in Hulun Lake ranged from 7.82 mg/L to 11.67 mg/L, and the average value was 9.67 mg/L. In Crulen River, DO was at a steady value of 7.54 mg/L. However, DO concentration in Orshen River ranged from 3.56 mg/L to 7.76 mg/L, with the lowest value observed in the estuary. The content of DO in Hailar River ranged from 7.30 mg/L to 9.56 mg/L, with a mean value of 8.17 mg/L. Hulun Lake was weakly alkaline with a pH value ranging from 8.83 to 9.40. In Crulen River, the average pH was 7.66, and higher values were found in the estuary. In Orshen River, the pH at sampling sites ranged from 8.34 to 8.78; there was no significant difference in pH across the sampling sites. The pH values measured in the estuary of Hailar River were much higher than levels recorded upstream.

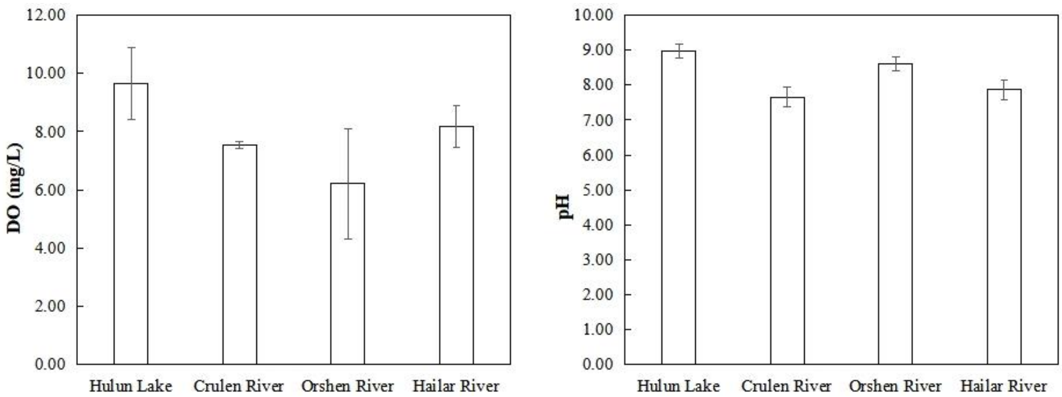

Figure 2. *Cont.*

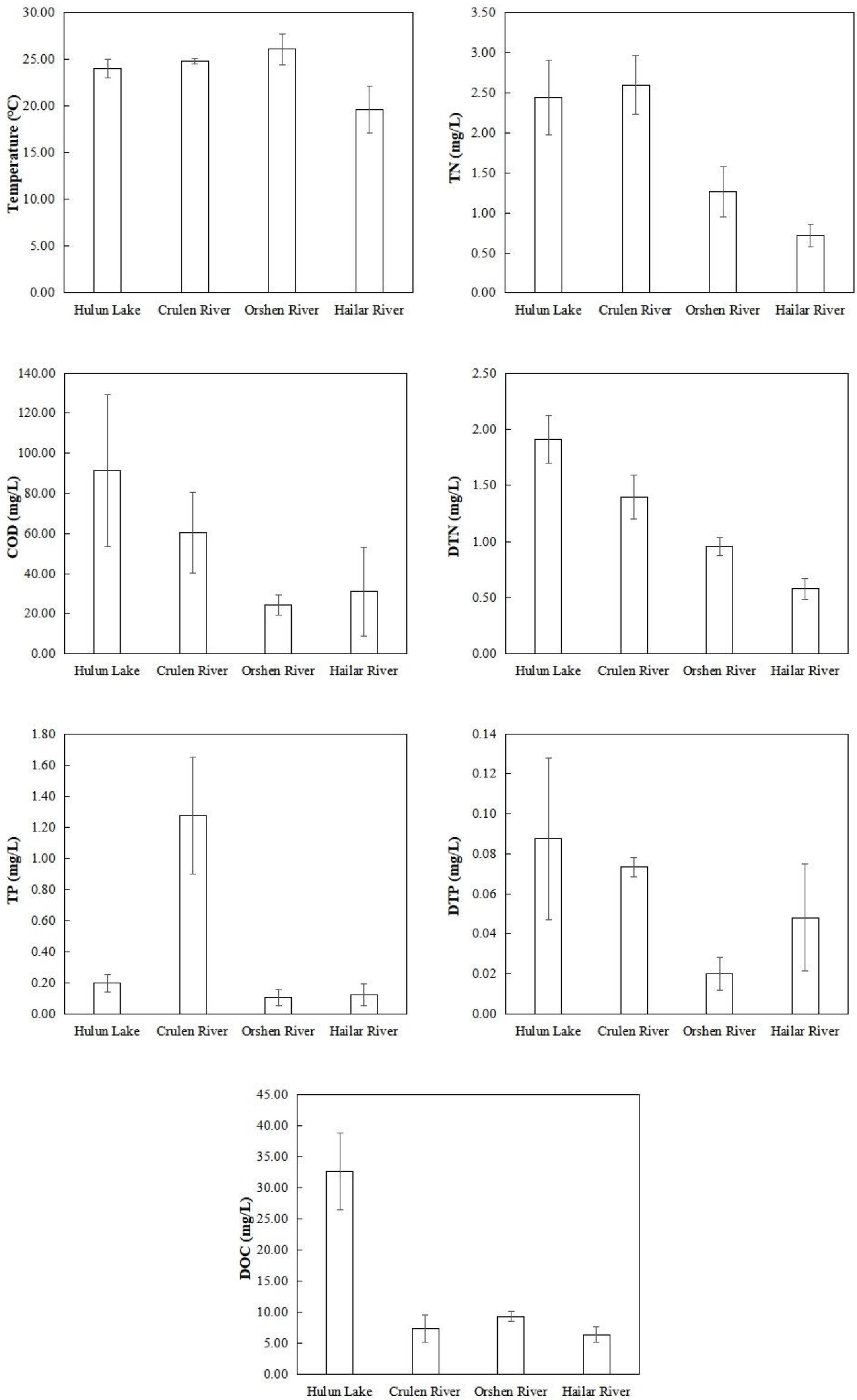

Figure 2. Physical and chemical parameters of sampling sites.

In Figure 2, the TN of Hulun Lake ranges from 2.01 mg/L to 3.57 mg/L, with an average value of 2.45 mg/L. The highest TN was in the northeast section of the lake (L6). DTN concentrations ranged from 1.72 mg/L to 2.37 mg/L, with a mean value of 1.91 mg/L. The dominant share of TN was DTN (the highest concentration of DTN being 88.92% of TN). The concentrations of TP and DTP ranged from 0.09 mg/L to 0.26 mg/L and from 0.03 mg/L to 0.14 mg/L, respectively. The level of TOC oscillated between 21.47 mg/L and 39.75 mg/L in Hulun Lake, with an average of 32.58 mg/L.

Nutrient loadings from Crulen River, Orshen River and Hailar River demonstrated the contrast in TN, TP and COD inflows during the study. In Crulen River, TN oscillated between 2.09 mg/L and 2.96 mg/L, with an average of 2.60 mg/L. The highest value occurred in the estuary. The highest concentration of DTN was 53.97% of TN. In Orshen River, the concentrations of TN and DTN ranged from 0.97 mg/L to 1.70 mg/L and from 0.88 mg/L to 1.07 mg/L, respectively. The highest value occurred in the estuary, which was consistent with the pattern seen in Crulen River. In Hailar River, TN and DTN ranged from 0.53 mg/L to 0.98 mg/L and from 0.40 mg/L to 0.73 mg/L, with averages of 0.72 mg/L and 0.58 mg/L, respectively. The mean values of TP in Crulen River, Orshen River and Hailar River were 1.27 mg/L, 0.11 mg/L and 0.12 mg/L, respectively. The dominant share of TP was PTP. TOC in Crulen River and Orshen River oscillated between 5.75 mg/L and 10.45 mg/L and between 8.58 mg/L and 10.48 mg/L, respectively, with maximum values occurring in the estuary of both rivers. In Hailar River, the level of TOC varied from 5.33 mg/L to 9.20 mg/L.

C/N atomic ratios and stable carbon isotopes were used to distinguish the contributions of terrigenous origin and those from phytoplankton. In Hulun Lake, the C/N ratios ranged from 6.01 to 17.90 (Figure 3). The lowest C/N atomic ratios were recorded in the northeast part of the lake (L6), which contained a high concentration of Chl.a (96 µg/L). At the sampling site, the DOM was mainly derived from phytoplankton due to the influence of cyanobacterial blooms. C/N levels in Crulen River varied from 2.15 mg/L to 3.53 mg/L, with a mean value of 2.81 mg/L. The mean values for C/N in Orshen River and Hailar River were 7.64 mg/L and 9.30 mg/L, respectively.

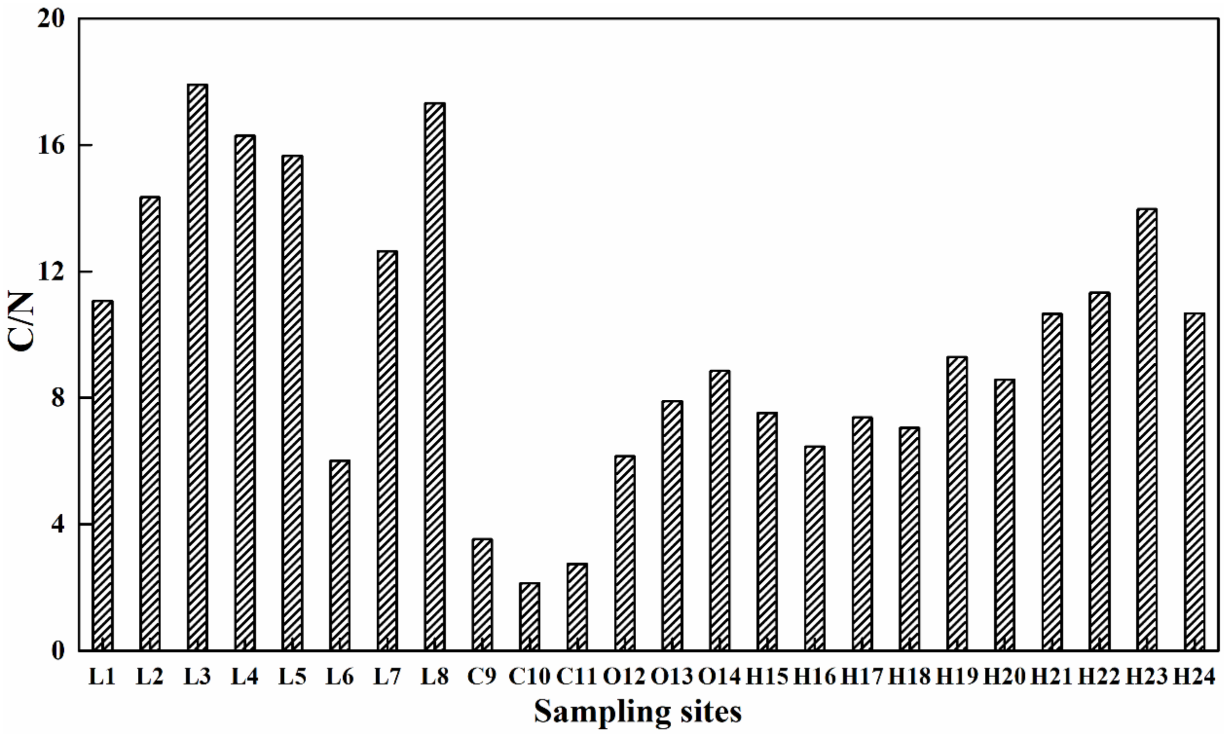

Figure 3. DOC/TN (C/N) ratios of water from Hulun Lake and its catchment.

3.2. Spectral Characteristics of DOM

The 3DEEM technique was used to identify the sources of DOM in Lake Hulun and the rivers. The peaks in the fluorescence EEMs of DOM in water of Lake Hulun and the rivers were similar to previously identified peaks (Figure 4, Table 2). Generally, these fluorescence EEMs featured two main peaks. One main peak was at the excitation/emission wavelengths (Ex/Em) of 325/425 nm (designated Peak C), which has been identified as visible fulvic-like fluorescence [8]. The other was observed at Ex/Em of 300/350 nm and is regarded as relating to tryptophan-like substances (designated Peak T).

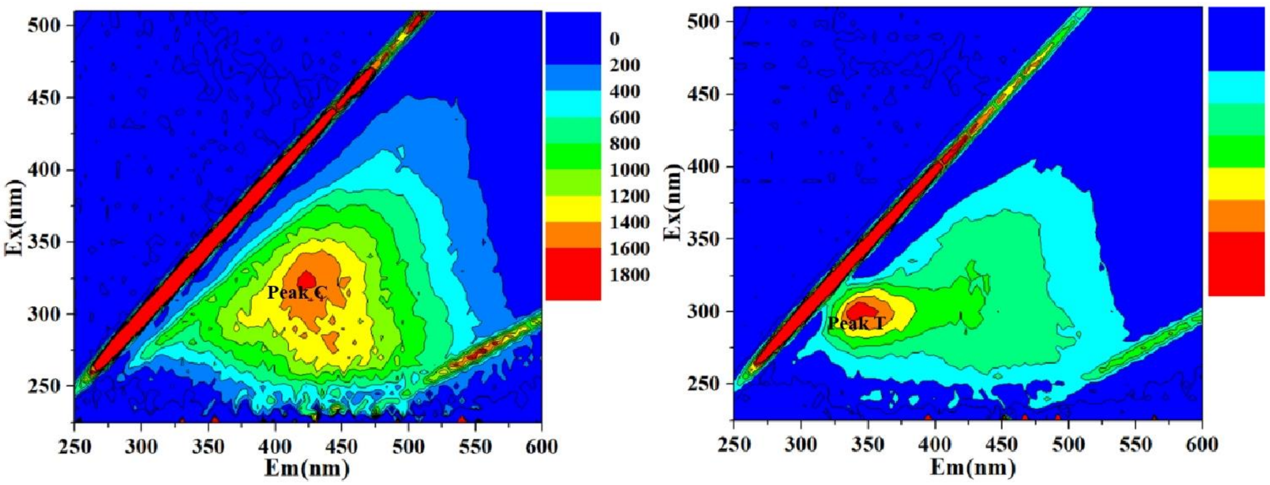

Figure 4. EEMs of DOM in Hulun Lake and the catchment.

Table 2. Major fluorescence peaks and their spectral positions for dissolved organic matter as stated in previous studies.

Label	Description	Excitation (nm)	Emission (nm)	Source
C	Visible Fulvic-like	320–360	420–460	[26]
C	Visible Fulvic-like	275–310	380–460	[27]
T	Tryptophan-like	270–290	320–370	[27]
T	Tryptophan-like	286	336	[28]

The FI in Hulun Lake ranged from 1.38 to 1.55, with an average of 1.43 (Figure 5). The mean values of FI in Crulen River, Orshen River and Hailar River were 1.53, 1.48 and 1.49, respectively.

3.3. Correlations between Spectral Characteristics of DOM and Water Quality Parameters

In this study, Orshen River and Hailar River were in a mild eutrophication state with TLI values of 56.42 and 50.23, respectively. The level of TLI in Hulun Lake was 64.91, indicating an intermediate nutrition status. Crulen River was in a severe eutrophication state, with TLI values exceeding 70 (Figure 6). There were strong positive correlations between TLI and water quality constituents, such as TN, TP, TOC and COD (Table 3). Significant correlations were also found between TN and DTN and between TOC and TN ($p < 0.01$). A positive correlation was also observed between TP and TN ($p < 0.05$). However, there were strong negative correlations between FI and C/N. The FI increases as the C/N ratio decreases [29]. In the current study, the COD and TOC showed a significant positive correlation ($p < 0.01$), indicating that TOC was the predominant organic matter in the water.

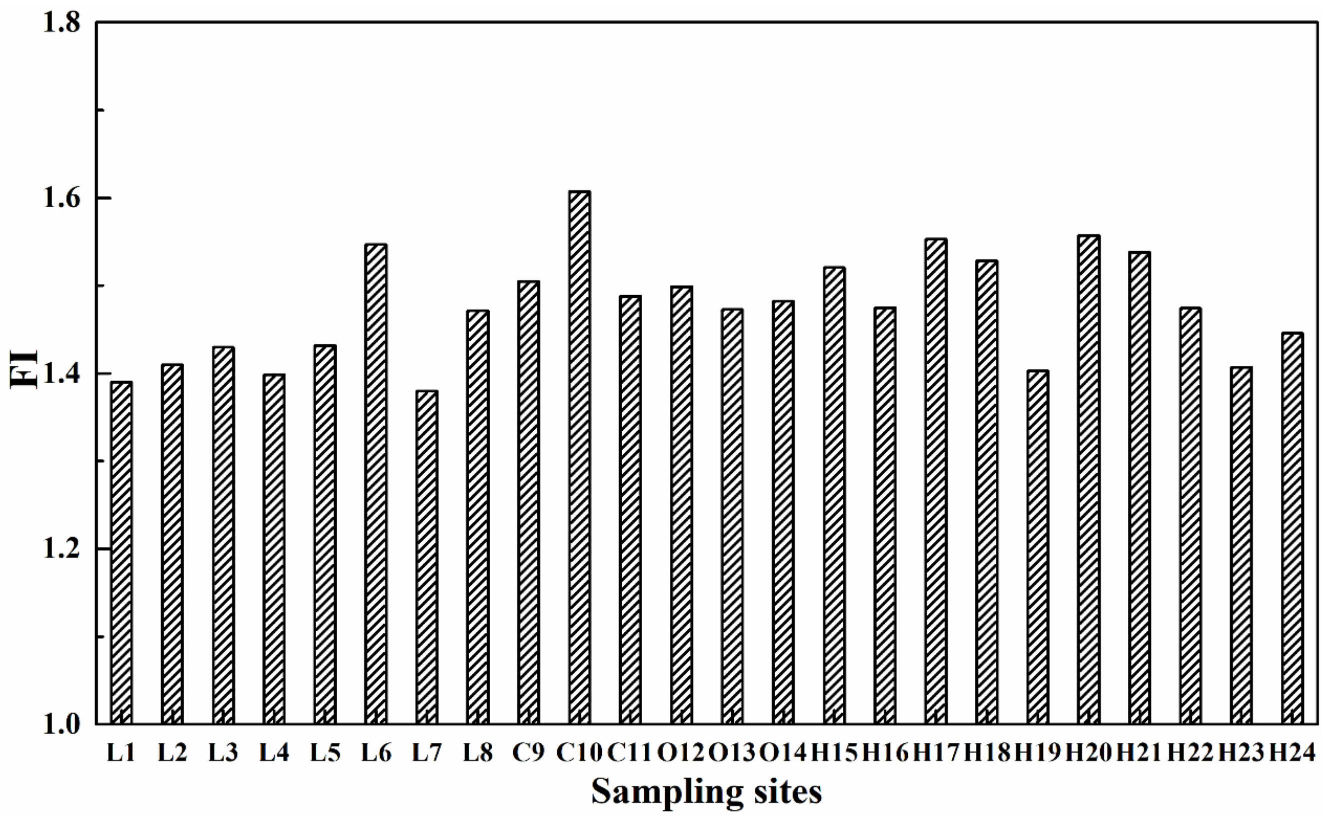

Figure 5. Fluorescence index (FI) in different samples from Hulun Lake and its catchment.

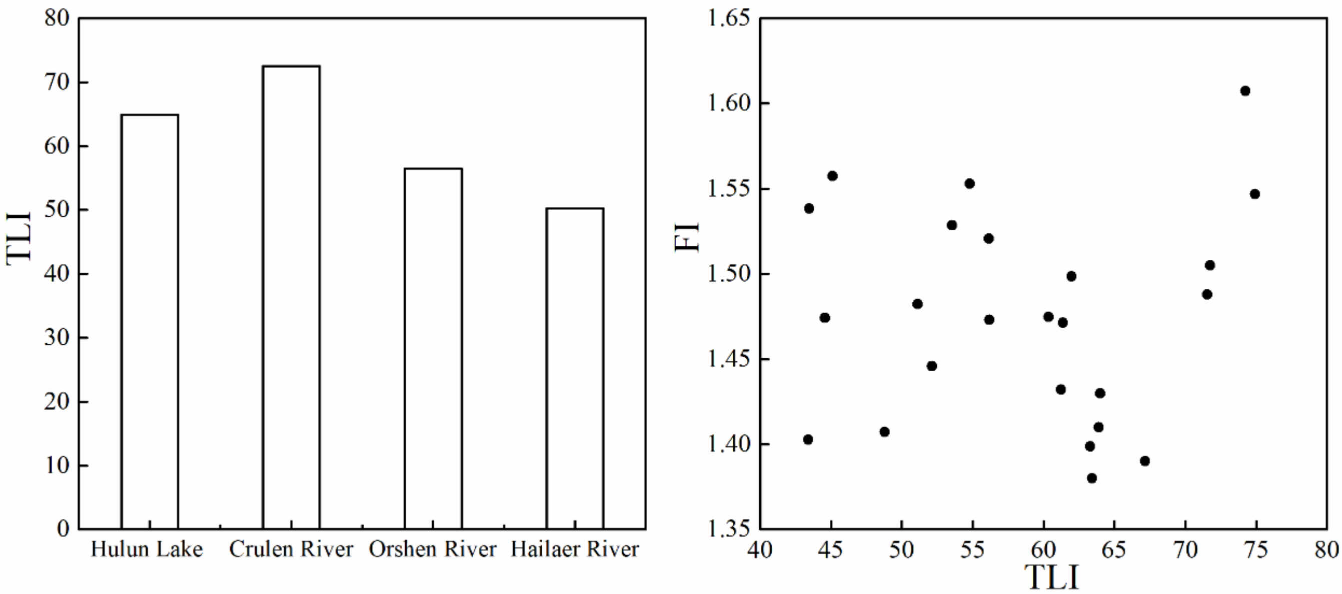

Figure 6. The trophic level index (TLI) and the fluorescence index (FI) of dissolved organic matter (DOM) at each sampling site.

Table 3. Pearson's correlation matrix of the water quality indices in samples.

	DO	pH	T	TP	DTP	TN	DTN	DOC	COD	C/N	FI	TLI
DO	1	0.391	−0.147	−0.129	0.335	0.339	0.492 *	0.465 *	0.552 **	0.404 *	−0.332	0.198
pH		1	0.593 **	−0.333	0.346	0.544 **	0.737 **	0.736 **	0.807 **	0.426 *	−0.393	0.378
T			1	0.316	0.229	0.602 **	0.556 **	0.366	0.453 *	−0.146	0.034	0.616 **
TP				1	0.232	0.479 *	0.227	−0.179	−0.054	−0.586 **	0.432 *	0.637 **
DTP					1	0.420 *	0.502 *	0.505 *	0.514 *	0.237	−0.162	0.463 *
TN						1	0.933 **	0.615 **	0.749 **	−0.008	−0.062	0.918 **
DTN							1	0.807 **	0.916 **	0.286	−0.278	0.794 **
DOC								1	0.936 **	0.756 **	−0.558 **	0.419 *
COD									1	0.561 **	−0.507 *	0.587 **
C/N										1	−0.662 **	−0.232
FI											1	0.062
TLI												1

Note: Significant correlation at the * $p < 0.05$ and ** $p < 0.01$ levels.

4. Discussion

4.1. The Source and Composition of DOM in Hulun Lake

There is widespread use of 3DEEM fluorescence spectroscopy to identify sources of organic matter in water or soil. The fluorescence index (FI) is the ratio of the emission intensity at a wavelength of 470 nm to the emission intensity at 500 nm, following an excitation wavelength of 370 nm. It has been found that the FI can be adopted as an important parameter to distinguish sources of CDOM. The FI in Hulun Lake ranged from 1.38 to 1.55, with an average of 1.43, which indicated that allochthonous organic matter is the main source of DOM in the lake.

C/N ratios have been widely used to determine OM sources and biogeochemical processing in natural lakes. The C/N ratio of the OM derived from terrigenous material was more than 20, while phytoplankton exhibits C/N ratios of between 4 and 10 [14]. Prahl et al. [30], however, considered the C/N ratio of terrigenous organic matter to be over 12 rather than over 20. In addition, a study has shown that the contribution of fresh vegetation to organic matter appears relatively small, meaning a C/N ratio of 14.6 may be representative of terrestrial sources [31]. Chappaz et al. [32]. found that the C/N atomic ratios of OM in certain lakes dominated by terrestrial materials were only 13–16. In this context, the average C/N ratio of 13.91 in Hulun Lake suggests that the DOM within it is largely dominated by terrigenous matter. The C/N ratios of all rivers were below 16; the C/N ratio of Crulen River was the lowest of the three rivers entering the lake, while the C/N ratios observed in Hailar River gradually decreased, moving upstream to downstream. The C/N values of different types of soils have also been investigated in the Hulun Lake Basin (unpublished). The average C/N ratios in grassland and forest soils were 10.35 and 15.61, respectively, which were similar to the values in Hulun Lake. Thus, the DOM in Lake Hulun is terrigenous matter originating from, for example, soil leaching and riverine inputs.

As shown in Figure 7, the spatial clustering analysis produced a tree diagram, which classified 24 sampling points into 5 clusters with statistical significance. Group I comprised sampling sites 1, 14, 19, 20, 21, 22 and 24; Group II contained sampling sites 6, 12, 13, 15, 16, 17 and 18; Group III consisted of the sites 9, 10 and 11; Group IV contained sampling sites 2, 7 and 23; and Group V included the remaining sampling sites. The results showed that the concentration of organic matter in Hulun Lake is affected equally by the different river inputs.

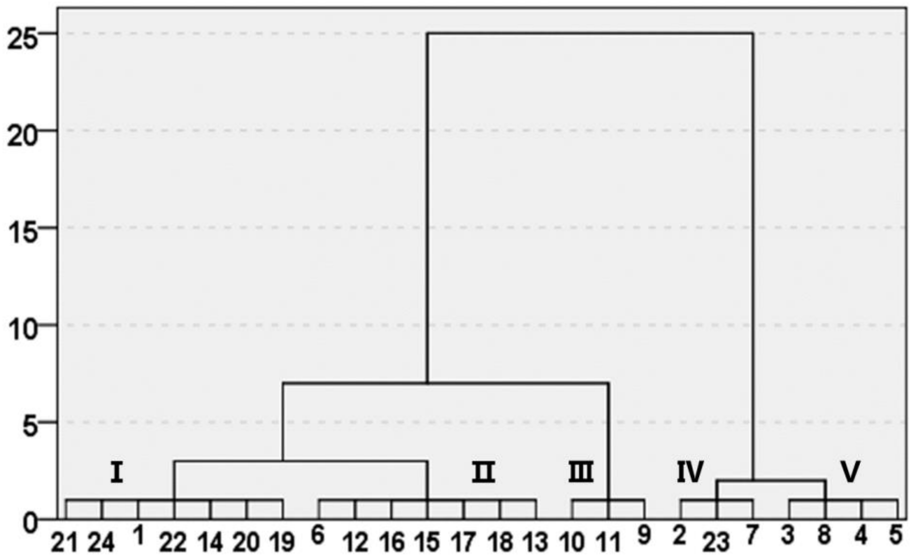

Figure 7. Dendrogram showing spatial correlation analysis of sampling sites. Hulun Lake (1–8), Crulen River (9–11), Orshen River (12–14) and Hailar River (15–24).

Additionally, the contribution of phytoplankton in summer cannot be ignored. At the L6 sampling site, the lower C/N value indicated that phytoplankton contributed a certain fraction of the DOM in Hulun Lake during the summer. The FI value (1.55) further confirmed this conclusion.

4.2. The Relative Contributions of Terrestrial DOM to Hulun Lake

DOM fluorescence spectra have been widely studied to investigate the age, source and reactivity of DOM from a variety of aquatic and marine sources [33]. The relative contributions of autogenic and terrestrial organic materials to DOM can be distinguished by analyzing the fluorescence properties of the fulvic acid share of DOM [25]. FI provides valuable information regarding the source and quality of DOM [34].

The mean values of FI in Hulun Lake, Crulen River, Orshen River and Hailar River were 1.43, 1.53, 1.48 and 1.49, respectively. The contributions of allochthonous DOM to Crulen River, Orshen River and Hailar River were determined to be 55.41%, 66.77% and 66.11%, respectively. In Hulun Lake, the contribution of allochthonous DOM could be calculated to range from 50.83% to 98.10%, with an average of 82.07%, which was similar to the results of Chen et al. [11].

4.3. The Factors Driving Terrigenous Organic Matter Input into Hulun Lake

An increase in DOM concentrations has been observed in lakes of the Northern Hemisphere, and is thought to have been caused by the climate and changes in land use [35,36]. Fellman et al. [37] found land use and land management to have an impact on the biochemistry of DOM in water systems. Hulun Lake, located on the Hulun Buir Steppe, is a lake in one of the cold regions of northern China. The number of livestock grazing on the Hulun Buir Steppe rose from 1.106 million in 1949 to 6.77 million in 2004, increasing more than sixfold in 55 years. This led to overgrazing [38]. Climate change and the increased impacts of human activities are directly or indirectly affecting grassland ecosystems [39]. The annual average air temperature of Lake Hulun gradually increased between 1958 and 2018 (Figure 8a). As the temperature rises, evaporation increases, making the surface evaporation increment much greater than the precipitation increment; grassland water loss becomes a serious issue, soil salinity increases, native vegetation growth is blocked and regional vegetation cover changes significantly [40,41]. The degradation of grassland is significant, and the proportion of weeds increases. Tumbleweed is common on Inner Mongolian grassland. In this study, C/N atomic ratios and spectral characteristics of DOM suggested that approximately 82% of the DOM in Lake Hulun is derived from terrigenous material. Tumbleweed is also a major source of terrestrial input. As shown in Figure 8b, the area of grassland desertification in 2015 was 2.06 times that in 2002 [42]. Grassland degradation and the desertification of land will increase dust storm erosion and aggravate eutrophication in Hulun Lake. Therefore, the stability of grassland ecosystems warrants meaningful consideration and attention.

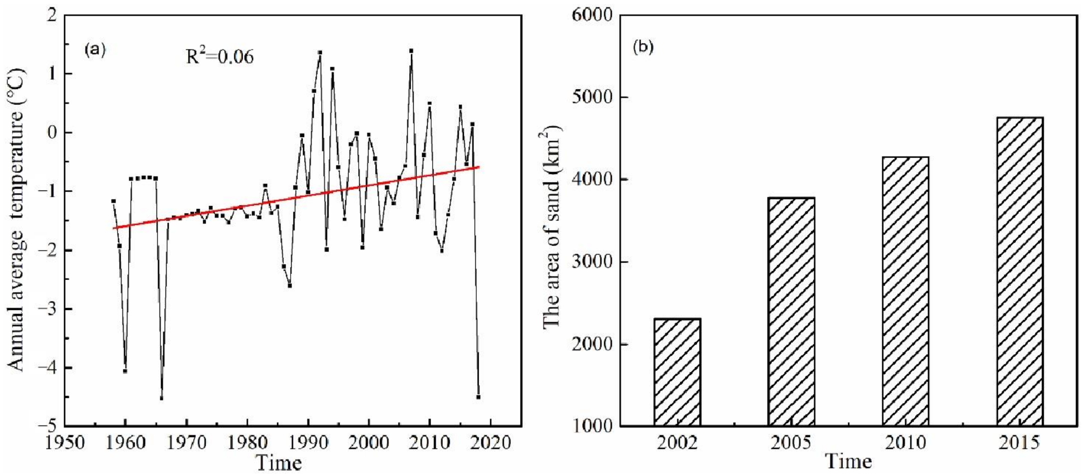

Figure 8. (**a**) Variation of the annual average air temperature of Lake Hulun (data from Meteorological Bureau of Hulun Buir City, China) and (**b**) the area of sand (figure from [41]).

5. Conclusions

This study investigated the concentrations and characteristics of CDOM and water parameters (TN, DTN, TP, DTP, TOC, COD, Chl.a, pH and DO) in Hulun Lake and its main inflow rivers. Two fluorescent components were identified from the DOM using the EEM-PARAFAC method: visible fulvic-like fluorescence (Ex/Em = 325/425 nm) and tryptophan-like substances (Ex/Em = 300/350 nm). The C/N ratio and spectral characteristics of DOM indicate that the majority (around 82%) of the DOM in Hulun Lake is the contribution of terrestrial materials such as those from river input. These findings provide technical support for the further analysis of pollution sources and for decision-making by parties involved in the environmental management of lakes.

Author Contributions: Conceptualization, W.W.; methodology, B.L.; software, S.Y.; investigation, W.D. and B.C.; resources, B.P.; data curation, H.D.; writing—original draft preparation, C.Z. and W.L.; writing—review and editing, W.A. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by the Cooperation and Guidance Project of Prospering Inner Mongolia through Science and Technology (2021CG0037); Project of Hulunbuir Science and Technology Planning (HZ2020003); Key Technology Research Projects for Strengthening Science and Technology Research Capacity of Hulun Lake (HSZCS-C-F-210094); Ecological Safety Investigation and Evaluation Project of Hulun Lake (HSZCS-G-F-210059); Budget Project of the Ministry of Ecology and Environment of the People's Republic of China: Supervision and Management of Surface Water Ecological Environment, Pilot Work on Water Ecological Assessment in the Yangtze River Basin, Investigation and Evaluation of Water Ecological Environment Quality of Key Lakes, Construction, Operation and Maintenance of Supporting Facilities of Hulun Lake Wetland field observation station (ZX2022QT040); and Innovation Training Program for College Students (202010304118Y).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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