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Research article

# Changes in dissolved organic matter chemistry of plateau lakes along gradients of human activity intensity in Yunnan Province, China

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ABSTRACT

Plateau lake systems are highly sensitive indicators and amplifiers of global climate change, while human activities are emerging as a growing threat to their ecological health and carbon cycling. As the dominant organic carbon pool, dissolved organic matter (DOM) plays essential roles in lake biogeochemical cycles and ecosystem health. However, the impacts of human activities along intensity gradients on DOM chemistry in plateau lakes remain unknown. Here we quantified human activity intensity (HAI) of nine plateau lakes in Yunnan Province (China) based on land use/cover data, coupled with DOM characterization using spectroscopic techniques and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to address these issues. The results showed that the pronounced spatial heterogeneity in the quantity and quality of DOM, and it was highly associated with HAI values across the nine plateau lakes. With increasing HAI values, the dissolved organic carbon, absorption coefficients at 355 nm, DOM aromaticity, humification degree, abundances of fluorescent components, and heteroatom-bearing compounds all significantly increased in plateau lakes. Anthropogenic-induced nutrient inputs along with allochthonous DOM, shifted the source of autochthonous DOM from submerged plant degradation to algal and/or phytoplankton metabolisms. This shift further significantly elevated the proportions of heteroatom molecules in DOM, and consequently complicated the organic matter pool in plateau lakes. We conclude that intensified human activities could significantly alter DOM quantity and quality, and potentially affect its biogeochemical function in plateau lake systems.

## 1. Introduction

Lakes constitute vital natural resources, occupying 1.8 % of the global land surfaces (Messager et al., 2016), furnish critical ecosystem services for human society, and regulate the global carbon cycling and climate (Woolway et al., 2020; Yao et al., 2023). Since the start of the 20th century, human activities (e.g., urbanization, agricultural production, and land exploitation) have significantly affected the structure and function of lake systems, leading to water quality deterioration, biodiversity reduction, and phytoplankton blooms globally (Grant et al., 2021; Ho et al., 2019; Zhang et al., 2024a). Particularly, plateau lakes, regarded as sensitive sentinel ecosystems (Zhang et al., 2024b), have the characteristics of insufficient supply water source, long water renewal cycle, and poor anti-interference ability due to their geographical and climatic conditions, which makes these systems more fragile and vulnerable to human activities (Lü et al., 2017; Zhang et al., 2024c). Evidence has confirmed that the ecosystem health and material cycles of plateau lakes are undergoing serious alterations due to the intensive anthropogenic impacts (Liu et al., 2017; Ran et al., 2023; Wu et al., 2021).

Dissolved organic matter (DOM), accounting for about 70 % of organic carbon pool (Du et al., 2023), plays important roles in food web

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dynamics, carbon cycling, and pollutant migration in aquatic systems (Lynch et al., 2019), and even has a close relationship with the freshwater health (Tanentzap and Fonvielle, 2024). The function, reactivity, and biogeochemical processes of DOM are largely determined by its inherent quality and quantity within lakes, which can be impacted by diverse anthropogenic activities (Dong et al., 2020; Kellerman et al., 2015). For instance, human-induced eutrophication was considered as the most severe environmental issue in lakes globally. Excessive nutrients in eutrophic lakes facilitate the production of autochthonous DOM (e.g., more aliphatic compounds), thereby altering the release pattern of $CO_2$ and $CH_4$ (Liu et al., 2022; Sun et al., 2021; Xiao et al., 2022). Given the sensitive ecosystems and serious anthropogenic impacts, the biogeochemical cycles and ecological function involving DOM may be more complicated and dynamic in plateau lakes. Therefore, investigating the DOM chemistry and its relationship with human activities in plateau lakes is essential for advancing the understanding of carbon cycling and ecosystem health.

The influence of human activities on DOM quality and quantity have been well investigated in lakes at a global scale, that the DOM chemistry and biogeochemical processes depend on the disturbance intensity and types of anthropogenic activity (Dong et al., 2020; Du et al., 2023; Luo et al., 2022; Williams et al., 2016). For example, agricultural practices facilitate substantial inputs of terrestrial organic matter into lake systems and result in the reproduction of phytoplankton, which may promote the release of autochthonous DOM inversely (Liu et al., 2022). Alternatively, urbanization with human population growth will introduce extra protein-like and sulfur-containing compounds to lakes through sewage and industrial wastewater discharges (Shang et al., 2022). In essence, the chemical composition and characteristics of DOM are integrally determined by various human activities in lake basins. However, previous studies predominantly focused on elucidating the impact of specific anthropogenic patterns (such as urbanization and distinct land use types) on lake DOM (Du et al., 2023; Williams et al., 2016), but critically lack an integrated quantification of multivariate anthropogenic drivers across entire lake watersheds. Therefore, to determine how DOM reactivity and biogeochemical processes will respond to anthropogenic interferences in plateau lakes, quantifying the intensity of human activities at the watershed scale and investigating its correlation with DOM chemistry is crucially important.

Yunnan Province, which belongs to one of China's five regions with the greatest natural lake abundance and has 31 freshwater lakes exceeding 1 $km^2$ in surface area (Liu et al., 2024; Yang et al., 2010). The lake ecosystems of Yunnan Province are of closed or semi-closed characteristics (rare outflows of lake) and have been significantly affected by anthropogenic activities (e.g., lake basin development and urbanization construction), leading to an array of problems such as water level depression, eutrophication, and biodiversity reduction in the past several decades (Yu et al., 2020). For instance, Dianchi Lake, ranking as the sixth largest freshwater lake in China, persistently exhibits moderate to severe eutrophication states and experiences frequent cyanobacteria bloom outbreaks (Zhan et al., 2017). The levels of anthropogenic disturbance differ significantly among these plateau lakes due to the variations in land cover patterns, population densities, and urbanization degree. Additionally, the water environment of these plateau lakes can effectively represent the overall characteristics under the influence of human activities over a long-time scale, because of their long water renewal cycles and weak self-purification capacities. Therefore, the lakes in Yunnan province provided an ideal area to examine how human activities along intensity gradients impact DOM chemistry in plateau lakes.

The goal of this study was to clarify the changes in DOM quantity and quality along human activity intensity (HAI) gradients in nine plateau lakes of Yunnan Province, China. The HAI values were quantified at the watershed scale based on land use/cover data. DOM sources, composition, and characteristics were investigated by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and spectroscopic techniques. Multivariate statistical analysis was used to reveal the relationship and driving forces between human activities and DOM chemistry in lake systems. Hypothetically, human activities alter DOM composition and diversity in plateau lakes by increasing terrestrial DOM inputs and facilitating the production of endogenous DOM. If confirmed, this alteration should evidence by DOM exhibiting increasingly allochthonous and autochthonous signatures (e.g., higher aromaticity, complexity) with intensified human activities. The findings of this study have important implications for the understanding of the biogeochemical cycles of DOM and the ecosystem conservation and management of plateau lakes.

## 2. Materials and methods

### 2.1. Study area and water sampling

Yunnan Province is situated in southwestern China (21°8′-29°15′N, 97°31′-106°11′E) with a subtropical or temperate climate. Nine typical lakes with surface area greater than 30 $km^2$ were selected (Dianchi Lake, Yangzonghai Lake, Fuxian Lake, Xingyun Lake, Qilu Lake, Yilong Lake, Erhai Lake, Chenghai Lake, and Lugu Lake) to investigate DOM chemistry (Fig. 1). Based on lacustrine morphometric features, six sampling sites were established per lake, and a total of 54 samples were obtained about 10 m from the lakeshore during August 2024. Detailed information of nine lakes and sampling locations is documented in Table S1.

Surface water samples (approximately 0.5 m deep) were collected using precleaned Nalgene bottles, transported to the laboratory within 6 h, and processed immediately upon arrival. Each sample was split into two subsamples: the first subsample was filtered through 0.7 μm precombusted GF/F glass fiber filter (480 °C for 4.5 h), then the filtrates were acidified to pH of 2 using HCl and maintained at 4 °C until further measurement. The second subsample was preserved at −20 °C in darkness and analyzed within two days.

### 2.2. Data sources and human activity intensity model

#### 2.2.1. Data sources

The primary datasets comprised digital elevation model (DEM) and land use/cover data. Specifically, the 30 m resolution DEM data, sourced from the Geospatial Data Cloud Platform of the Chinese Academy of Sciences (https://www.gscloud.cn/) and used to identify and extract the lake watershed boundary on the ArcGIS 10.3 platform. Land use/cover data with a spatial resolution of 30*30 m were acquired from the Resource and Environment Data Center of the Chinese Academy of Sciences (https://www.resdc.cn/).

#### 2.2.2. Human activity intensity model

Human activity intensity (HAI) refers to the magnitudes of anthropogenic pressures on the regional natural complexes through socioeconomic interventions (Qing et al., 2024). The utilization, transformation, and exploitation of natural land cover are considered as the mainstay of human activities, based on that, Xu et al. (2016) proposed a HAI measurement model using the construction land equivalent (CLE) as the basic measurement unit, which has been widely used in previous researches (Hu et al., 2023; Li et al., 2024). The main principle of this model is to convert the surface area of different land use/cover into CLE values according to corresponding conversion coefficients (Table S2), and then calculated the proportions of total CLE values. The HAI index is calculated using the following formula:

$$HAI = \frac{S_{CLE}}{S}$$

$$S_{CLE} = \sum_{i=1}^{n} SL_i * CI_i$$

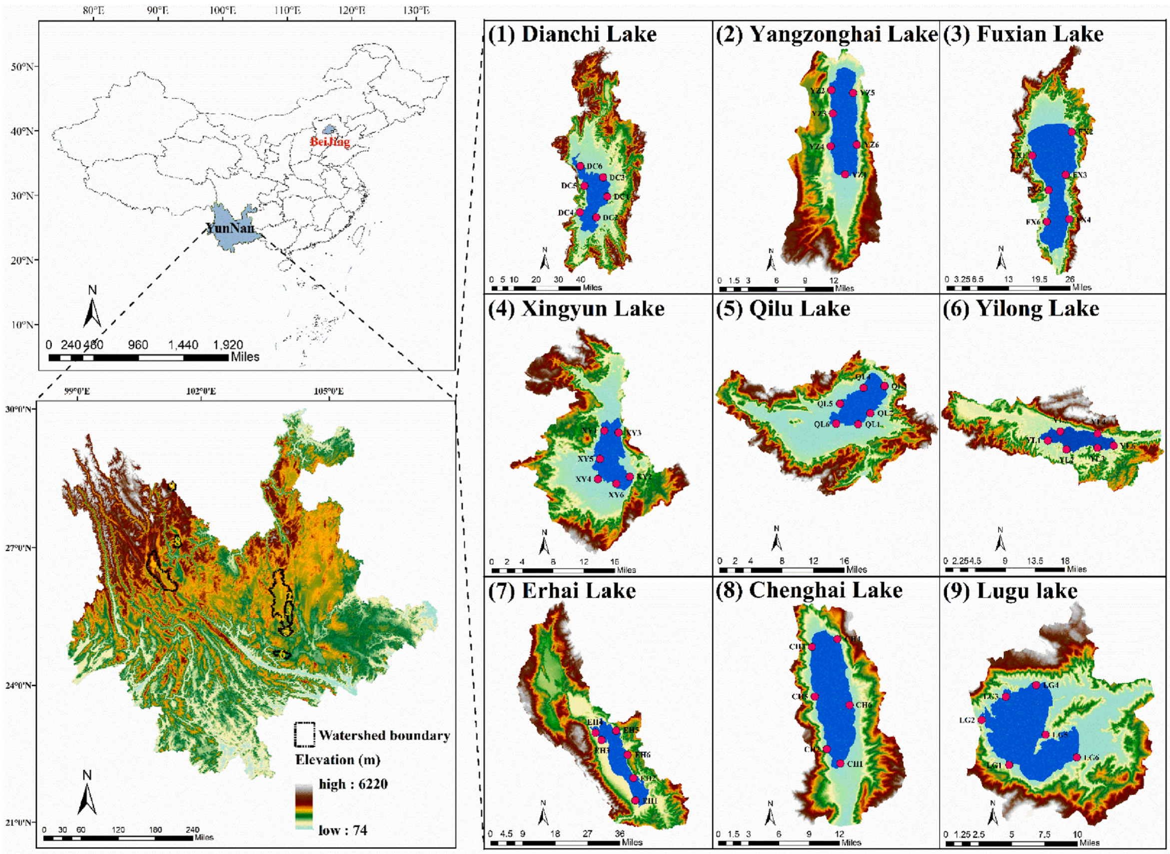


**Fig. 1.** Location of nine plateau lakes in Yunnan Province (China). Enlarged figures show the sampling sites in individual lakes, including: (1) Dianchi Lake, (2) Yangzonghai Lake, (3) Fuxian Lake, (4) Xingyun Lake, (5) Qilu Lake, (6) Yilong Lake, (7) Erhai Lake, (8) Chenghai Lake, and (9) Lugu Lake.

Where *HAI* is the human activity intensity for each lake basin; $S_{CLE}$ is the area of construction land equivalent; $S$ is the total surface land area; $CI_i$ is the conversion coefficient of land use/cover type $i$ for construction land equivalent, and $i$ is the numbers of land use/cover types.

### 2.3. Analytic methods

#### 2.3.1. Bulk geochemical analysis

Water temperature (WT), dissolved oxygen (DO), electrical conductivity (EC), pH, and chlorophyll *a* (Chl *a*) of water samples were recorded *in situ* using a portable YSI EXO2 multiparameter water quality probe (YSI Inc., USA). Total nitrogen (TN) and total phosphorus (TP) concentrations were measured by corresponding standard spectrophotometric method, respectively. Permanganate index ($COD_{Mn}$) was determined using the $KMnO_4$ oxidation method in acidic conditions. Concentration of dissolved organic carbon (DOC) was determined via a TOC-L CPH analyzer (Shimadzu, Japan) using the non-purgeable organic carbon method (NPOC). Details for the above parameters have been described in our previous publications (Xu et al., 2024) and the results are displayed in Table S3.

#### 2.3.2. Spectroscopic measurements and PARAFAC modeling

Ultraviolet–visible absorbance spectra of filtrated DOM samples were measured from 200 to 600 nm (1 nm interval) with a 1 cm quartz cuvette on a Shimadzu UV-2700i spectrophotometer (Japan). Milli-Q water was employed as the reference and each absorbance spectrum was baseline corrected by subtracting the absorbance at 700 nm (Xu et al., 2024). DOM excitation-emission matrices were analyzed using an Aqualog fluorescence spectrometer (Horiba, Japan). The excitation (Ex: 250–650 nm, 2 nm increment) and emission (Em: 246.08–827.571 nm, 2 pixels) wavelengths were scanned at 1200 nm/min with integration time of 2.0 s. All EEM spectra were blank-subtracted, calibrated for Raman scattering, corrected for inner-filter effects, and normalized into the Raman units (R.U.) (Murphy et al., 2010). Several spectroscopic indexes were employed to characterize DOM optical properties from the established equations as previously described. Specifically, the absorption coefficient at 355 nm ($a_{355}$) is generally used to represent chromophoric DOM (CDOM) abundance. The specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) is an index to indicate DOM aromaticity (Li and Hur, 2017). Fluorescence index (FI) was the indicator of DOM source (>1.8: microbial source; 1.2–1.8: terrestrial origin and microbial source; <1.2: terrestrial origin) (Cory and Mcknight, 2005). Humification index (HIX), corresponds to the humic substances content or humification degree of DOM, and increases with increasing DOM aromaticity (Fellman et al., 2010). Biological index (BIX), an index to evaluate the contribution of freshly produced autochthonous origin to DOM. Higher BIX values (>1) suggest a predominantly biogenic sources in DOM whereas lower values (0.6–0.7) indicate the higher input from terrestrial sources (Huguet et al., 2009).

Parallel factor analysis (PARAFAC) was conducted to study the fluorescent DOM components using the drEEM toolbox in MATLAB R2019b. A four-component model was validated based on split-half validation, random initialization, and residual analysis (Stedmon and Bro, 2008; Zhou et al., 2023b). All fluorescent components were further compared with the previous publications using the OpenFluor database (Murphy et al., 2014). Component-specific maximum fluorescence intensity (Fmax) quantified the relative contribution of each fluorescent component in the DOM (Tank et al., 2011).

#### 2.3.3. Solid-phase extraction of DOM and FT-ICR MS analysis

Equal volumes from six water samples were taken to mix up thoroughly to form a typical DOM sample of each lake for FT-ICR MS measurement. Nine filtrated samples (50 mL–100 mL, depending on the DOC concentration) underwent solid-phase extraction (SPE) with Agilent Bond Elut PPL cartridges (500 mg, 6 mL) as previously described (Dittmar et al., 2008). The extracted DOM samples were eluted with 1 mL of 1:1 (v/v) methanol: water and then analyzed using a 15 T FT-ICR MS equipped with a negative electrospray ionization source (Apollo II), detailed measurement settings and calibration followed those described in our previous studies (Xu et al., 2022, 2024). After calibration, a low absolute mass error (less than 1 ppm) was achieved over the entire mass range (Cao et al., 2015).

Mass peaks with a signal-to-noise ratio (S/N) above 4 were identified using Bruker Data Analysis Software with elements constraints of $C_{0\text{-}100}H_{0\text{-}200}O_{0\text{-}50}N_{0\text{-}1}0S_{0\text{-}2}$, details of molecular formulae assignment were the same as previously reported (Xu et al., 2022). Molecular characteristics of DOM were revealed through various intensity-weighted parameters, including chemical elements (C, H, O, S, N), atomic ratios (H/C, O/C), formulae (CHO, CHOS, CHON, CHONS), molecular weight (m/z), double bond equivalents (DBE), and aromatic index ($AI_{mod}$). DOM lability were evaluated via the indexes of molecular lability boundary ($MLB_L$%) (D'Andrilli et al., 2015) and the island of stability (IOS%) (Lechtenfeld et al., 2014). In addition, several biochemical compound groups were defined: polycyclic condensed aromatics ($AI_{mod} > 0.66$), polyphenols ($0.66 \geq AI_{mod} > 0.50$), highly unsaturated and phenolics ($AI_{mod} \leq 0.50$, $H/C < 1.5$), aliphatics ($2.0 > H/C \geq 1.5$), saturated compounds ($H/C \geq 2.0$, or $O/C \geq 0.9$), and carboxylic-rich alicyclic molecules (CRAMs; DBE/C = 0.30–0.68, DBE/O = 0.77–1.75, DBE/H = 0.20–0.95) (Kellerman et al., 2018; Xu et al., 2024).

### 2.4. Statistics analysis

The distribution of the lakes and sampling sites was mapped using ArcGIS 10.1 software. Pearson correlations were conducted to explore the relationships between HAI values and parameters of water quality, optical properties, and molecular compositions in SPSS 26. Spearman's rank correlations were performed between HAI values and the relative intensity of each identified formula from FT-ICR MS analysis in R 4.0.2. Statistical significance thresholds were $p < 0.05$* and $p < 0.01$**. Principal component analysis (PCA) was employed to reveal the differences in the quantity and quality of DOM across plateau lakes with various HAI levels using Origin 2021.

## 3. Results

### 3.1. HAI values in the nine plateau lakes

The nine plateau lake basins were classified into six primary land use/cover types based on the watershed features, including farmland, grassland, forest land, construction land, unused land, and water bodies. The HAI values exhibited great inter-lake variability across the nine lakes, ranging from 7.15 % to 42.10 % (Fig. 2; Table S2), where more enriched HAI values corresponded with higher human impact and more depleted values related to weak human disturbance. The type of construction land (4.75 %–38.79 %) has the highest contribution to the HAI values in each lake, followed by grassland (1.57 %–4.72 %) and farmland (0.71 %–3.14 %; Fig. 2).

### 3.2. Bulk geochemical characteristics

The nine plateau lakes encompassed broad variations in nutrient concentrations, phytoplankton biomass, and oxygen balance indices (Table S3). The average values of TN, TP, Chl *a*, DO, $COD_{Mn}$, DOC, and EC respectively were in the range of 0.13–2.69 mg/L, 0.008–0.106 mg/L, 1.31–15.67 μg/L, 5.26–8.37 mg/L, 1.01–10.22 mg/L, 8.36–35.61 mg/L, and 215.32–1361.58 μS/cm. The DOC concentrations showed positive correlations with TN ($r^2 = 0.63$, $p < 0.01$**), TP ($r^2 = 0.60$, $p < 0.01$**), and Chl *a* ($r^2 = 0.38$, $p < 0.05$*; Fig. S1a–c). Additionally, it was noted that the average TN ($r^2 = 0.75$, $p < 0.01$**), TP ($r^2 = 0.65$, $p < 0.05$*), Chl *a* ($r^2 = 0.58$, $p < 0.05$*), and DOC ($r^2 = 0.67$, $p < 0.01$**) concentrations increased with the increasing HAI values across the sampled lakes (Fig. 3a–d).

### 3.3. Optical properties of DOM

Obvious variations in optical parameters and fluorescent components of DOM with increasing HAI values were observed among nine plateau lakes (Table S3). The average $a_{355}$ values ranged from 0.46 to 4.61 $m^{-1}$, were positively correlated with DOC concentration ($r^2 = 0.84$, $p < 0.01$**; Fig. S1d), and also increased with increasing HAI values ($r^2 = 0.65$, $p < 0.01$**; Fig. 3e). The averaged values of $SUVA_{254}$ (0.46–0.99 L $mg^{-1}$-C $m^{-1}$) showed a positive correlation with HIX values (0.37–0.80; Fig. S2), and both the indexes increased with increasing HAI values ($SUVA_{254}$: $r^2 = 0.53$, $p < 0.05$*; HIX: $r^2 = 0.52$, $p < 0.05$*; Fig. 3f–i). The average FI and BIX values ranged from 1.53 to 2.03 and 0.89–1.27, respectively, and had no significant correlation with the HAI values (FI: $r^2 = 0.07$, $p = 0.48$; BIX: $r^2 = 0.15$, $p = 0.16$; Fig. 3g and h).

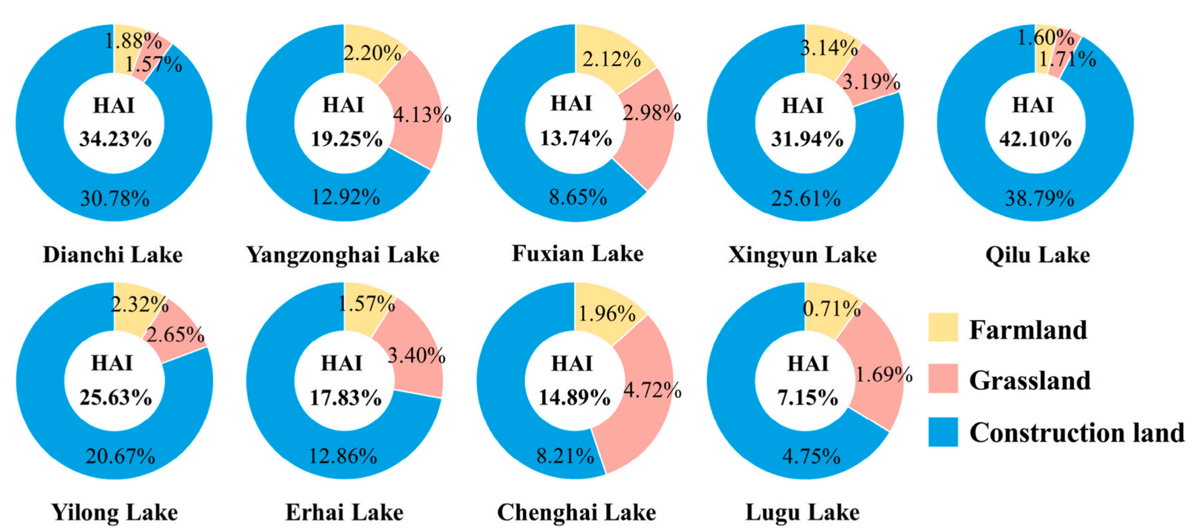


**Fig. 2.** Contributions of farmland, grassland, and construction land types to HAI values for the nine plateau lake basins.

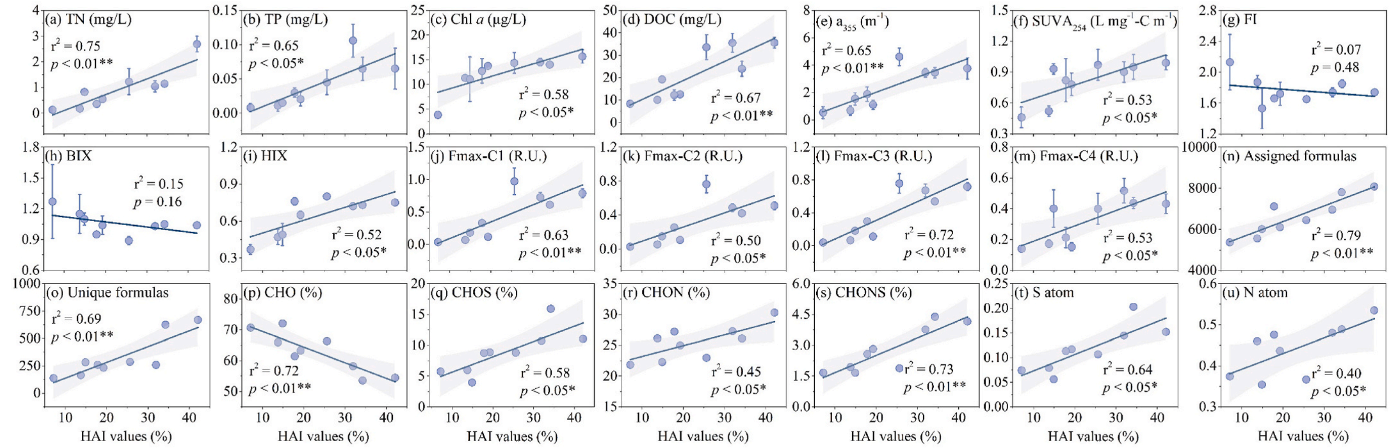


**Fig. 3.** Regression of general water quality, optical properties, and molecular composition indexes against a HAI value gradient for the nine plateau lakes. Parameters include (a) total nitrogen (TN), (b) total phosphorus (TP), (c) chlorophyll-a (Chl *a*), (d) dissolved organic carbon (DOC), (e) absorption coefficient at 355 nm ($a_{355}$), (f) specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), (g) fluorescence index (FI), (h) biological index (BIX), (i) humification index (HIX), (j) Fmax of C1, (k) Fmax of C2, (l) Fmax of C3, (m) Fmax of C4, (n) the number of assigned formulae, (o) the number of unique formulae, (p) relative abundance of CHO formulae; (q) relative abundance of CHOS formulae, (r) relative abundance of CHON formulae, (s) relative abundance of CHONS formulae; (t) N atom; (u) S atom. The values of water quality and optical parameters are the averaged values, and the error bar represents the standard deviations (n = 6).

Four fluorescent components (humic-like components: C1-C3; protein-like component: C4) were resolved through EEM-PARAFAC modeling (Fig. 4a). Both C1 and C2 are categorized as terrestrial humic-like components, however, C1 is commonly found in wetlands, forest streams, and agriculturally influenced streams (Hosen et al., 2014), whereas C2 is frequently detected in high-nutrient and wastewater-impacted environments (Fellman et al., 2010; Murphy et al., 2011). C3 corresponds to an autochthonous marine humic-like component, prevalent in marine environments and associated with biological processes (Beggs and Summers, 2011; Fellman et al., 2010). C4 is a tyrosine-like component, and usually related to the degradation products of DOM (Murphy et al., 2011). The total fluorescence intensity varied widely, spanned from 0.24 to 2.90 R.U. across nine lakes (Fig. 4b). The averaged proportions of humic-like components ranged from 41.97 % to 86.24 %, and were more predominant in strong human-impacted lakes (e.g., Qilu Lake). In contrast, the average percentage of the protein-like component ranged from 13.76 % to 58.03 %, and was the most abundant component in the lakes with lower HAI values, such as Lugu Lake (Fig. 4c). Notably, all DOM fluorescent components were significantly correlated with DOC concentrations (Fig. S1f–i) and increased with increasing HAI values (Fig. 3j–m).

### 3.4. *Molecular composition and characteristics of DOM*

FT-ICR MS showed a powerful advantage in deciphering the chemodiversity of DOM compositions. A total of 11,758 molecular formulae and 3638 common formulae were identified by FT-ICR MS among the nine plateau lakes, the numbers of CHO, CHOS, CHON, and CHONS were 3398, 1671, 4852, and 1837, in all molecular formulae, respectively, and 1835, 286, 1419, and 98, in common formulae, respectively (Fig. S3a and b). The numbers of molecular formulae detected in each DOM sample were in the range of 5366–8086, and the unique formulae ranged from 138 to 669 (Table S4). The number of assigned formulae ($r^2 = 0.79$, $p < 0.01$**) and unique formulae ($r^2 = 0.69$, $p < 0.01$**) in each lake both increased with increasing HAI values (Fig. 3n and o). The relative intensity of CHO formulae was dominant in each lake, ranged from 53.58 % to 72.40 %, and decreased with increasing HAI values ($r^2 = 0.72$, $p < 0.01$**; Fig. 2p). The proportions of CHOS, CHON, and

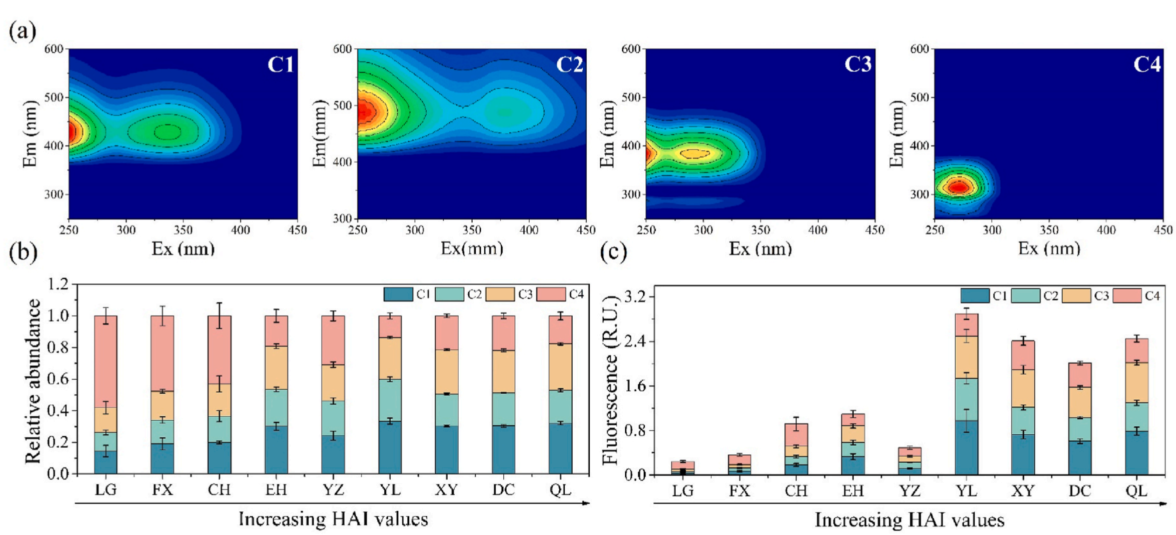


**Fig. 4.** Spectral proporties of fluorescence components identified through PARAFAC modeling: (a) four split-half validated components, (b) fluorescence intensity distribution of four components, (c) Relative abundance distribution of four components.

CHONS ranged from 3.95 % to 15.92 %, 21.85 %–30.31 %, and 1.66 %–4.61 %, respectively, and all increased with increasing HAI values (CHOS: $r^2 = 0.58$, $p < 0.05$*; CHON: $r^2 = 0.45$, $p < 0.05$*; CHONS: $r^2 = 0.73$, $p < 0.01$**; Fig. 3q–s). Additionally, the values of nitrogen (N) and sulfur (S) atoms also increased with increasing HAI values (N: $r^2 = 0.40$, $p < 0.05$*; S: $r^2 = 0.64$, $p < 0.05$*; Fig. 3t and u).

The values of molecular parameters and proportions of compound groups showed great variability in DOM of the nine lakes (Table S4). The values of m/z, DBE, and $AI_{mod}$ ranged from 406.86 Da to 431.62 Da, 7.77 to 8.60, and 0.19 to 0.24, respectively. The $MLB_L$ and IOS were 9.50 %–15.99 % and 10.99 %–12.89 %, respectively. Highly unsaturated and phenolic compounds were predominant (79.86 %–87.04 %), followed by aliphatic compounds (9.46 %–15.91 %), polyphenols (1.02 %–5.19 %), polycyclic condensed aromatics (0.43 %–1.67 %), and saturated compounds (0.13 %–0.30 %) across the samples lakes. Molecular correlations (Spearman's rank) between individual molecules and HAI values indicate that HAI values were associated with the three compound groups of DOM (Fig. 5a). In general, the HAI values were positively correlated with the relative abundance of polyphenols, highly unsaturated high-oxygen phenolic compounds (O/C > 0.5), and aliphatics and negatively correlated with highly unsaturated low-oxygen phenolic compounds (O/C < 0.5). Specifically, the vast majority of these molecules that exhibited positive relationships with HAI values are S- and N-containing compounds, while the negatively correlated molecules are mostly CHO compounds (Fig. 5e). Moreover, the water quality parameters such as TN, TP, and Chl *a*, which serve as the indicators of human impact on lakes, presented parallel correlation patterns with DOM molecules when compared to HAI values (Fig. 5b–d, f-h).

### 3.5. PCA results

PCA results of HAI values, water chemistry, and DOM optical properties and molecular characteristics across the nine plateau lakes revealed that the first two principal components (PC1 and PC2) accounted for 46.6 % and 21.0 % of the total variance of all parameters (Fig. 6a). PC1 exhibited strong positive loadings that were related to HAI values, water quality indices (DOC, TP, TN, COD, and Chl *a*), humification- or aromaticity-associated indexes ($SUVA_{254}$, HIX, DBE, and $AI_{mod}$), fluorescent components (C1 - C4), molecular parameters (CHOS, CHON, CHONS, polycyclic condensed aromatics, and polyphenols). In contrast, negative PC1 loadings were mainly linked to the indicator of autochthonous features, including FI, BIX, H/C, CHO, aliphatic compounds, and saturated compounds. Additionally, relatively elevated PC1 scores were observed in those lakes with greater HAI values (Fig. 6b). Specifically, Qilu Lake, Dianchi Lake, Yilong Lake, and Xingyun Lake showed relatively positive PC1 scores, whereas Lugu Lake, Fuxian Lake, Chenghai Lake, and Yangzong Lake presented negative PC1 scores.

## 4. Discussions

### 4.1. Multiple sources of DOM in the nine plateau lakes

DOM originating from allochthonous, autochthonous, and anthropogenic sources were identified across the nine plateau lakes through the analysis of DOM using optical and FT-ICR MS methods, and these sources are mainly controlled by regional coverage of farmland, forest, grass, and urbanization within the lake basin (Table S2). The values of FI (1.53–2.13) and BIX (0.89–1.27) were both used to distinguish terrestrial or microbial sources, suggesting a combined origin of DOM from allochthonous and autochthonous sources in the plateau lakes. The $SUVA_{254}$ and HIX values typically ranged from 0.6 to 5.3 L $mg^{-1}$-C $m^{-1}$ and 0.6–0.9 in natural waters, respectively (Hansen et al., 2016; Wang et al., 2021), and $SUVA_{254}$ (>6) and HIX (<0.9) could indicate the dominance of terrestrial features and fresh plant and/or algae inputs in DOM, respectively (Hansen et al., 2016; Jaffé et al., 2008). Thus, our $SUVA_{254}$ (0.46–0.99 L $mg^{-1}$-C $m^{-1}$) and HIX (0.37–0.80) values suggested terrestrial inputs and autogenous production as well. Many previous studies have reported similar ranges of these optical parameters in plateau lakes (Zhang et al., 2018, 2022). Additionally, fluorescent component C1 and two components (C3 and C4) have been widely identified in lake ecosystems (Dong et al., 2020; Ejarque et al., 2018), and could be used to infer terrestrial signatures and autochthonous productions in DOM, respectively. In terms of molecular groups, the presence of polyphenols (1.02 %–5.19 %) and highly unsaturated compounds (79.86 %–87.04 %), which originate from vascular plants and soil-derived products of lignin degradation (Seidel et al., 2015), respectively, demonstrated the terrestrial DOM origin. The detection of aliphatics (9.46 %–15.91 %), typically from products of bacterial and algal metabolism (Kellerman et al., 2018), indicates the autochthonous contributions to DOM. These results align with prior studies documenting both allochthonous and autochthonous sources in DOM from other plateau lakes (Shang et al., 2022; Zhou et al., 2023a).

The humic-like component C2, which was observed in wastewater-impacted systems (Murphy et al., 2011), and it could indicate the anthropogenic inputs. Besides, multiple CHOS formulae belonging to $O_3S$ and $O_5S$ classes with unexpected intensity were identified in nine plateau lakes, resulting in the non-Gaussian distribution patterns (Fig. S4). It was reported that the abnormally high abundances of $O_3S$

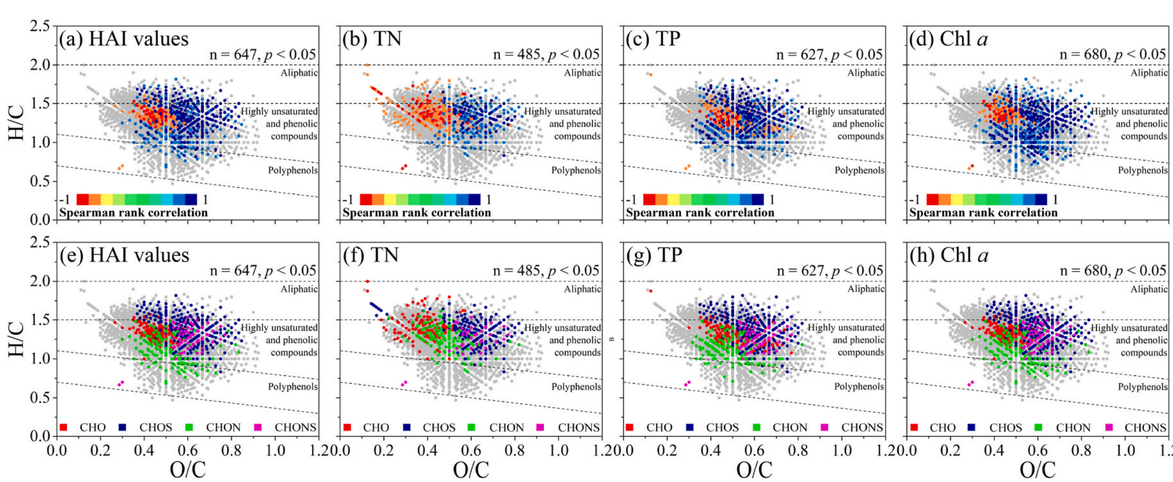


**Fig. 5.** van-Krevelen diagrams of molecular formulae significantly correlated with HAI values (a, e), TN (b, f), TP (c, g), and Chl *a* (d, h) using Spearman rank correlations ($p < 0.05$). Positively and negatively correlated formulae are colored in blue and red, respectively (a–d). Molecular formulae are colored according to their heteroatomic composition: CHO formulae, red; CHOS formulae, royal; CHON formulae, green; CHONS formulae, magenta (e–h).

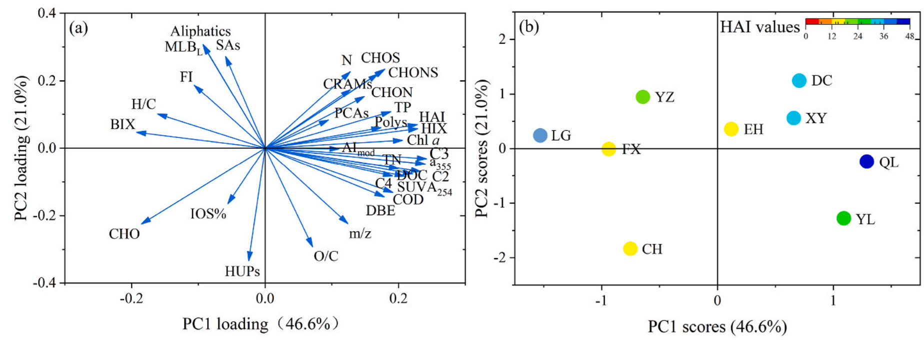


**Fig. 6.** (a) PCA results of HAI values, water quality, and DOM characteristics. (b) PC loading of lake samples in various HAI values. Colors denote the values of HAI. PCAs, Poly, and, HUPs, SAs were short for polycyclic condensed aromatics, polyphenols, highly unsaturated and phenolic compounds, and saturated compounds.

and $O_5S$ are widely observed in human-impacted waters and domestic wastewater (Gonsior et al., 2011). The possible formulae (e.g., $C_{17}H_{27}O_3S$, $C_{18}H_{29}O_3S$, $C_{19}H_{31}O_3S$) in the $O_3S$ class were likely related to linear alkylbenzene sulfonates (LAS), which are a kind of widely utilized surfactants and have been detected in domestic wastewater (Melendez-Perez et al., 2016). Collectively, these findings denote anthropogenic sources of DOM in plateau lakes as well. This interpretation is reinforced by recent evidence that frequent human activities (e.g., urbanization) enriched the exogenous and endogenous organic carbon pool in the sediments of these nine plateau lakes, corroborating the contribution of anthropogenic inputs in DOM (Yin et al., 2024).

### 4.2. Changes of DOM chemistry along HAI values gradient in plateau lakes

Significant differences in DOM quality and quantity with increasing HAI values were revealed based on bulk, optical, and molecular composition, which demonstrated pronounced relationships between the degree of human activities and DOM chemistry in plateau lakes. The DOM in plateau lakes exhibited an increasing trend of DOC, $a_{355}$, $SUVA_{254}$, HIX, humic-like and protein-like substances, molecular diversity, CHOS, CHON, and CHONS with increasing HAI values, substantiating that intensified human activities have promoted the increase in DOM quantity, aromaticity and humification degree, and molecular complexity in plateau lakes. PCA results further confirmed the differences in sources and chemical composition of DOM in plateau lakes at distinct levels of HAI values. These observations suggest that the DOM in plateau lakes with higher HAI values showed both features of terrestrial inputs and autochthonous production, whereas DOM in those with lower HAI values displayed predominantly autochthonous signature.

As has been reported for other inland lakes, our findings demonstrate severe impacts of human activities on DOM chemistry in plateau lakes. A conceptual diagram was developed to elucidate the potential transformation processes in DOM chemistry under different HAI levels of plateau lakes (Fig. 7). Our results revealed that lower HAI values were observed in lakes like Lugu Lake (7.15 %) and Fuxian Lake (13.74 %), and the DOM in those lakes mainly exhibited autochthonous features. Historical records indicate the nine studied plateau lakes previously sustained extensive submerged macrophyte communities, though anthropogenic pressures (e.g., urbanization, agricultural intensification) have differentially degraded these ecosystems since the 1950s (Ley et al., 1963; Li, 1980). For instance, the submerged vegetation in Dianchi Lake has sharply declined, while Lugu Lake still retains relatively intact macrophyte communities (Dong et al., 2014; Zhao et al., 2016). Therefore, we deduce that organic matter released from healthy submerged plants during their growth and metabolisms, along with those from senescing tissues during their degradations, constitute the primary endogenous DOM inputs in these plateau lakes with lower HAI values. Conversely, the DOM in low-altitude lakes with limited anthropogenic disturbances exhibited predominant allochthonous features as previously reported (Liu et al., 2022). This difference likely arises from the geomorphological characteristic of plateau lakes that restricts significant terrestrial organic matter transport pathways (e.g., riverine inputs). Some publications on lakes of the Qinghai-Tibetan Plateau also reported that the DOM predominantly exhibits autochthonous features (Du et al., 2021; Zhou et al., 2023a). Moreover, the observed increase in DOC, $SUVA_{254}$, HIX, humic-like components (C1, C3, and C4), molecules in polyphenols, and highly unsaturated high-oxygen phenolic compounds with the increase of HAI values, in addition to the positive relationships between DOC and these above terrestrial inputs indicators (Fig. S1), imply that human activities facilitate the terrestrial organic matter inputs into plateau lakes. It was thus that the quantity, humification degree, and aromaticity of DOM were all enhanced in plateau lakes with higher HAI values, consistent with prior research (Dong et al., 2020; Du et al., 2023). On the other hand, more nutrient inputs accompanied by increasing terrestrial organic matter inputs may potentially contribute

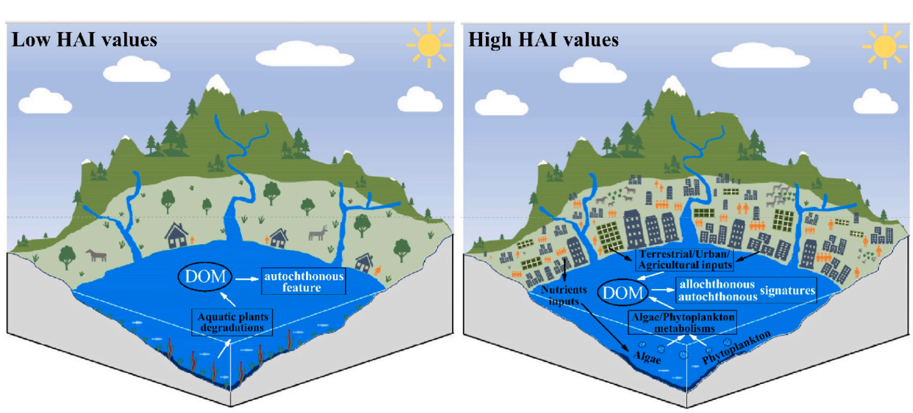


**Fig. 7.** The proposed conceptual diagram for elucidating the impacts of human activities on DOM chemistry in plateau lakes.

to the proliferation of phytoplankton and algae, and thereby facilitating the production of autochthonous DOM. This could be evidenced by the increase of TN, TP, Chl *a*, protein-like substances (C2), and molecules in aliphatic compounds with increasing HAI values, as well as positive correlations between Chl *a* and DOC or molecules in aliphatic compounds. Hence, these results revealed that human activities shift the DOM source from autochthonous-dominated features to the coexistence of allochthonous and autochthonous signatures in plateau lakes as hypothesized. More importantly, the autochthonous DOM likely derived from phytoplankton and algal metabolisms in those lakes with higher HAI values. These findings agree with previous studies indicating that abundant anthropogenic inputs of terrestrial DOM from the watershed contribute to metabolisms of algae and phytoplankton and stimulate the production of endogenous DOM in human-induced eutrophic lakes (Liu et al., 2022; Wen et al., 2022).

A significant trend of change in the DOM molecular composition was observed along the gradients of HAI values (Fig. 3n–u). Human activities altered the molecular structure of the DOM pool by reducing the proportion of CHO formulae while enhancing heteroatom S- and N-containing formulae (CHOS, CHON, and CHONS), and consequently increasing the DOM molecular diversity in plateau lakes. It is widely recognized that intensive anthropogenic activities, including urbanization, agricultural activities, and sewage discharge, could export terrestrial protein-like and humic-like substances into aquatic systems, particularly the S- and N-containing compounds (Qu et al., 2024; Shang et al., 2022; Williams et al., 2016). For example, farmland expansion and fertilizer usage contribute abundant N-containing compounds (Qu et al., 2024), while elevated S-containing formulae were associated with domestic sewages (Gonsior et al., 2011). Currently, some other investigations proposed that algae blooms in human-induced eutrophic lakes may stimulate the production of S-containing compounds to autochthonous DOM (Pang et al., 2020). Therefore, we speculated that various anthropogenic disturbances across plateau lake watersheds synergistically complicated the DOM pool and increased its chemical diversity.

The dynamic changes in DOM chemistry are crucial for the health of lake ecosystems (Tanentzap and Fonvielle, 2024). Compared to plain (low-altitude) lakes, although plateau lakes differ in the original source and chemical composition of DOM, the influenced patterns of human activities on both are highly similar (e.g., source shifts, molecular composition) (Dong et al., 2020; Liu et al., 2022; Shang et al., 2022). It is noteworthy that pronounced differences in the formation processes and ecosystem characteristics between the plateau and plain lakes likely lead to distinct ecological effects from DOM dynamics. Plain lakes, characterized by strong hydrological connectivity and short water renewal times (typically less than 1 year), facilitate the downstream transport of terrestrial inputs (e.g., nutrients, organic matter). In contrast, plateau lakes usually have slow water renewal (e.g., Fuxian Lake with a water renewal time as long as 167 years) (He et al., 2022), making them more prone to accumulating those above substances. Concurrently, suitable light and temperature conditions on the plateau areas may further accelerate in-lake biochemical processes, such as nutrient transformation and algal photosynthesis (Jia et al., 2021). These differences possibly result in a more intense and complex DOM transformation process in plateau lakes, accompanied by more pronounced changes in its quantity and quality. Therefore, human-induced changes in DOM may exert a more profound potential impact on plateau lake ecosystems than on plain lakes.

### 4.3. *Implications for carbon cycling in plateau lakes and future considerations*

Impacted by human activities, many freshwater lakes tend to enter high trophic states or even eutrophic ones. As important components of global carbon cycling, the effects of human activities on the carbon cycling of plateau lakes have been increasingly emphasized (Du et al., 2023; Yin et al., 2024). Based on the variations in source, composition, and characteristics of DOM with increasing HAI values here, the results could suggest that intensified human activities in plateau lakes can remarkably impact the processes of carbon release and carbon burial. Firstly, the increased DOC concentrations with increasing HAI values revealed more organic matter is accumulated and participated in the biogeochemical processes in plateau lakes with intensive anthropogenic disturbances. Secondly, with intensified human activities, both autochthonous and allochthonous DOM increased, and the sources of autochthonous DOM potentially shifted from submerged plants to algal biomass, suggesting human activities have facilitated the inputs of terrestrial organic matter into lakes and in turn promoted the production of endogenous DOM. This may facilitate the in-lake transformation of DOM and alter its biogeochemical processes because of the different natures of autochthonous (e.g., more bio-labile) and allochthonous DOM (e.g., more photoreactive) (Kellerman et al., 2018). Thirdly, intensified human activities not only increased the organic matter chemo-diversity but also complicated the structure of organic carbon pool within plateau lake systems. Research indicates that heteroatoms (S- and N-containing) compounds within DOM are considered as highly bio-labile molecules and are rapidly metabolized by microorganisms (Ni et al., 2024), which may enhance the DOM mineralization and potentially promote the release of greenhouse gas. In conclusion, human activities could shape the migration and transformation of organic carbon through re-building the DOM quantity and quality, and further accelerates organic carbon dynamics in plateau lakes.

This study has provided novel insights into how human activities affects the chemistry of DOM within plateau lake systems. Nevertheless, some limitations still exist in the current study. On the one hand, this investigation was undertaken exclusively in summer, while the DOM quality and quantity in plateau lakes exhibit seasonal variations, as observed in other inland lakes, future seasonal studies should be carried out to enable a more holistic understanding of the relationship between human activities and DOM chemistry. On the other hand, microorganisms are recognized as central drivers in DOM biogeochemical cycling within lake ecosystems as previously reported, the dynamic interactions between DOM chemistry and microbial communities in plateau lakes warrants focused attention in future research, particularly in the context of intensified anthropogenic disturbances.

## 5. Conclusion

By combining the UV–Vis, EEM, and FT-ICR MS techniques, the spectroscopic properties and molecular characteristics of DOM in nine plateau lakes (Yunnan Province, China) along a HAI gradient were investigated to thoroughly assess the relationship between HAI levels and DOM chemistry. This study showed that various sources (allochthonous, autochthonous, and anthropogenic) of DOM were revealed within plateau lakes, and the variations of DOM quality and quantity were highly associated with HAI values. The allochthonous and autochthonous DOM both increased with increasing HAI values, as indicated by significantly elevated DOC concentration, $a_{355}$, $SUVA_{254}$, HIX, abundances of four fluorescent components, and heteroatoms (S- and N-containing) compounds. Enhanced inputs of nutrients and terrestrial DOM likely facilitate the algal growth and accumulation of algal biomass, which subsequently altered the potential sources of autochthonous DOM from submerged plant degradations to algal metabolisms in plateau lakes. In addition, intensified human activities greatly modified the chemo-diversity and complexity of DOM pools in plateau lakes, and thus may further accelerate the carbon cycling and affect the ecosystem health of these plateau lakes. Collectively, this study emphasized the substantial effects of intensified human activities on DOM chemistry in plateau lakes, and provided insights into the DOM dynamics and biogeochemical cycles within these aquatic systems amidst growing anthropogenic disturbances.

## CRediT authorship contribution statement

**Lei Xu:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Wenjun Zhong:** Methodology, Investigation, Formal analysis. **Zetian Liu:** Visualization, Software. **Qian Hu:** Writing – review & editing. **Xin Xiong:** Supervision. **Jianlin Tang:** Investigation. **Tao Chen:** Data curation. **Wei Liao:** Writing – review & editing, Funding acquisition. **Aiwen Zhong:** Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.127513.

## Data availability

Data will be made available on request.

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