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Deciphering the optical properties and molecular composition of dissolved organic matter in estuarine constructed wetlands of Erhai Lake, Southwest China

Lei Xu [a], Jianlin Tang [b], Gang Wu [b], Bangyao Zhong [b], Chongqing Yu [b], Aiwen Zhong [a], Qian Hu [a], Wei Liao [c], Tao Chen [b,*], Yansong Peng [d,*]

[a] Jiangxi Provincial Key Laboratory of Wetland Plant Resources Conservation and Utilization, Lushan Botanical Garden, Jiangxi Province and Chinese Academy of Sciences, Jiujiang, 332900, China

[b] China Railway Tenth Group of the Fifth Engineering Co., Ltd., Suzhou, 215011, China

[c] Wetland Research Center, Jiangxi Academy of Forestry, Nanchang, 330032, China

[d] Jiangxi Provincial Key Laboratory of Carbon Neutrality and Ecosystem Carbon Sink, Lushan Botanical Garden, Jiangxi Province and Chinese Academy of Sciences, Jiujiang, 332900, China

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ABSTRACT

Estuarine constructed wetlands (ECWs) are critical ecological buffers for mitigating non-point source pollutants transported via riverine systems into downstream lakes. Dissolved organic matter (DOM), as the largest organic matter pool, has variations in quantity and quality that directly regulate pollutant treatment efficiency and processes of wastewater within these wetlands. However, the DOM chemistry and its changes as it traverses the ECWs remain poorly understood. Here, we investigated variations in DOM optical properties and molecular characteristics between influent and effluent in the six ECWs along the Yonganjiang River, located upstream of the Erhai Lake basin (Yunnan, China), using ultraviolet-visible (UV–Vis) spectroscopy, excitation-emission matrix (EEM) fluorescence spectroscopy, and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). The results showed that ECWs effectively reduce the DOM abundance in wastewater, with preferential removal of protein-like substances and aliphatic compounds. The DOM pool undergoes significant changes as the wastewater passes through wetlands, exhibiting pronounced terrestrial signatures characterized by higher unsaturation degree, aromaticity, humification degree, and recalcitrance. Additionally, a marked decrease in CHOS molecules within DOM was observed. The reduction in O_3S and O_5S classes, which are associated with linear alkylbenzene sulfonates (LAS) and sulfophenyl carboxylic acids (SPCs), respectively, demonstrates the purification capacity of ECWs against organic pollutants. The synergistic interactions among plant-derived organic matter inputs, photodegradation, and microbial degradation are likely the primary mechanisms governing the changes in DOM chemistry from influent to effluent within ECWs. Our findings offer novel insights into DOM removal and transformation during ECWs treatment, providing valuable guidance for optimizing wetland design and management strategies to strengthen the control of external pollution within large lake systems.

1. Introduction

Excessive pollutants (e.g., nutrients, organic contaminants) from domestic sewage, agricultural runoff, and industrial wastewater enter lakes through riverine transport, leading to severe issues in lake ecosystems, such as ecological degradation and eutrophication [1–3]. Empirical studies have demonstrated that controlling external pollution inputs is a crucial prerequisite for lake ecological restoration and sustainable management [4,5]. In this context, established estuarine constructed wetlands (ECWs) are considered critical measures for reducing exogenous pollutant loads into lakes and have been widely implemented in the ecological protection of aquatic ecosystems globally [6–8].

The purification efficiency of constructed wetlands for pollutants largely depends on their internal biogeochemical processes, in which

* Corresponding authors.
E-mail addresses: chentao45@crecg.com (T. Chen), pengys@lsbg.cn (Y. Peng).

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dissolved organic matter (DOM) plays a critical role in the transport and removal of pollutant (e.g., nutrients, heavy metals) [9,10]. It has been reported that investigating the dynamic changes in the quantity and quality of DOM in wetlands can effectively reveal the sources of organic substances and elucidate the removal mechanisms and biochemical processes of pollutant [11–13]. For example, the organic matter in the influent of constructed wetlands often displays endogenous characteristics (e.g., more protein-like substances), while the effluent frequently exhibits significant terrestrial features (e.g., more humic-like substances) [14]. Meanwhile, microbial processes (e.g., adsorption) serve as important pathways for the removal of pollutants, such as nitrogen, phosphorus, and heavy metals [10]. As the primary carbon source for microbial metabolism, DOM directly regulates the progression of these processes. Previous studies have suggested that the DOM composition and characteristics are primarily regulated by its origins and diverse biogeochemical processes [15,16]. On the one hand, DOM in ECWs is typically derived from multiple sources (e.g., agricultural tailwater, domestic sewage) and undergoes simultaneous transformation processes (e.g., photodegradation, biodegradation) within the wetlands. On the other hand, although aquatic plants can absorb a portion of the organic matter, the decomposition of their litter also introduces extra organic matter into the system [13,17]. The synergistic interplay of these factors may induce complicated and dynamic variations in DOM chemistry within ECWs. Therefore, exploring the chemical composition and transformation processes of DOM in these ECWs has profound significance for optimizing wetland management and pollution control strategies for lake systems.

Conventional evaluations on DOM changes in constructed wetlands have primarily relied on bulk parameters (e.g., DOC) and spectrometric techniques, such as ultraviolet visible (UV–Vis) absorbance spectroscopy and excitation-emission matrix (EEM) fluorescence spectroscopy [12,14,18]. However, these traditional methods provide only ambiguous information on DOM sources, chemical composition, and characteristics [19]. Precisely investigating the changes in DOM chemistry within constructed wetlands remains challenging due to their tremendous heterogeneity and complicated treatment processes. Recently, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has demonstrated powerful capabilities for characterizing DOM chemistry at the molecular level [20,21] and has been widely applied to investigate the sources, chemical composition, and transformation processes of DOM in combination with optical techniques and bulk chemical analysis across various environmental systems [22–25]. Although recent study has elucidated the molecular composition and evolutionary patterns of DOM in constructed wetlands, these findings are mainly derived from laboratory-scale systems [26]. Given the significant advantages of ECWs in intercepting non-point source pollution and the multiple influencing factors on their operation in the natural environment, deciphering the chemical composition and transformation processes of DOM in ECWs at the molecular level holds significant practical relevance and is therefore needed.

Erhai Lake, the second-largest plateau lake in Yunnan Province, is a vital regional drinking water source and a renowned tourist attraction in China. Over the past decades, intensified anthropogenic activities (e.g., agricultural expansion and livestock production) within the lake watershed have facilitated the substantial entry of exogenous pollutants into Erhai Lake, resulting in eutrophication [27,28]. To restore the lake's ecological functions and mitigate external pollution loads, the government has implemented ecological engineering measures, including the establishment of hundreds of constructed wetland systems (primarily retrofitted natural wetlands) along the inflow rivers of the Erhai Lake basin [29]. Yonganjiang River, one of the three main northern tributaries discharging into Erhai Lake, contributes approximately 5.2 % of the total inflow volume [6]. The non-point source pollution from agricultural activities and livestock production is of great concern in this river watershed, where most wastewater from these processes is frequently discharged into the river without treatment [30]. Furthermore, inadequate sewage collection systems and outdated sewage treatment facilities in surrounding watershed villages result in the direct discharge of domestic wastewater into the Yonganjiang River [31]. Previous studies have confirmed that the ECWs along this river effectively remove nutrients and organic matter from wastewater [6]. However, the molecular composition and transformation processes of DOM remain poorly characterized.

In this study, we investigated the optical properties and molecular characteristics of DOM in six constructed wetland systems within the Yonganjiang River basin using bulk chemical analysis, spectroscopy methods, and FT-ICR MS technique. The objective of this study is to explore the source, composition, and characteristics of DOM in the influent and effluent in ECWs and unravel its changes as it traverses the wetlands. This study provides important insights into the management strategies of ECWs that can ultimately contribute to the pollution prevention and protection of large lake ecosystems.

2. Materials and methods

2.1. Study area description

Yonganjiang River (25°25′-26°16′N, 99°32′-100°27′E) is situated in the Dali Bai Autonomous Prefecture, Yunnan Province, China (Fig. 1). It originates from Eryuan County and flows into the northern part of Erhai Lake. The river spans 18.35 km in length, with a drainage area of 110 km^2 and an annual runoff of 0.38×10^8 m^3 [6]. The region is characterized by a subtropical plateau monsoon climate, with an average annual temperature of 13.9 °C and annual precipitation of 732 mm. Land use data for the Yonganjiang River basin, with a spatial resolution of 30 × 30 m, was acquired from the Resource and Environment Data Center of the Chinese Academy of Sciences (https://www.resdc.cn/). Through visual interpretation and field investigation, land use within this watershed was classified into five categories: water bodies (3.93 %), vegetation (37.70 %), residential area (16.59 %), farmland (22.14 %), and other land types (19.65 %).

Multiple constructed wetlands, initially established in 2013, are distributed along the Yonganjiang River. A marked decline in treatment efficiency was observed in these wetlands, primarily attributed to the death of aquatic vegetation and inadequate management practices. Consequently, these wetlands underwent comprehensive reconstruction in March 2023 and have maintained stable operation for 17 months as of the sampling time. Based on differences in treatment processes and capacities, six representative ECWs along the Yonganjiang River were selected for focused analysis in this study. These include the Yongan West (YAW) wetland, Yongan East (YAE) wetland, Tuanshan North (TSN) wetland, Tuanshan South (TSS) wetland, Dengbeiqiao I (DB I) wetland, and Dengbeiqiao II (DB II) wetland. All six wetlands were designed to treat wastewater with a low pollution load, and detailed information on their primary treatment units and designed parameters can be found in the Supporting Information.

2.2. Sample collection and processing

The sampling was conducted on 11 August 2024. A total of 11 surface water samples (labeled S1, S2, etc.) were collected from six ECWs to investigate the variability of DOM composition and characteristics (Fig. 1). All samples of 1 L were taken using an acid-precleaned glass bottle and transported to the laboratory on ice within 4 h. In the laboratory, a certain volume of samples was filtered through a precombusted 0.7 μm Whatman GF/F glass fiber membrane, then the filtrates were acidified with hydrochloric acid to pH = 2 and maintained at 4 °C in the dark for subsequent processing. The remaining water was stored at −20 °C and analyzed for water chemistry within 2 days.

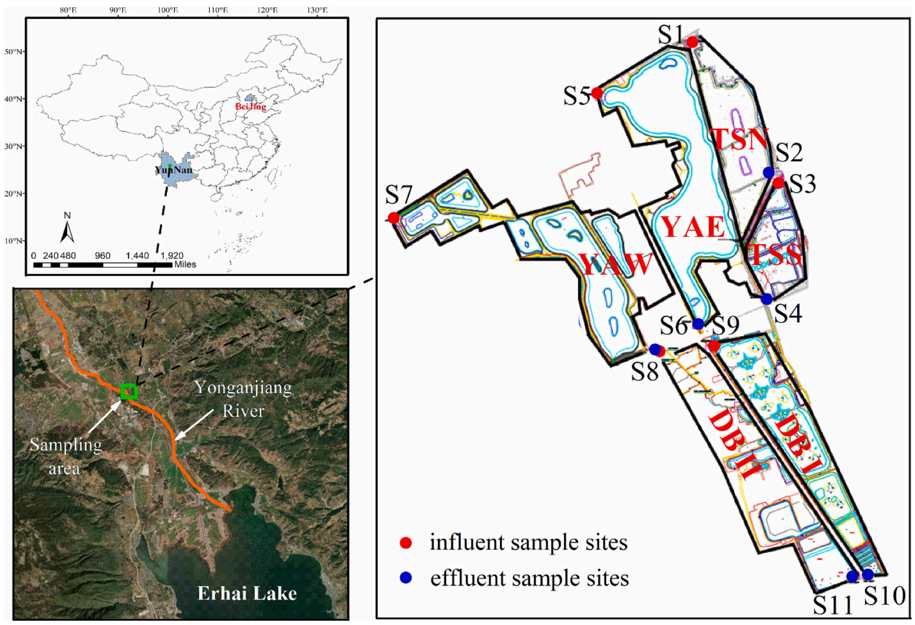

Fig. 1. Study area and sampling sites of ECWs along the Yonganjiang River. Note: the sample site of S8 serves simultaneously as the effluent of YAW wetland and the influent of DB II wetland.

2.3. Water quality parameters measurement

The concentrations of water quality parameters, including total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH_3−N), and chemical oxygen demand (COD_{Mn}), were measured according to Chinese standard methods. Dissolved organic carbon (DOC) concentrations were determined via high-temperature catalytic oxidation on a TOC-L CPN analyzer (Shimadzu, Japan). All parameters were calculated as mean values of triplicate measurements. Detailed analytical procedures are described in our previous studies [16,22].

2.4. Spectroscopic analysis and processing

The ultraviolet-visible (UV–Vis) absorbance spectra of all DOM samples were recorded between 200 and 800 nm at 1 nm intervals using a UV-2700i spectrophotometer (Shimadzu, Japan) with a 1 cm quartz cuvette. The fluorescent DOM excitation-emission matrices were acquired on an Aqualog spectrofluorometer (Horiba, Japan), and the wavelength ranges of excitation (Ex) and emission (Em) were set to 200–450 nm (2 nm increments) and 250–600 nm (approximately 1 nm increment), respectively. The ultrapure water (Milli-Q) was used as a blank for both absorbance and fluorescent spectra of DOM. Several spectroscopic indices were calculated to characterize the DOM optical properties. The absorption coefficient at a wavelength of 254 nm (A_{254}) was commonly applied as a proxy for the abundance of chromophoric DOM (CDOM) [32]. The specific ultraviolet absorption of DOC at 254 nm ($SUVA_{254}$) was widely used to indicate DOM aromaticity [33]. The fluorescence index (FI), which is defined as the ratio of the fluorescence intensity at Ex 470 nm to that at 500 nm when Ex was 370 nm, serves as an indicator of DOM source (approximately 1.8 for microbial and 1.2 for terrestrial origin) [34]. The biological index (BIX), a metric for the contribution of freshly produced autochthonous DOM (> 1: autochthonous origin; < 0.6: terrestrial source), was determined as the ratio of fluorescence intensity at Em 380 nm to the maximum values between 420 and 435 nm when Ex was 310 nm [35]. The humification index (HIX), used to indicate the humification degree of DOM, was calculated as the fluorescence intensity integration at Em 435–480 nm divided by the sum of integration at 300–345 nm and 435–480 nm when Ex was 254 nm [36]. Additionally, fluorescence regional integration (FRI) analysis was employed as a quantitative method to divide the EEM spectra into five regions by wavelength ranges of Ex and Em (Table 1). Each region indicates a specific fluorescent substance and detailed descriptions of this method have been reported previously [37].

2.5. Solid phase extraction and FT-ICR MS analysis

To explore the changes of DOM chemistry as it traversed the wetlands at the molecular level, the influent and effluent (S8, S9, S10, and S11) from the DB I and DB II wetlands were selected for FT-ICR MS analysis. These two wetlands were chosen due to their representative and complex design configurations, which encompass all wetland types in this study. The analysis was performed via electrospray ionization in negative ion mode using a 15 T Bruker SolariX FT-ICR MS instrument. Before FT-ICR MS analysis, DOM samples were extracted via solid-phase extraction (SPE) using an Agilent Bond Elute PPL cartridge (500 mg, 6 mL), following the method in Dittmar et al. [38]. The detailed measurement procedures, parameter settings, and calibration of FT-ICR MS have been reported previously [39,40].

Molecular formulas were assigned to peaks with a signal-to-noise ratio (S/N) above four under the following constraints: $^{12}C_{0-100}\,^{1}H_{0-200}\,^{16}O_{0-50}\,^{14}N_{0-10}\,^{32}S_{0-2}$, $0.2 < H/C < 2.3$, $0 < O/C < 1.2$, $N/C < 0.5$, $S/C < 0.2$, and $-10 \leq$ DBE-O ≤ 10 (DBE was short for double bond equivalent) [41]. The intensity-weighted parameters were calculated to reveal the basic molecular characteristics of DOM. Specifically, DBE and modified aromaticity (AI_{mod}) were calculated to evaluate the degree of unsaturation and aromaticity of each compound, respectively [42]. The molecular lability boundary (MLB_L%) and the island of stability (IOS%) were identified to assess the overall lability of DOM [43,44]. In addition, several biochemical compound groups were defined as follows [23,45]:

Table 1
FRI analysis for the five defined regions [37].

Region	Excitation (nm)	Emission (nm)	Substances
I	200–250	250–330	Tyrosine-like protein
II	200–250	330–380	Tryptophan-like protein
III	200–250	380–600	Fulvic acid-like
IV	250–450	250–380	Microbial-like
V	250–450	380–600	Humic-like

1) condensed or polycyclic condensed aromatics ($AI_{mod} > 0.66$); 2) polyphenolic compounds ($0.66 \geq AI_{mod} > 0.50$); 3) highly unsaturated and phenolic compounds ($AI_{mod} \leq 0.50$, $H/C < 1.5$); 4) aliphatic compounds ($2.0 > H/C \geq 1.5$); 5) saturated compounds ($H/C \geq 2.0$, or $O/C \geq 0.9$); 6) carboxylic-rich alicyclic molecules (CRAMs, DBE/C = 0.30–0.68, DBE/H = 0.20–0.95, DBE/O = 0.77–1.75) [46].

2.6. Statistical analyses

The distribution of the sampling area was mapped using ArcGIS 10.2 software. Significant differences in water quality, optical properties, and molecular composition between the influent and effluent of ECWs were assessed using Student's *t*-test (for normally distributed variables) and nonparametric Mann-Whitney *U* test (for non-normally distributed variables) in SPSS Statistics 26. The significance level was reported as $p < 0.05$.

3. Results and discussions

3.1. Bulk water quality parameters

The concentrations of bulk water quality parameters in the influent and effluent across the six ECWs are summarized in Fig. 2. The influent concentrations of TN and NH_3-N ranged from 0.90 to 3.15 mg/L and 0.36 to 0.97 mg/L, respectively, and both were significantly higher than those in the effluent with the concentrations ranging from 0.54 to 1.0 mg/L and 0.146 to 0.196 mg/L, respectively ($p < 0.05$; Fig. 2a, c). Although the difference in TP concentrations between influent (0.04–0.16 mg/L) and effluent (0.02–0.07 mg/L) was not statistically significant, a clear decreasing trend was observed (Fig. 2b). The concentrations of COD_{Mn}, an indicator of organic pollution levels in waters [47], also decreased significantly from 6.05 to 10.96 mg/L in the influent and to 3.96 to 6.61 mg/L in the effluent ($p < 0.05$; Fig. 2d). Consequently, the average removal efficiencies of 54.42 %, 43.75 %, 51.24 %, and 44.19 % were achieved for TN, TP, NH_3-N, and COD_{Mn} across the six ECWs, respectively (Fig. 2). These results confirm the severe non-point source pollution within the Yonganjiang River basin, and demonstrate the capacities of ECWs in removing the nutrients and organic matter in wastewater. The removal levels of these pollutants are consistent with previous studies [48,49]. The intensive crop activities and livestock production have undergone extensive development around the Yonganjiang River basin [6], which might explain the greater concentrations of nutrients and organic matter in the influent of the ECWs.

3.2. DOC concentrations and optical properties of the DOM

The concentrations of DOC could represent the abundance of DOM in various aquatic systems. The influent DOC concentrations ranged from 9.45 to 18.88 mg/L, and were significantly higher than those in the effluent samples (7.43–13.42 mg/L) in ECWs ($p < 0.05$; Fig. 3a), indicating that DOM in waters could be eliminated partially when traversing the ECWs, similar findings were also observed in other studies [12,13]. For example, Sardana et al. [13] reported a substantial decrease in DOC concentrations (from 43.82 to 9.42 mg/L) as the wastewater passed through the constructed wetlands. Generally, the observed DOC reduction was primarily due to the DOM absorbed by aquatic plants and/or degraded by microorganisms in constructed wetlands [11]. These ECWs were all cultivated with abundant aquatic plants (e.g., lotus) and likely maintained diverse microbial communities (distributed in plant roots, sediments, and gravel substrates). In addition, these wetland types are predominantly free water surface flow (YAW, YAE, and TSN wetlands)

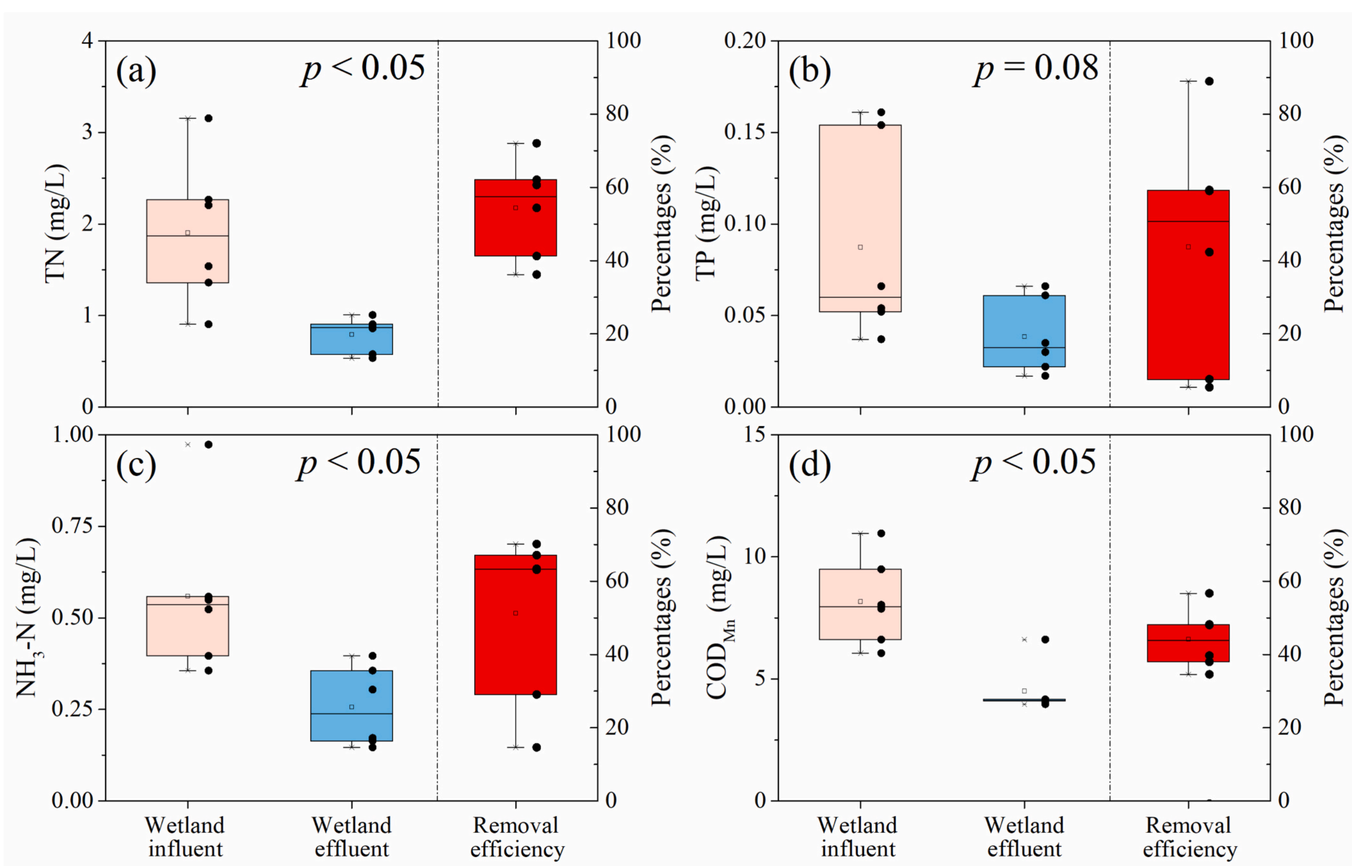

Fig. 2. Water quality parameters of the influent and effluent and their removal efficiency across the six ECWs: (a) total nitrogen (TN), (b) total phosphorus (TP), (c) ammonia nitrogen (NH_3-N), (d) chemical oxygen demand (COD_{Mn}).

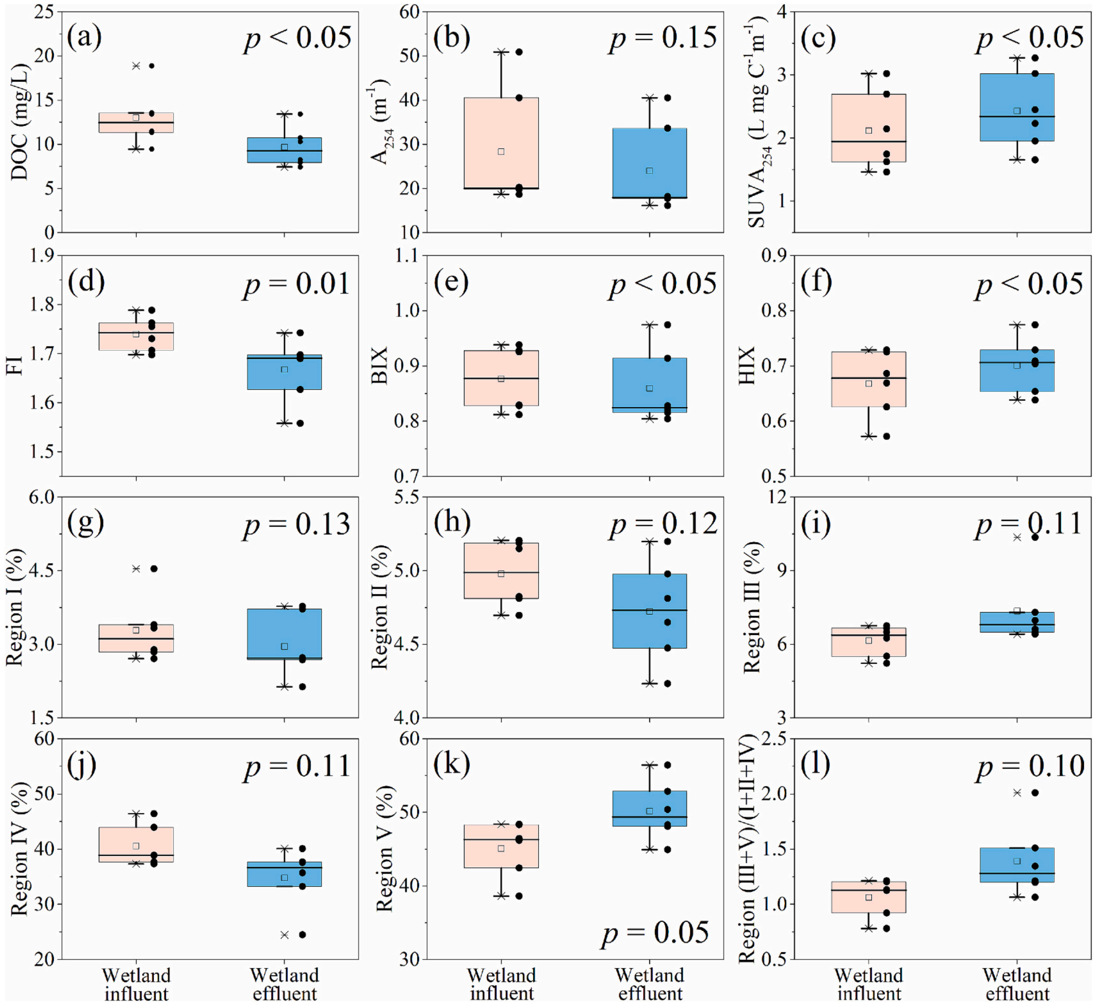

Fig. 3. DOC concentrations and DOM optical properties of the influent and effluent across six ECWs: (a) dissolved organic carbon (DOC), (b) absorption coefficient at 254 nm (A_{254}), (c) specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), (d) fluorescence index (FI), (e) biological index (BIX), (f) humification index (HIX), (g) volume percentage of region I (region I), (h) volume percentage of region II (region II), (i) volume percentage of region III (region III), (j) volume percentage of region IV (region IV), (k) volume percentage of region V (region V), (l) ratio of region (III + V)/(I + II + IV).

and hybrid surface-subsurface flow constructed wetlands (TSS, DB I, and DB II wetlands). Their open-water characteristics and plateau topography (about 2000 m) significantly facilitate the photodegradation process of DOM. Therefore, taken together, the removal of DOC in these ECWs can be attributed to the combined effects of aquatic plant uptake, microbial degradation, and photodegradation.

The absorption coefficient A_{254} was used to indicate the content of CDOM, which decreased from the wetland influent (18.65–50.90 m^{-1}) to the effluent (16.12–40.53 m^{-1}; Fig. 3b), suggesting that the CDOM was likely removed by the ECWs. Moreover, the A_{254} values were positively related to DOC concentrations across all samples ($r^2 = 0.58$, $p < 0.05$; Fig. S1a), implying that CDOM constitutes an important fraction of DOM in these wastewaters. As noted previously, the parameters of $SUVA_{254}$ and HIX can reveal the aromaticity and humification degree of DOM [33,36]. The $SUVA_{254}$ values for the influent and effluent ranged from 1.46 to 3.02 and 1.65 to 3.27 L mg C^{-1} m^{-1}, respectively, and HIX values from 0.57 to 0.73 and 0.64 to 0.77, respectively (Fig. 3c, f). Interestingly, a positive correlation was observed between $SUVA_{254}$ and HIX in this study ($r^2 = 0.37$, $p < 0.05$; Fig. S1b), and both parameters significantly increased from influent to effluent across all six ECWs ($p < 0.05$). This indicates that the wetland treatment processes enhanced DOM aromaticity and humification degree. The FI and BIX were usually used to distinguish the potential sources of DOM in waters, with lower values of FI and BIX commonly indicating a greater contribution of terrestrial sources in DOM. In this study, the values of FI and BIX for the influent (FI: 1.74 ± 0.03; BIX: 0.88 ± 0.05) were higher than those for the effluent (FI: 1.67 ± 0.05; BIX: 0.86 ± 0.06) in ECWs (Fig. 3d, e). These findings suggest that while DOM in the influent and effluent derived from both autochthonous and allochthonous sources, and the effluent DOM exhibits more significant terrestrial features than influent DOM. It is worth noting that remarkable variations in the values of the DOM optical properties of constructed wetlands were observed in different research. For instance, compared to our study, Xuan et al. [50] found similar FI values for the influent and effluent that were 1.72 ± 0.022 and 1.68 ± 0.023, respectively, whereas significantly higher values (influent: 2.322, effluent: 2.095) were reported by Yao et al. [51]. These disparities are attributed to various factors, such as wastewater pollution load, wetland type, operation time, and effective management. However, more importantly, the change patterns of these DOM optical parameters from influent to effluent in ECWs were similar in the present study and most previous studies.

Based on the FRI method, each fluorescence EEM spectrum was separated into five regions (I, II, III, IV, and V) to distinguish the DOM compositions in the influent and effluent of ECWs (Fig. S2). Region I (tyrosine-like), region II (tryptophan-like), and region IV (microbial-like) can be considered as protein-like substances, while region III (fulvic acid-like) and region V (humic acid-like) are classified as humic-like substances [16,52]. Fig. 3g-k illustrates the volume percentage of each fluorescent substance, microbial-like, and humic acid-like compounds were predominant in all influent and effluent DOM. It was reported that the DOM was mainly dominated by humic-like substances in natural inland water [53], and human activities could greatly promote the proportions of protein-like substances in the water [47,54]. This relevance is evident in our study area, where land use data show that approximately 40 % of the land within the Yonganjiang River watershed is dedicated to human activities (e.g., residential area, farmland), indicating substantial anthropogenic influence on the influent DOM. Furthermore, a decrease in protein-like substances and an increase in humic-like substances were observed in DOM from influent to effluent in constructed wetlands. Particularly, the ratio of region (III + V)/(I + II + IV) was further used to analyze the alteration of the DOM compositional structure between the influent and effluent. The ratio of DOM for the influent (1.06 ± 0.15) was greatly lower than that of effluent (1.39 ± 0.31) (Fig. 3l), indicating that more humic-like and less protein-like substances existed in effluent DOM compared to influent DOM. These results further evidence that the protein-like substances in DOM were preferentially removed by wetlands treatment. This finding agrees with previous reports that the labile components (e.g., proteins) of DOM were preferentially removed compared to refractory components (e.g., aromatic substances) in constructed wetlands [14,51]. Moreover, the leaching of humic-like materials derived from aquatic plants could enhance the terrestrial signatures of DOM in wetland waters [13], which also contributes to increasing the proportions of humic-like substances in effluent DOM.

3.3. Molecular-level characteristics of DOM revealed using FT-ICR MS

FT-ICR MS provided a significant advantage for investigating the changes in DOM composition and characteristics at the molecular level. As shown in Fig. 4, thousands of molecules were detected in the mass range of 100–800 Da in the influent and effluent DOM, indicating the complexity of the DOM pool within the two ECWs. In total, the number of molecular formulae identified in the DB I wetland were 6428 (influent) and 6732 (effluent), whereas those in the DB II wetland were 7846 and 7707, respectively (Table 2). Notably, 5449 and 6569 formulae were shared between the DB I and DB II wetlands, respectively. The numbers of removed and produced molecules in DB I wetland were 979 and 1283, respectively, while in DB II wetland, the numbers were 1277 and 1138, respectively (Fig. S3). These results demonstrate that although the DOM composition of the influent and effluent was overall similar in each ECW, there were non-negligible changes in the DOM pool through the treatment processes. The molecular weight (*m/z*) of influent DOM in both the DB I (416.64 Da) and DB II wetlands (429.01 Da) was slightly lower than that of effluent DOM (425.91 Da and 429.42 Da, respectively). Additionally, the parameters of O/C, H/C, DBE, and AI_{mod} values, were also lower in the influent (O/C: 0.49 and 0.50, H/C:1.19 and 1.16, DBE: 9.05 and 9.58, AI_{mod}: 0.29 and 0.30) than those of effluent (O/C: 0.50 and 0.52, H/C: 1.16 and 1.15, DBE: 9.52 and 9.60, AI_{mod}: 0.31 and 0.31) in the two ECWs, which revealed that the effluent DOM has relatively more unsaturated, aromatic, and oxygen-containing compounds with higher molecular weight compared with the influent DOM in ECWs, consistent with the results observed from $SUVA_{254}$ and HIX parameters. Similar findings were also reported previously that microbial activities could utilize some specific DOM materials (e.g.,

Table 2
Intensity-weighted molecular parameters of DOM samples based on FT-ICR MS.

Parameters	DB I wetland		DB II wetland	
	influent	effluent	influent	effluent
Number	6428	6732	7846	7707
m/z	416.64	425.91	429.01	429.42
C	19.46	19.82	19.92	19.79
H	23.27	23.03	23.10	22.83
O	9.42	9.75	9.82	9.97
N	0.45	0.44	0.42	0.46
S	0.11	0.11	0.16	0.13
O/C	0.49	0.50	0.50	0.52
H/C	1.19	1.16	1.16	1.15
DBE	9.05	9.52	9.58	9.60
AI_{mod}	0.29	0.31	0.30	0.31
MLB_L (%)	9.74	8.60	10.31	7.41
IOS (%)	12.04	12.05	10.30	11.01
CRAMs (%)	63.78	63.93	61.75	62.46

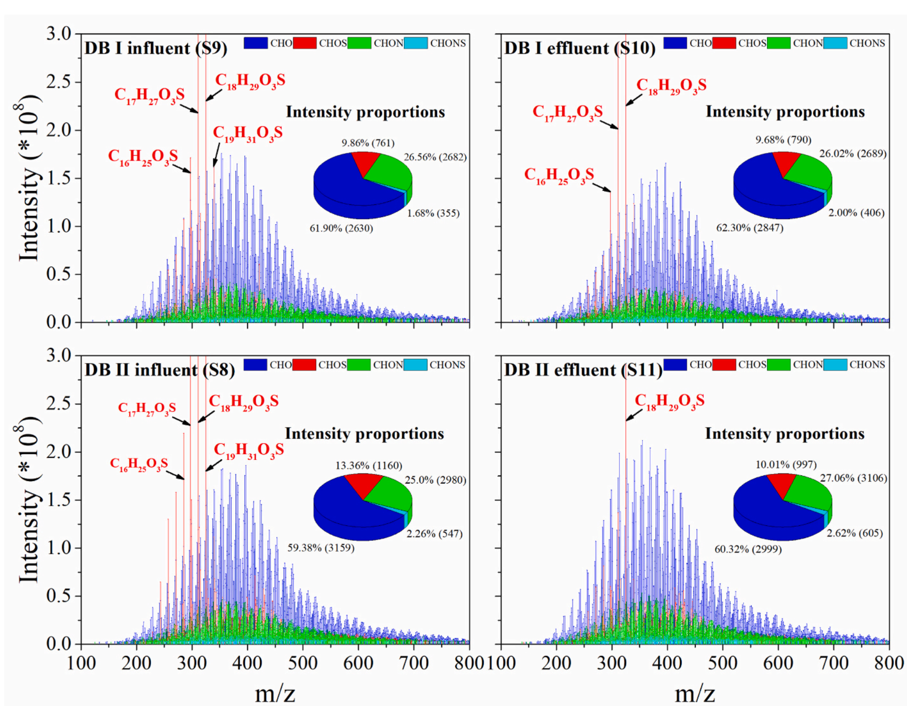

Fig. 4. Broadband negative ion ESI FT-ICR MS spectra across the *m/z* range 200–800 and intensity proportions of CHO, CHOS, CHON, and CHONS formulas in DOM samples.

aromatic protein tyrosine and tryptophan substances) and produce new compounds in DOM pools, resulting in the effluent DOM characterized by a greater unsaturation degree, aromaticity, and oxygen contents [26,55].

The assigned molecules were divided into four compound classes based on the elemental composition, including CHO, CHOS, CHON, and CHONS. Overall, the differences in relative proportions of each elemental group between influent and effluent were all less than 4 % (Fig. 4). As the most abundant compounds, CHO formulas were slightly lower in the influent (DB I: 61.90 %, DB II: 59.38 %) than in the effluent (DB I: 62.30 %, DB II: 60.32 %). Conversely, the proportions of CHOS compounds in the influent of DB I and DB II wetland were 9.86 % and 13.36 %, respectively, which decreased to 9.68 % and 10.01 % in the effluents. Many studies have proposed that DOM is mainly dominated by CHO formulas in various natural waters [23,56], and human activities can remarkably facilitate the relative abundance of sulfur-containing compounds [47,54]. For example, higher proportions of CHOS groups (11.48 ± 2.1 %) were observed in the Daliao River (China), which was reported under severe anthropogenic impacts and heavily polluted from wastewater [57]. Specifically, the O_3S and O_5S classes in CHOS formulas, likely associated with linear alkylbenzene-sulfonates (LAS, commonly used surfactants in detergents) and sulfophenyl carboxylic acids (SPC, degradation products of LAS), respectively, were widely detected with high abundance in wastewater and other severely polluted inland waters [15,24]. In this study, the abnormally high abundance of O_3S classes (e.g., $C_{16}H_{25}O_3S$, $C_{17}H_{27}O_3S$, $C_{18}H_{29}O_3S$, and $C_{19}H_{31}O_3S$) was identified in the influent DOM, and the proportions of O_3S and O_5S were both decreased in the effluent (Fig. 4 and Fig. S4). This finding is further supported by land use characteristics, as a high proportion of residential area was observed in the Yonganjiang River watershed. Taken together, these results confirm that the Yonganjiang River is severely impacted by human activities and highlight the important roles of ECWs in pollutant treatment.

Complicated molecules in the DOM can be visualized into five compounds by van-Krevelen diagrams (H/C and O/C ratios of the formulae; Fig. 5). In both wetlands, highly unsaturated and phenolic compounds (HUPs) constituted the largest relative proportions of both influent and effluent DOM, followed by aliphatic, polyphenolic, polycyclic aromatic, and saturated compounds. This composition is consistent with the compositions reported in natural lakes and rivers [22,58]. According to previous studies in aquatic systems, polyphenolics and HUPs, as the terrestrial indicators, are usually derived from vascular plant-sourced organic matter and products of lignin degradation, respectively [45], and these highly aromatic substances are sensitive to photodegradation [59,60]. In the DB I wetland, the relative abundance of polyphenolics increased from 8.21 % to 9.99 % between the influent and effluent, while that of HUPs decreased from 79.30 % to 78.00 %. In contrast, the change patterns of these two compounds were reversed in the DB II wetland (polyphenolics: decreased from 10.87 % to 10.42 %, HUPs: increased from 75.51 % to 78.34 %). Generally, the terrestrial organic matter in constructed wetlands mainly originates from the leachates of aquatic plants. There are no further results in this study that could explain this variation well. Still, we speculate that it is likely related to the wetland's structures and aquatic plant configuration. On the one hand, the quantities and types of plants in DB I and DB II wetlands were different (Table S1). The DB I wetland contained four species of aquatic plants and exhibited relatively higher vegetation coverage compared to DB II, which contained only two species. It has been reported that the composition of DOM leached from different wetland plants varies (e.g., proportions of polyphenols), suggesting that the variations in plants may affect the DOM chemistry leached into the water [61]. On the other hand, the DB II wetland has a larger surface area (6.81×10^4 m^2) than the DB I wetland (2.72×10^4 m^2). This greater area enhances photodegradation processes. For instance, prolonged photo exposure has been shown to decrease the abundance of polyphenols in lake DOM [60], which is consistent with the lower levels we observed in DB wetland. Additionally, aliphatic compounds (e.g., protein/peptides materials), which are considered bio-labile [16], decreased from the influent (9.52 % and 10.15 %) to the effluent (8.44 % and 7.33 %) in both ECWs. This finding is highly consistent with the decrease in protein-like substances in EEM-FRI results. It indicates microbial degradation may also be a significant process of DOM

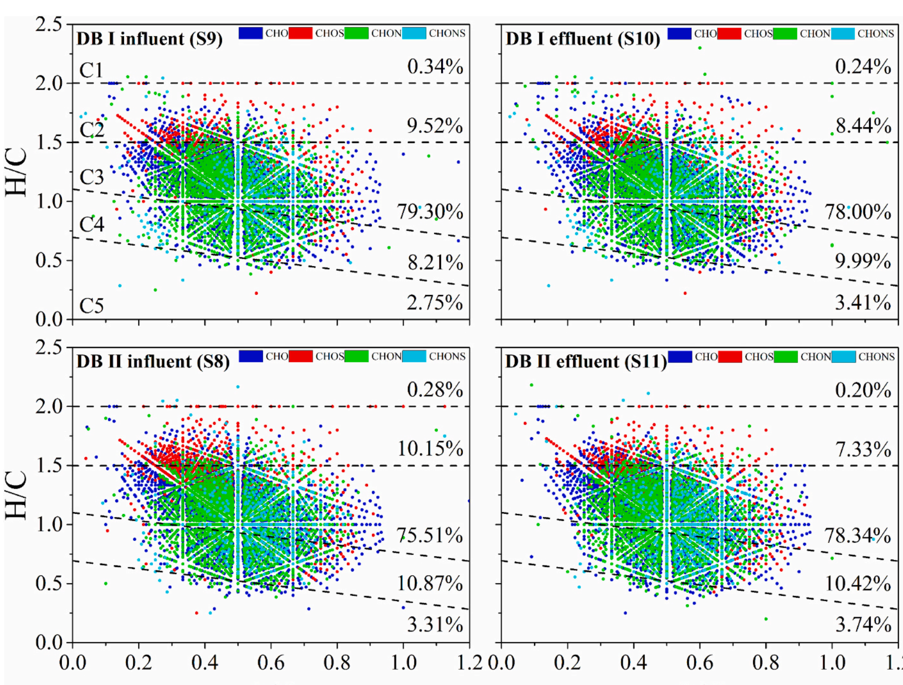

Fig. 5. Van-Krevelen diagrams of four formulas (CHO, CHOS, CHON, and CHONS) in DOM samples. C1, C2, C3, C4, and C5 were saturated compounds, aliphatics, highly unsaturated and phenolic compounds, polyphenolics, and polycyclic aromatics, respectively. (Numbers in the diagram represents the proportions of each corresponding compound).

compositional changes in these treatment wetlands. Previous studies have reported that aliphatics usually contain abundant S-containing molecules, and a significant positive correlation has been observed between the abundance of aliphatics and CHOS formulas [22,62]. As shown in Fig. S3, we also observed the elimination of some CHOS molecules occurring in aliphatics, which may explain the reduction in CHOS formulas from influent to effluent. Moreover, since aliphatics often originate from the bacterial and algal metabolites [63], the presence of highly aromatic substances and aliphatic compounds in the DOM further confirms the conclusion we deduced based on FI and BIX parameters.

It is worth noting that the treatment processes not only altered the DOM composition but also changed its lability and recalcitrance in ECWs. The CRAMs and IOS are typically proposed to evaluate the abundance of recalcitrant molecules, whereas the MLB_L is used to represent the proportions of labile molecules in DOM [43,44,46]. As shown in Table 2, an increase in CRAMs and IOS accompanied by a decrease in MLB_L values was observed between the influent and effluent of both ECWs, demonstrating that the DOM became more stable and recalcitrant after passing through the wetlands. Previous studies have suggested different reactivity between autochthonous (generally more bio-labile) and allochthonous (more photoactive) DOM [23], while recent research has shown that combined photo and microbial processing can facilitate the enrichment of refractory DOM (e.g., CRAMs) [64]. The intense solar radiation and suitable temperatures in the plateau ECWs provide favorable conditions for these biogeochemical processes in the wastewater DOM. Furthermore, existing studies have indicated that CHOS compounds are a major component of the labile DOM pool and possibly contribute to DOM bioavailability in aquatic systems. The observed decrease in CHOS abundance in this study further supports the enhanced stability of the effluent DOM. This finding aligns with previous studies reporting that more labile and low molecular weight organic materials are removed through the wetlands treatment [51,65].

The dynamic changes in optical properties and molecular composition of DOM are crucial for elucidating intricate biogeochemical processes within constructed wetland ecosystems. Although the original sources and chemical composition of DOM in the influent of high-altitude constructed wetlands may differ from those in low-altitude systems, the impact patterns of wetland treatment on both are highly consistent (e.g., a decrease in labile components and an elevation of terrestrial signatures) [12,14]. While most current studies attribute DOM transformation to a combination of mechanisms, including photodegradation, microbial degradation, and plant uptake, the relative contributions of these processes likely differ substantially between plateau and plain constructed wetlands. In low-altitude constructed wetlands, microbial metabolism and plant assimilation have been identified as the dominant pathways in DOM transformation [13,65]. In contrast, in high-altitude systems, intense solar radiation (particularly in the ultraviolet spectrum) enables photodegradation to play a far more substantial role than previously recognized (e.g., through the photochemical mineralization of aromatic compounds) [66], potentially making it the critical process governing DOM dynamics in these environments. This distinction underscores the importance of fully accounting for environmental drivers and their dominant mechanisms when evaluating the performance of constructed wetlands across different altitudes, while also providing a theoretical basis for the design and management of plateau constructed wetlands.

3.4. Environmental implications and future considerations

The findings of this study provide significant implications for the application of ECWs as engineered systems for mitigating non-point source pollution in large aquatic ecosystems. The transformation patterns of different DOM components within ECWs accurately reflect the diverse and complex internal biogeochemical processes, offering critical guidance for optimizing the design and management of wetland engineering systems. From a practical engineering perspective, labile components within DOM (such as protein-like substances and aliphatic compounds) are preferentially removed, whereas refractory components (e.g., highly aromatic compounds) tend to accumulate. The significant differences in degradation behavior between autochthonous and allochthonous components in DOM indicate that tailored treatment strategies are required to achieve effective removal of distinct organic matter fractions. For instance, regulating microbial community structure and dissolved oxygen levels may enhance the biodegradation of labile components, while increasing the surface area or hydraulic retention time of ECWs can improve the removal efficiency of highly aromatic compounds. Although the quantitative contribution of aquatic plants to the DOM pool requires further clarification, they likely represent an important source of freshly aromatic compounds. This suggests that selecting plant species and implementing seasonal biomass harvesting could help control the accumulation of terrestrial DOM in wetlands. Furthermore, effluent DOM from ECWs exhibits strong terrestrial signatures (e.g., higher abundance of recalcitrant aromatic compounds), which may significantly affect downstream ecological processes. For example, recalcitrant DOM compounds (e.g., CRAMs) can bind to metals on sediment surfaces [67], thereby promoting carbon sequestration in Erhai Lake. However, these terrestrial DOM components are also known precursors of disinfection byproducts, including trihalomethanes (THMs) and haloacetic acids (HAAs) [68]. Given that Erhai Lake serves as a critical drinking water source, the increase in allochthonous DOM may elevate the formation potential of DBPs during water treatment and underscores the need for advanced treatment of ECW effluent. Overall, integrating DOM characterization into management practices can provide a more accurate basis for optimizing treatment efficiency and evaluating ecological effects.

Under natural conditions, the performance of ECWs is significantly modulated by seasonal variations, such as temperature, solar radiation, rainfall, and aquatic plant life cycles. These environmental factors profoundly influence the chemistry and transformation of DOM within wetlands. Here, this study was designed to decipher the dynamics of DOM quantity and quality from influent to effluent within ECWs during summer, and the above research findings could be directly applicable to other constructed wetland systems. However, some limitations still remain. Firstly, while DOM chemistry in constructed wetlands is known to vary seasonally, its seasonal variations in DOM removal and transformation within ECWs were not considered. Secondly, although most ECWs are integrated wetland systems, this work focused only on DOM in the original influent and final effluent of the systems. As a result, the DOM composition in the effluent from different internal treatment units (e.g., surface flow units, aeration contact oxidation units) and the corresponding laboratory incubation experiments were not characterized. Therefore, future investigations should address the above aspects to enable a more comprehensive understanding of DOM transformation processes and purification mechanisms of ECWs.

4. Conclusions

This study comprehensively deciphers the intricate dynamic changes in the quantity and quality of wastewater DOM as it traverses the ECWs. Significant transformations were revealed in DOM chemistry facilitated by ECWs through the analyses of bulk water chemistry, spectroscopic techniques, and FT-ICR MS. The results showed a significant decrease in DOC concentration after wetland treatment, along with an increased aromaticity, humification degree, and unsaturation in the effluent DOM, exhibiting stronger terrestrial signatures. During the treatment processes, various biogeochemical pathways (e.g., photo and microbial degradation) within the ECWs preferentially removed labile components (e.g., protein-like/aliphatic compounds) while accumulating refractory humic-like and highly aromatic compounds, as evidenced by both optical and molecular parameters. These changes resulted in a more stable and recalcitrant DOM pool in the effluent. Additionally, the reduction in O_3S and O_5S classes (indicators of surfactant-derived pollutants) within the CHOS molecules demonstrates the effectiveness of ECWs not only in improving water quality but also in potentially purifying specific organic pollutants. Collectively, our findings emphasize the pivotal role of ECWs in regulating the DOM chemistry and enhancing water quality, and provide significant insights into the removal processes and mechanisms of contaminants within ECWs.

CRediT authorship contribution statement

Lei Xu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Jianlin Tang:** Investigation, Data curation. **Gang Wu:** Software, Data curation. **Bangyao Zhong:** Investigation. **Chongqing Yu:** Investigation. **Aiwen Zhong:** Supervision. **Qian Hu:** Writing – review & editing. **Wei Liao:** Writing – review & editing. **Tao Chen:** Writing – review & editing, Supervision. **Yansong Peng:** Supervision, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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