

Article

Dynamics of Dissolved Organic Matter During Summertime Deoxygenation in a Shallow Coastal Sea

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Abstract

Deoxygenation in marine ecosystems has received increasing attention. The variation of dissolved organic matter (DOM) during deoxygenation has been investigated in hypoxic and anoxic waters in coastal seas and open oceans. In the present study, the dynamics of DOM were investigated during summertime deoxygenation in the bottom water of the shallow coastal Bohai Sea, China. Dissolved organic carbon (DOC), chromophoric and humic-like fluorescent DOM (CDOM and FDOM) gradually increased in the bottom water during summer, which was mainly induced by biological activities in the water column and/or the surface sediment. The estimated net accumulation rates of DOC, CDOM and humic-like FDOM from June to early August in the bottom water were $0.11 \pm 0.04 \mu\text{mol L}^{-1} \text{d}^{-1}$, $0.002 \pm 0.0002 \text{ m}^{-1} \text{d}^{-1}$ and $0.0004 \pm 0.0001 \text{ R.U. d}^{-1}$, respectively. Moreover, the ratio of DOC to CO_2 accumulations was 0.08 in this duration in the bottom water. Compared with DOM in the bottom water prior to deoxygenation, the newly accumulated DOC contained more CDOM and humic-like FDOM that are considered to be microbially refractory.

Keywords: dissolved organic matter; accumulation; deoxygenation; Bohai Sea

1. Introduction

Bottom water deoxygenation in estuarine and coastal waters is aggravating and expanding worldwide [1,2]. It is usually attributed to the eutrophication-enhanced downward flux of particulate organic matter (POM), combined with restricted oxygen replenishment from surrounding waters often due to stratification [3–5]. Therefore, POM is usually considered as the dominant substrate in oxygen consumption in estuarine and coastal waters [6–8], and more attention has been paid to microbial POM degradation in oxygen-depleted waters [9,10].

Along with microbial POM consumption, accumulations of dissolved organic matter (DOM), in the form of dissolved organic carbon (DOC), chromophoric DOM (CDOM) and humic-like fluorescent DOM (FDOM), have been observed in the anoxic deep waters of the Cariaco Basin [11] and the Black Sea [12], and in hypoxic waters of the Peruvian coastal sea [13] and the western continental shelf of India [14]. Moreover, a higher chemodiversity of DOM, including protein-like FDOM components, has been observed in hypoxic waters



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than in oxic waters [15–17], suggesting that hypoxic/anoxic conditions improve organic carbon preservation [18,19]. Notably, all the above studies were conducted in relatively deep coastal waters (depth > 40 m) and open oceans. A recent study revealed that POM is firstly transformed to microbial labile DOM (LDOM), and then this fraction of LDOM can be further remineralized to CO₂ with a small fraction being refractory DOM [19,20]. In shallower estuarine and coastal ecosystems, the interactions between surface and bottom waters are much stronger, including the water masses mixing and vertical transportation of nutrients, oxygen and organic matter [21,22]. More importantly, these systems are usually strongly influenced by riverine input and anthropogenic activities, and thus the primary production is high [23,24]. Consequently, the downward flux of POM should be high. Additionally, the residence time of POM in the water column in shallower waters should be shorter than in deeper waters, and more POM can be buried in surface sediments before degradation. All the above factors should have substantial impact on DOM dynamics in shallow waters, while little is known about this. To our knowledge, Marcinek et al. (2020) [25] reported the only study of DOM cycling during deoxygenation in the shallower Krka River estuary (~10 m depth), Croatia, revealing accumulations of DOC, CDOM and humic-like FDOM in the bottom water.

The Bohai Sea is a semi-enclosed shallow coastal sea in northeast China, with an average water depth of 18 m [26] (Figure 1). Eutrophication in the Bohai Sea has been common in the past 30 years due to industrialization and urbanization along its coast [27–29]. Like other coastal seas, eutrophication, combined with strong water column stratification, has led to seasonal deoxygenation in the bottom water of the Bohai Sea in the past decades [30–33]. Further studies have suggested that pelagic degradation of fresh organic matter produced by smaller-sized marine plankton is mainly responsible for deoxygenation during summer [15,34,35]. However, the dynamics of DOM during deoxygenation in the bottom water of the Bohai Sea remain unknown.

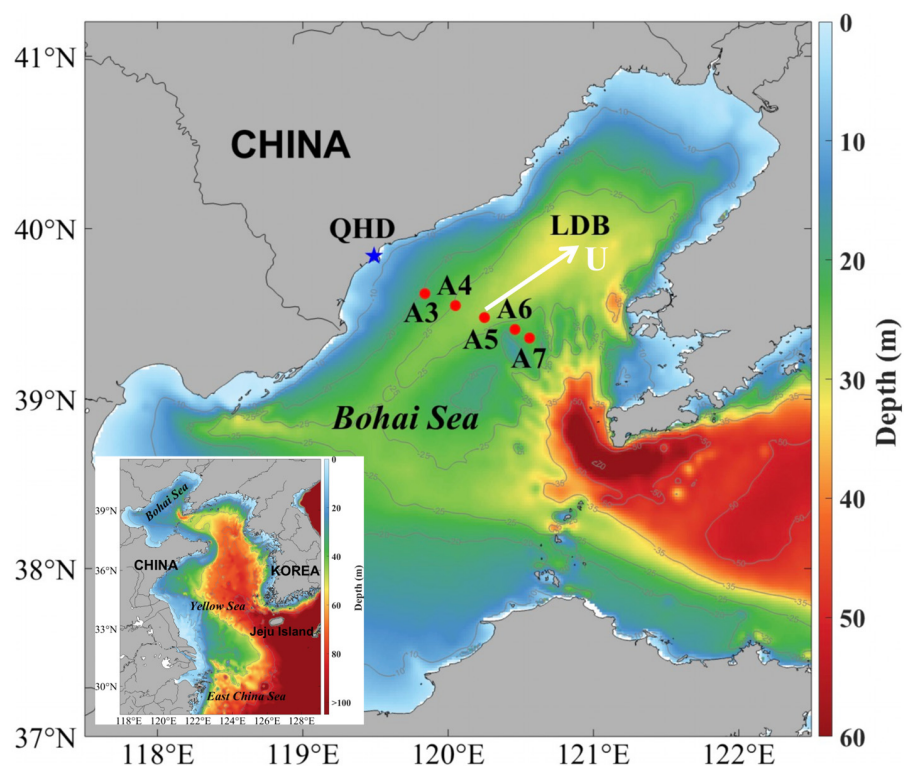


Figure 1. Bathymetry of the Bohai Sea with the sampling stations indicated. QHD and LDB denote Qinhuangdao City and Liaodong Bay, respectively. The white arrow denotes the direction of the residual current in summer [36].

In this study, the contents of DOC, CDOM and FDOM in the water column off Qinhuangdao, Bohai Sea, were measured during the summer of 2018 to (1) monitor the temporal variations and the vertical distributions of DOM; (2) ascertain if DOC, CDOM and FDOM are accumulated in bottom water during deoxygenation; (3) derive the properties of these DOM components. Results from this study lead to a better understanding of biogeochemical DOM cycling during deoxygenation in shallow coastal systems.

2. Materials and Methods

2.1. Sample Collection

DOC, CDOM, FDOM and dissolved oxygen (DO) were investigated on 11th June (day of the year 162, DOY 162), 19th July (DOY 200), 8th August (DOY 220) and 25th August (DOY 237) 2018 along an inshore–offshore transect across the well-known oxygen depletion area (Figure 1). The transect, located off Qinhuangdao City, crossed a bowl-shaped depression extending from northeast to southwest. The detailed sampling dates, station coordinates and water depths are shown in Table S1. Briefly, water samples were collected from four to six depths using 5 L Niskin bottles. After collection, DO was collected immediately and titrated on board using the Winkler method [37], with the relative standard deviation for all triplicate samples being <2% [34]. The remaining water samples were transferred into 1 L glass bottles and filtered through 0.2 μm polyethersulphone (PES) filters (Pall Life Sciences). The filtrates were collected into 20 mL and 100 mL acid-cleaned and pre-combusted clear-glass bottles with Teflon-lined screw caps for DOC concentration and CDOM absorption and fluorescent spectra determinations. DOC samples were stored at $-20\text{ }^{\circ}\text{C}$ and CDOM samples at $4\text{ }^{\circ}\text{C}$ in the dark before analysis. The vertical profiles of temperature, salinity and chlorophyll *a* (Chl *a*) fluorescence were simultaneously acquired using a RBR maestro multi-parameter.

2.2. Sample Analysis

The procedures for DOC concentration, CDOM absorption and fluorescent spectra were previously reported by Li et al. (2019) [38] and Yang et al. (2020) [39]. DOC concentrations were quantified on a Shimadzu TOC-L_{CPH} analyzer, which was calibrated with standard potassium hydrogen phthalate solution. To track the analytical stability of the analyzer, two reference materials sources from Hansell's research group (University of Miami) were run every 10 test samples: a low-carbon standard ranging from $1\text{--}2\text{ }\mu\text{mol L}^{-1}$ and a deep-water reference with a target concentration of $41\text{--}44\text{ }\mu\text{mol L}^{-1}$. Repeated measurements yielded an average DOC value of $2.3 \pm 0.1\text{ }\mu\text{mol L}^{-1}$ for the low-carbon reference and $42.8 \pm 1.2\text{ }\mu\text{mol L}^{-1}$ for the deep-water reference. For five consecutive replicate injections of identical samples, the coefficient of variation remained below <2%.

Absorption spectra of CDOM were acquired over the wavelength range of 200–800 nm with 1 nm resolution using a Shimadzu UV-2550 dual-beam UV-Visible spectrophotometer. Ten-centimeter quartz cuvettes were deployed for all scans and Milli-Q water served as the blank reference through measurements. All water samples were equilibrated to ambient temperature in darkness prior to spectral acquisition [39]. A baseline correction was made by subtracting the average absorbance between 683 and 687 nm from all the spectral values [40]. The Napierian absorption coefficient at wavelength λ (nm), $a_{\text{CDOM}}(\lambda)$ (m^{-1}), was calculated as 2.303 times the corrected absorbance at wavelength λ divided by the pathlength of the cuvette in meters (0.1 m). $a_{\text{CDOM}}(330)$ was used to represent the content of CDOM [38,41,42]. The spectral slope of CDOM between 275 nm and 295 nm ($S_{275-295}$, μm^{-1}), a proxy of CDOM molecular weight [43], was derived from nonlinear fitting adapted from the method of Helms et al. (2008) [43]. The specific UV absorption coefficient of CDOM at 254 nm ($a_{\text{CDOM}}^*(254)$, $\text{L mg}^{-1}\text{ m}^{-1}$), an indicator of DOM aromatic-

ity [44], is calculated as the CDOM absorption coefficient at 254 nm ($a_{\text{CDOM}}(254)$) divided by DOC concentration.

Fluorescence excitation–emission matrices (EEMs) were acquired using a Hitachi F-4600 fluorescence spectrophotometer fitted with a 1 cm quartz cell to characterize FDOM [45,46]. Emission spectra were recorded at excitation wavelengths from 230 nm to 450 nm at 2 nm intervals and emission wavelengths from 450 nm to 600 nm at 5 nm increments. Blank Milli-Q water was measured intermittently during sample analysis and was subtracted to remove Raman scattering. The spectral fluorescence intensities were normalized to Raman Units (R.U.) following the Raman Scatter Peak correction reported by Lawaetz and Stedmon (2009) [47]. Potential inner-filtering effects were corrected using the obtained absorbance spectra [48]. PARAFAC modeling was performed to decompose the EEMs into a set of underlying fluorescent components [49,50]. The humification index (HIX) and biological index (BIX) were calculated as the ratio of the fluorescence intensity integrated over 435–480 nm to that over 300–345 nm with excitation at 254 nm [48], and the ratio of the fluorescence intensity at 380 nm to that at 430 nm with excitation at 310 nm [51], respectively. HIX and BIX serve as indicators of the degree of DOM humification and freshness, respectively [48,51,52].

3. Results

3.1. General Physical, Biological and Chemical Settings

The water column was strongly stratified during the first three cruises, while the stratification became much weaker during the last cruise on DOY 237 (Figure S1) due to the passage of Typhoon Rumbia [34]. The water temperature decreased while salinity increased with depth during all four cruises (Figure S1a–h), with the pycnocline located between 7 and 15 m depth [34]. In the entire water column, temperature increased from DOY 162 to DOY 220, while salinity decreased. During the first three cruises, the Chl *a* concentration ([Chl *a*]) peaked at 7–12 m (Figure S1i–k). To facilitate the investigation of DOM dynamics in the bottom water, the water column was separated into the surface layer (SL, depths < 15 m) and bottom layer (BL, depths ≥ 15 m) [34]. Thus, the SL included the surface mixed layer and the pycnocline. The transect-mean [Chl *a*] ([Chl *a*]_{T-M}) in the SL were $2.47 \pm 0.74 \mu\text{g L}^{-1}$ on DOY 162, $1.88 \pm 0.85 \mu\text{g L}^{-1}$ on DOY 200 and $2.02 \pm 0.63 \mu\text{g L}^{-1}$ on DOY 220, which were 1.7–3.7 times those in the BL (Figure S2). Owing to the strong typhoon-induced turbulent mixing on DOY 237, [Chl *a*] distribution in the water column was more homogenous (Figure S1), and [Chl *a*]_{T-M} in the SL was only 1.5 times that in the BL on that day (Figure S2). In the SL, DO was mainly controlled by seawater temperature and photosynthesis, while the transect-mean DO concentration ([DO]_{T-M}) in the BL linearly decreased from $270.1 \pm 7.3 \mu\text{mol L}^{-1}$ on DOY 162 to $150.0 \pm 4.9 \mu\text{mol L}^{-1}$ on DOY 220 [34]. The BL was thus experiencing substantial oxygen depletion during the sampling season.

3.2. Spatiotemporal Variations of DOM Quantity

Four fluorescent components (C1–C4) were identified using the PARAFAC model. C1 (250(215)/418 nm) was designated as humic-like FDOM, while C2 (230/344 nm), C3 (225(275)/324 nm) and C4 (295(245)/344 nm) as protein-like FDOM (Figure 2). Specifically, C1 was a mixture of peaks A and M, C2 and C4 were tryptophan-like component and C3 was tyrosine-like component [37]. To simplify discussion, C_P was used to represent protein-like FDOM, i.e., the sum of C2, C3 and C4.

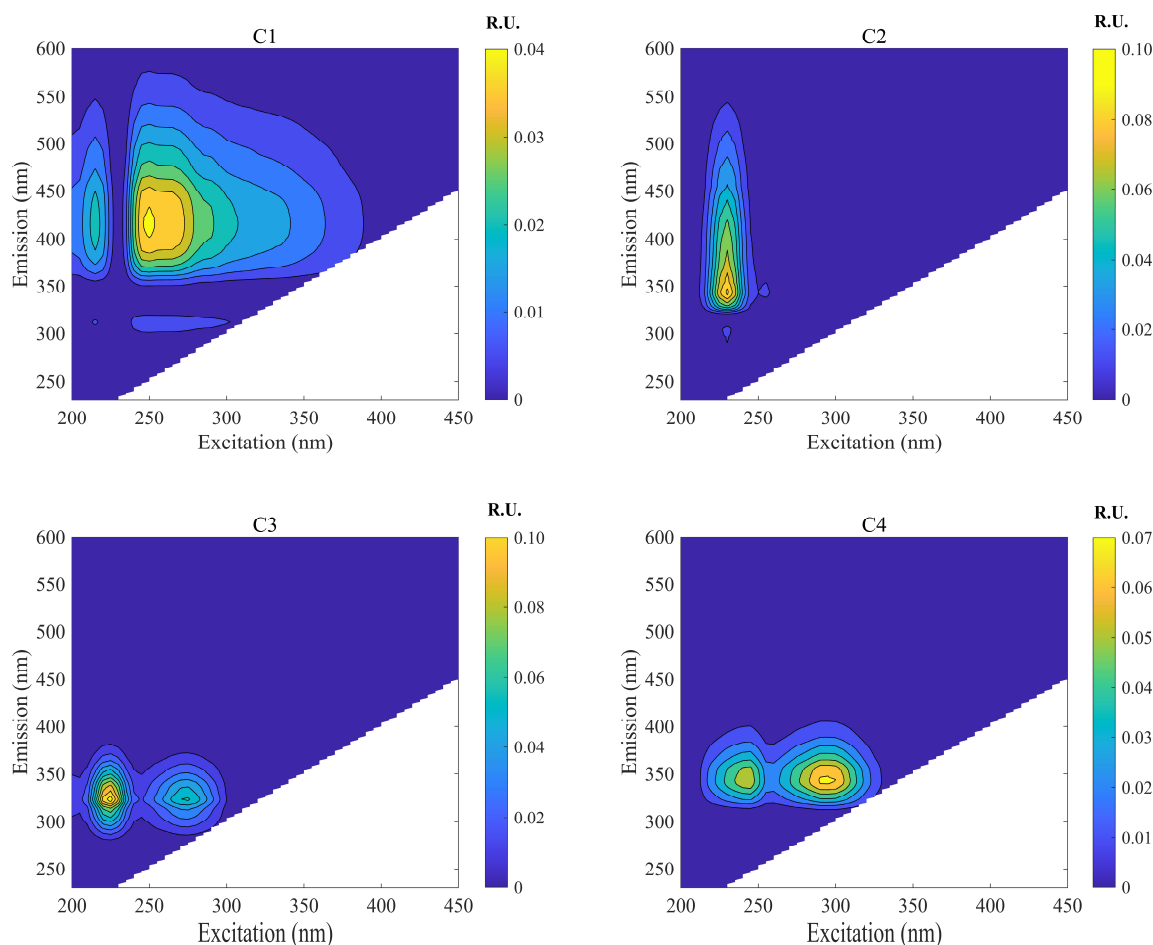


Figure 2. Excitation–emission contours of four components identified by PARAFAC model.

Overall, DOC concentration ($[\text{DOC}]$) decreased from the surface to the bottom and from inshore to offshore along the transect (Figure S3a–d). Notably, $[\text{DOC}]$ in the bottommost water (depth > 20 m) was significantly higher than that within the depth range of 15–20 m on DOY 220 (Figure S3c), suggesting that sediment release was an important source of DOC in bottom water during this period. Along the transect, $a_{\text{CDOM}}(330)$ generally decreased with depth and from inshore to offshore on DOYs 162 and 200, while the vertical trend inversed on DOYs 220 and 237 (Figure S3e–h), suggesting substantial photobleaching in the upper layer. Both C1 and C_P varied with depth, but no consistent vertical distribution pattern during the four cruises (Figure S3i–p).

The transect-mean DOC concentration ($[\text{DOC}]_{\text{T-M}}$) in the SL during the first three cruises was consistently higher than that in the BL (t -test, $p < 0.01$), while it was comparable between the SL and BL on DOY 237 (Figure 3a). The higher $[\text{DOC}]$ s in the SL were mainly located in the surface mixed layer (<7 m), and $[\text{DOC}]$ s within the pycnocline (7–15 m) and the depth range of 15–20 m were comparable (t -test, $p > 0.05$) (Figure S3a–d, Table S2). There was no obvious difference for the transect-mean $a_{\text{CDOM}}(330)$ ($a_{\text{CDOM}}(330)_{\text{T-M}}$) between the SL and BL on DOYs 162 and 237, but it was higher in the SL on DOY 200 ($p < 0.05$), and higher in the BL on DOY 220 ($p < 0.01$) (Figure 3b). No significant differences were found for the transect-mean abundances of the FDOM components ($C1_{\text{T-M}}$, $C_{P\text{T-M}}$) between the SL and BL during all four cruises, excluding DOY 200 on which $C_{P\text{T-M}}$ in the SL was 8% lower than that in the BL ($p < 0.05$) (Figure 3c,d).

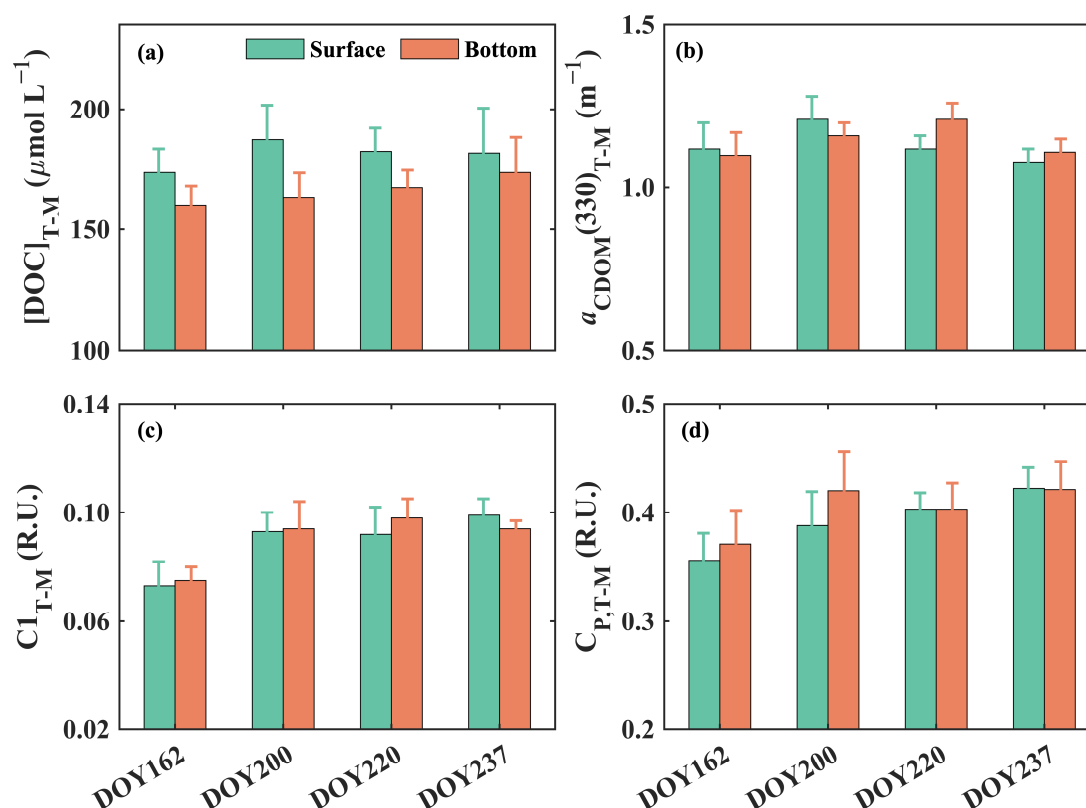


Figure 3. The $[\text{DOC}]_{\text{T-M}}$ (a), $a_{\text{CDOM}(330)}_{\text{T-M}}$ (b), $C1_{\text{T-M}}$ (c) and $C_{\text{P,T-M}}$ (d) in the SL and BL during each cruise. The error bars denote the one standard deviations.

In the SL, the $[\text{DOC}]_{\text{T-M}}$ significantly increased from $173.8 \pm 9.9 \mu\text{mol L}^{-1}$ on DOY 162 to $187.5 \pm 14.2 \mu\text{mol L}^{-1}$ on DOY 200 (ANOVA, $p < 0.05$), and then slightly decreased to $182.7 \pm 9.6 \mu\text{mol L}^{-1}$ on DOY 220, and $182.0 \pm 18.5 \mu\text{mol L}^{-1}$ on DOY 237 (ANOVA, $p > 0.05$) (Figure 3a). The temporal variation of $a_{\text{CDOM}(330)}_{\text{T-M}}$ in the SL was similar to that of $[\text{DOC}]_{\text{T-M}}$, increasing from $1.12 \pm 0.08 \text{ m}^{-1}$ on DOY 162 to $1.21 \pm 0.07 \text{ m}^{-1}$ on DOY 200 (t -test, $p < 0.05$) and then decreasing to $1.12 \pm 0.04 \text{ m}^{-1}$ on DOY 220 and $1.08 \pm 0.04 \text{ m}^{-1}$ on DOY 237 (ANOVA, $p < 0.05$) (Figure 3b). The increases in $[\text{DOC}]_{\text{T-M}}$ and $a_{\text{CDOM}(330)}_{\text{T-M}}$ from DOY 162 to DOY 200 could result from both riverine input [53,54] and in situ biological production [55,56], while photomineralization and photobleaching could be mainly responsible for the slight decrease in DOC and CDOM from DOY 200 to DOY 237. Unlike $[\text{DOC}]_{\text{T-M}}$ and $a_{\text{CDOM}(330)}_{\text{T-M}}$, $C1_{\text{T-M}}$ and $C_{\text{P,T-M}}$ generally increased from DOY 162 to DOY 237 (ANOVA, $p < 0.05$). In the BL, $[\text{DOC}]_{\text{T-M}}$ gradually increased from DOY 162 to DOY 237 (ANOVA, $p < 0.05$), while $a_{\text{CDOM}(330)}_{\text{T-M}}$ and $C1_{\text{T-M}}$ increased from DOY 162 to DOY 220 (ANOVA, $p < 0.05$) and then decreased on DOY 237. $C_{\text{P,T-M}}$ did not show consistent temporal trends in the BL (Figure 3d).

3.3. Spatiotemporal Variations of DOM Quality

Contrast to DOC, $a^*_{\text{CDOM}(254)}$ generally increased with depth and from inshore to off-shore (Figure S4a–d), and the sectional distribution of $S_{275-295}$ mirrored that of $a_{\text{CDOM}(330)}$ during all four cruises (Figure S4e–h). Like $C1$ and C_{P} , no consistent spatial and temporal distribution pattern for HIX and BIX along the transect (Figure S4i–p). The transect-mean $a^*_{\text{CDOM}(254)}$ ($a^*_{\text{CDOM}(254)}_{\text{T-M}}$) in the BL was consistently higher than that in the SL during the first three cruises but comparable between the two layers on DOY 237 (Figure 4a). Contrary to $a^*_{\text{CDOM}(254)}_{\text{T-M}}$, the transect-mean $S_{275-295}$ ($S_{275-295,\text{T-M}}$) in the BL was comparable to that in the SL on DOY 162 and DOY 237 ($p > 0.05$) but significantly lower than that in the SL on DOY 200 and DOY 220 ($p < 0.01$) (Figure 4b). The differences in the transect-mean

HIX ($\text{HIX}_{\text{T-M}}$) and BIX ($\text{BIX}_{\text{T-M}}$) between the two layers were not significant over the entire sampling period ($p > 0.05$), except for $\text{BIX}_{\text{T-M}}$ on DOY 162 on which $\text{BIX}_{\text{T-M}}$ in the BL was significantly higher than that in the SL ($p < 0.01$) (Figure 4c,d).

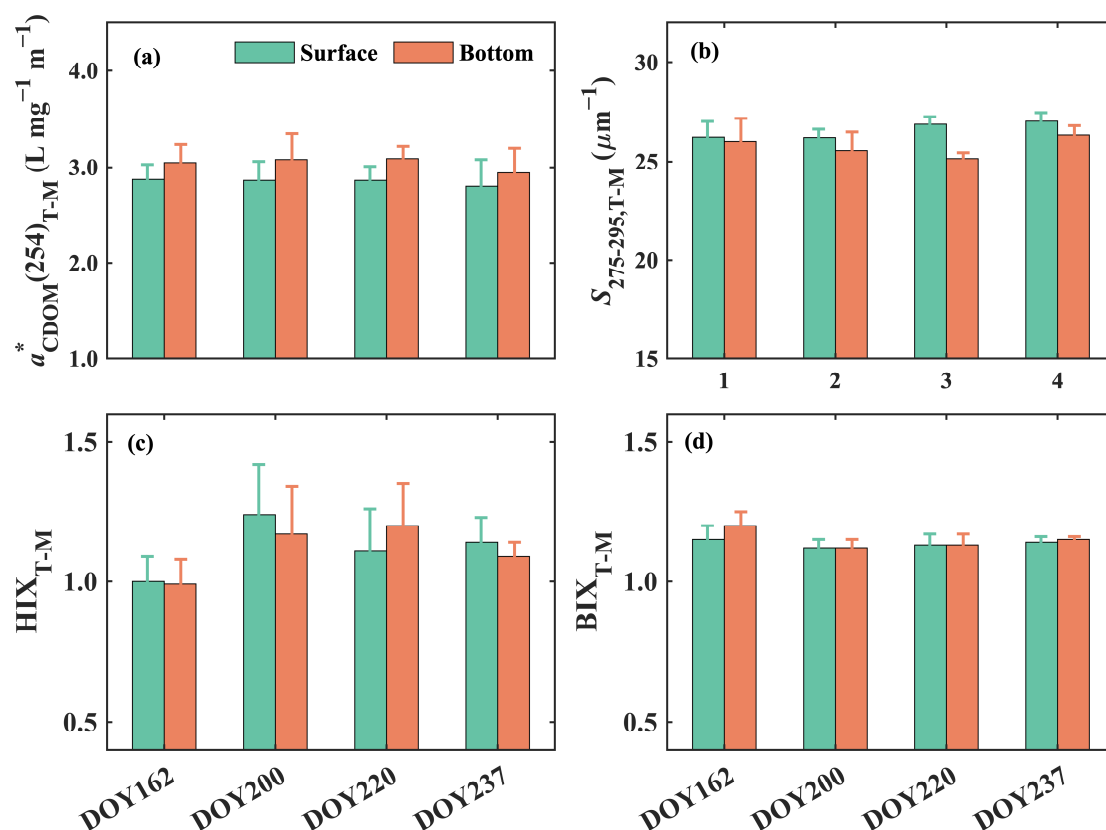


Figure 4. The $a^*_{\text{CDOM}(254)_{\text{T-M}}}$ (a), $S_{275-295, \text{T-M}}$ (b), $\text{HIX}_{\text{T-M}}$ (c) and $\text{BIX}_{\text{T-M}}$ (d) in the SL and BL during each cruise. The error bars denote the one standard deviation * denote significant difference between the SL and BL at $p < 0.05$.

Albeit not statistically significant (ANOVA, $p > 0.05$), $a^*_{\text{CDOM}(254)_{\text{T-M}}}$ gradually decreased from DOY 162 to DOY 237 in the SL, while $S_{275-295, \text{T-M}}$ increased. Meanwhile, $\text{HIX}_{\text{T-M}}$ in the SL fluctuated across the four cruises. $\text{BIX}_{\text{T-M}}$ in the SL kept relatively constant throughout the four cruises (1.14 ± 0.01). In the BL, $a^*_{\text{CDOM}(254)_{\text{T-M}}}$ gradually increased from $3.05 \pm 0.19 \text{ L mg}^{-1} \text{ m}^{-1}$ on DOY 162 to $3.09 \pm 0.13 \text{ L mg}^{-1} \text{ m}^{-1}$ on DOY 220 (though not significantly, ANOVA, $p > 0.05$) and then decreased to $2.94 \pm 0.26 \text{ L mg}^{-1} \text{ m}^{-1}$ on DOY 237 (ANOVA, $p < 0.05$). From DOY 162 to DOY 220, $S_{275-295, \text{T-M}}$ in the BL decreased consistently (though not significantly, ANOVA, $p > 0.05$), while $\text{HIX}_{\text{T-M}}$ increased significantly (ANOVA, $p < 0.05$). $\text{BIX}_{\text{T-M}}$ sharply decreased from 1.20 ± 0.05 on DOY 162 to 1.12 ± 0.03 on DOY 200 and then kept relatively constant, with the average value of 1.13 ± 0.02 (Figure 4d).

4. Discussion

4.1. DOM Sources in the BL

In the BL, DOC, CDOM and C1 increased from DOY 162 to 220 (Figure 3a–c). Possible pathways resulting in the accumulation of DOM in the BL included vertical diffusion from the SL, lateral transport, release from surface sediments and certain biological processes in the water column. From DOY 162 to DOY 220, $[\text{DOC}]_{\text{T-M}}$, $a_{\text{CDOM}(330)_{\text{T-M}}}$ and $\text{C1}_{\text{T-M}}$ within the pycnocline were comparable to or lower than those just beneath the pycnocline (Table S2), ruling out a significant contribution of DOM to the BL through vertical diffusion.

Consistent with the present study, DOC, CDOM and FDOM slightly decreased from inshore to offshore in summer, with the gradient parallel to the coastline [56,57]. Moreover, the direction of the residual current in the bottom layer in this season was northeast [36], almost perpendicular to the DOM gradient. Therefore, lateral transport could move DOM from the central Bohai Sea to this area and would have insignificant influence on DOM content in the BL, either. Photosynthetically driven DOM production in the BL was also likely negligible because of the shallow euphotic zone depth (<15.5 m) along the transect during summer [36], though the [Chl *a*] were still moderate in the BL (Figure S2). In conclusion, microbial degradation of POM and other biological processes in the water column [58], along with sediment release (see Section 3.2, Figure S3c,k), were likely the dominant processes controlling DOM accumulation in the BL.

A plausible sedimentary DOM release into the overlying water can be inferred from the observation on DOY 220 showing a higher [DOC] in the bottommost water than that in the overlying bottom water ($171.9 \pm 8.1 \mu\text{mol L}^{-1}$ vs. $165.8 \pm 5.8 \mu\text{mol L}^{-1}$) (Figure S3c), with DO in the bottom water reaching the minimum value of $151.0 \pm 4.9 \mu\text{mol L}^{-1}$ [34]. Previous studies demonstrated that the sediment-to-seawater flux of DOM is usually higher under hypoxic conditions than under oxic conditions [59,60] due mainly to the incomplete remineralization of sedimentary POM under oxygen deficiency. However, the exact contribution of sediments to DOM accumulation in the SL remains unknown, due to the lack of direct in situ measurements. Our earlier study indicates that bacterial respiration in surface sediments accounted for 19.2–34.6% of total oxygen uptake in the BL of sampling transect [34]. Notably, this contribution is not equivalent to that of DOM accumulation, since organic matter remineralization (i.e., production of CO_2) and nitrification can also consume oxygen. POM in surface sediments in the central Bohai Sea are mainly algal-derived [55], thus the characteristics of DOM released from surface sediments should be likely similar to those of DOM produced in the overlying water. Therefore, in this study, DOM accumulation from both pelagic and sedimentary POM is considered together.

4.2. Accumulation and Properties of DOM in the BL During Deoxygenation

Further analysis demonstrates that $[\text{DOC}]_{\text{T-M}}$, $a_{\text{CDOM}}(330)_{\text{T-M}}$, and $\text{C1}_{\text{T-M}}$ in the BL linearly increased from DOY 162 to DOY 220 (Figure 5). The passage of Typhoon Rumbia substantially weakened stratification and homogenized the water column (Figure S1), which strengthened the vertical mixing of DOM. Hence, DOM data collected on DOY 237 were excluded in this section. Notably, the results revealed a relatively weak linear accumulation of DOC with time in the BL, with a low coefficient of determination ($R^2 = 0.850$, $p = 0.12$, Figure 5b, Table 1). This is expected because DOC sources might be non-linear, including episodic sediment release and variable POM input. Our result is also consistent with that in the Black Sea, where DOC only increases slightly under anoxic conditions [12]. The estimated DOC accumulation rate (i.e., the slope of the linear fit) was $0.11 \pm 0.04 \mu\text{mol L}^{-1} \text{d}^{-1}$ (Table 1). The CO_2 production rate in the BL was previously estimated as $1.41 \pm 0.40 \mu\text{mol L}^{-1} \text{d}^{-1}$ [34]. Thus, the DOC/ CO_2 accumulation ratio is 0.08, at the lower bound of those observed in sediments in the East China Sea and Yellow Sea (0.11–0.72) [61]. The higher DOC/ CO_2 ratio in sediments has been ascribed to the anoxic conditions under which POC is prone to being transformed into DOC or methane, other than thoroughly remineralized to CO_2 [62]. Moreover, the freshly algal-derived POC in the present study (Section 4.1) could also somewhat lower the DOC/ CO_2 ratio, since DOC produced from fresher POC can be relatively more easily remineralized to CO_2 than DOC formed from aged sedimentary POC [19,63].

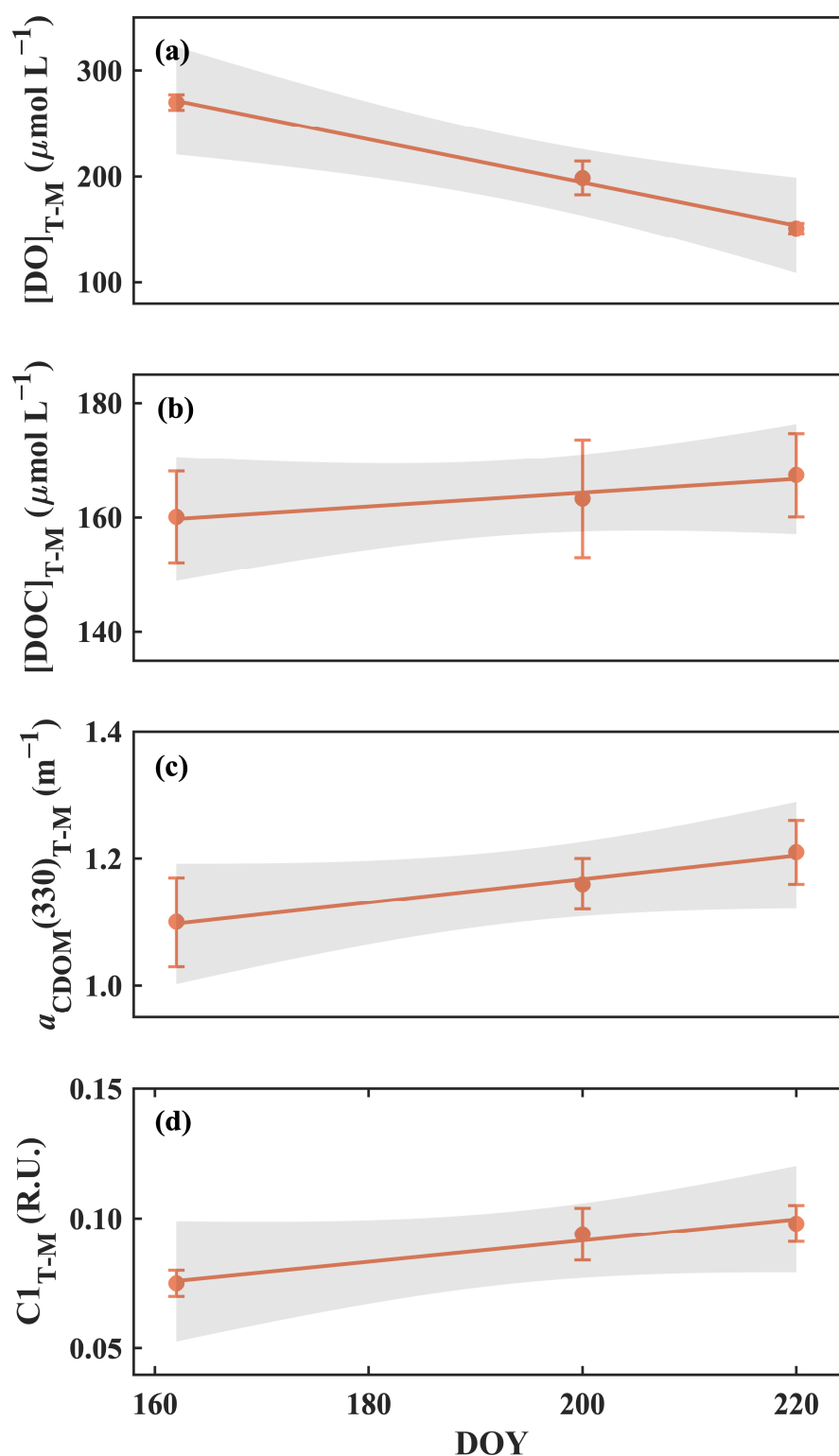


Figure 5. Temporal evolution of the [DO]_{T-M} (a), [DOC]_{T-M} (b), *a*_{CDOM(330)}_{T-M} (c) and C1_{T-M} (d) in the BL. The solid line in each panel is the linear fit to the data between DOY 162 and 220 and the grey band is the 95% confidence interval. The regression results were presented in Table 1.

Table 1. Results of linear regression ($Y = a \times X + b$) of DO, DOC, $a_{\text{CDOM}(330)}$ and C1 against DOY. SE denotes standard error.

	$a \pm \text{SE}$	$b \pm \text{SE}$	R^2	p
DO	-2.03 ± 0.12	600.6 ± 24.4	0.995	0.03
DOC	0.11 ± 0.04	140.6 ± 9.7	0.850	0.12
$a_{\text{CDOM}(330)}$	0.002 ± 0.0002	0.80 ± 0.05	0.985	0.04
C1	0.0004 ± 0.0001	0.009 ± 0.019	0.951	0.05

The estimated accumulation rates of $a_{\text{CDOM}(330)}$ and C1 were $0.002 \pm 0.0002 \text{ m}^{-1} \text{ d}^{-1}$ and $0.0004 \pm 0.0001 \text{ R.U. d}^{-1}$ (Table 1), respectively. Therefore, the $a_{\text{CDOM}(330)}/\text{DOC}$ and $\text{C1}/\text{DOC}$ ratios for the “new DOM” were $1.52 \text{ L mg}^{-1} \text{ m}^{-1}$ and $0.30 \text{ R.U. L mg}^{-1}$, respectively, which are 2.6 and 7.7 times the corresponding background values of $0.57 \text{ L mg}^{-1} \text{ m}^{-1}$ and $0.039 \text{ R.U. L mg}^{-1}$ on DOY 162. Meanwhile, the $\text{C1}/a_{\text{CDOM}(330)}$ ratio for this “new CDOM” (0.20 R.U. m) was 2.9 times the background value (0.07 R.U. m). Here, we define “new DOM” as DOM accumulated in the BL between DOY 162 and DOY 220. DOY 162 was chosen as the background, since previous study [34] suggested the water column starts to be stratified before DOY 150, and the saturation degree of DO in the BL becomes 100% around DOY 165, and after that DO gradually becomes unsaturated. The newly accumulated DOM by biological processes and sediment release during this period is thus enriched with chromophores and humic-like fluorophores (Figure 6), consistent with the finding in the anoxic water of the Black Sea [12]. The increase of $a^*_{\text{CDOM}(254)}_{\text{T-M}}$ and $\text{HIX}_{\text{T-M}}$ and decrease of $S_{275-295, \text{T-M}}$ in this period in the BL (Figure 4a–c) indicate this fraction of DOM possesses higher aromaticity [44], molecular weight [43] and humification degree [48,51].

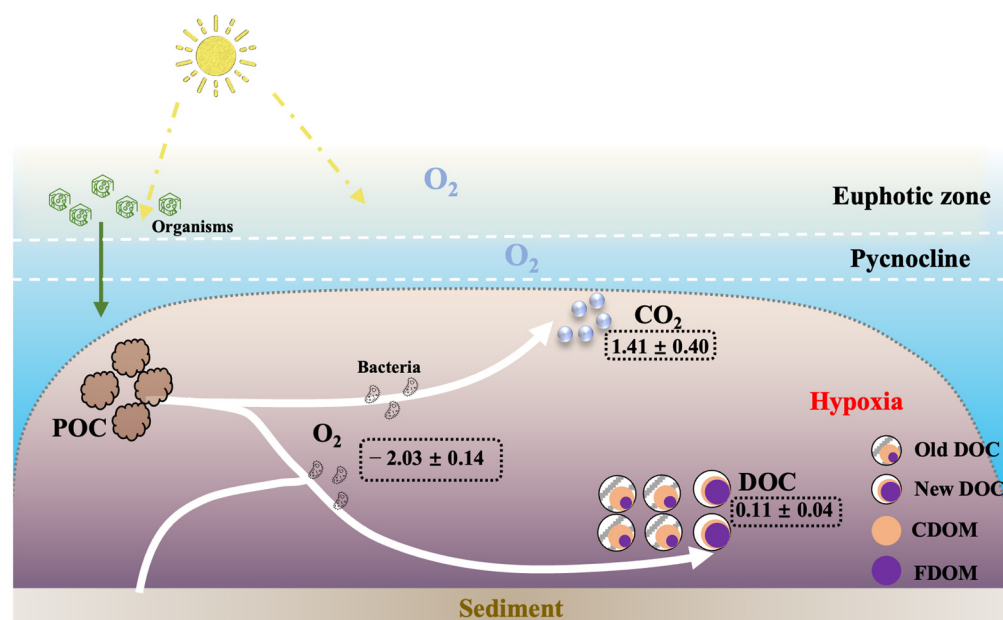


Figure 6. Conceptual diagram of DOC (i.e., DOM) and CO_2 accumulations during deoxygenation below the pycnocline in the Bohai Sea. The numbers in the dashed boxes denote the O_2 consumption rates (negative values) and DOC and CO_2 accumulation rates (positive values) (unit: $\mu\text{mol L}^{-1} \text{ d}^{-1}$). The “newly accumulated DOM” contains more CDOM and humic-like FDOM. The CO_2 production rate was from Song et al. (2020) [34].

The relative accumulation efficiency for a given DOM fraction (here, DOC, $a_{\text{CDOM}(330)}$, and C1) is defined as the ratio of its accumulation rate to the DO consumption rate. Table 2

summarizes the accumulation efficiencies obtained from this study and those previously reported for other ocean areas. The DOC accumulation efficiency in the Bohai Sea is 54.2×10^{-3} , 24 times that in the anoxic deep water in the Black Sea [12]. The accumulation efficiency of humic-like FDOM (i.e., FDOM component C1) in the Bohai Sea is 3–7.5 times that in the Black Sea and global oceans. Hence, relatively more DOC and FDOM are accumulated per unit of DO consumed in the Bohai Sea. The shallow and productive features could be responsible for the higher DOC and FDOM accumulation efficiencies, since high primary production in the SL, as indicated by the high [Chl *a*] (Figure S2), can continually supply plentiful labile POM to heterotrophic bacteria in the BL. Indeed, the mean POC concentration in the BL during the sampling period was $\sim 23 \mu\text{mol L}^{-1}$ (Song, unpublished data), which is ~ 5 – 10 times that in the anoxic deep water in the Black Sea (~ 2 – $5 \mu\text{mol L}^{-1}$) [64]. Unlike the DOC and humic-like FDOM accumulation efficiencies, the CDOM accumulation efficiency in the Bohai Sea is comparable to those in other regions (Table 2). The exact reasons for this discrepancy are unclear. It might be partly caused by different communities of heterotrophic bacteria responsible for transforming POM into DOM imposed by factors such as oxygen level and water temperature (anoxic and ~ 8.5 – 9.0 °C in the Black Sea vs. oxic and 9.4 – 23.3 °C in the Bohai Sea) [12,65].

Table 2. Accumulation efficiencies of DOC, CDOM and humic-like FDOM in terms of per unit DO consumed. The values from references are the linearly regressed slopes of DOC, CDOM and humic-like FDOM versus apparent oxygen utilization (AOU), except those from Margolin et al. (2018) [12], which are converted from apparent carbon mineralization (ACM) to AOU (ACM = AOU $\times$ 117/170). To compare our data with previous studies, a_{320} and a_{325} are also presented.

Region	Efficiency $\times 10^3$	References
DOC		
Bohai Sea	54.2	This study
Black Sea	2.22	Margolin et al., 2018 [12]
CDOM		
Bohai Sea	0.99 ($a_{\text{CDOM}}(330)$)	This study
Bohai Sea	0.64 ($a_{\text{CDOM}}(325)$)	This study
Bohai Sea	0.54 ($a_{\text{CDOM}}(320)$)	This study
Black Sea	0.36 ($a_{\text{CDOM}}(325)$)	Margolin et al., 2018 [12]
Central Pacific	0.53 ($a_{\text{CDOM}}(320)$)	Yamashita and Tanoue, 2009 [66]
Pacific	0.57 ($a_{\text{CDOM}}(325)$)	Nelson et al., 2010 [67]
Indian Ocean	0.63 ($a_{\text{CDOM}}(325)$)	Nelson et al., 2010 [67]
Global Ocean	0.60 ($a_{\text{CDOM}}(325)$)	Catalá et al., 2015 [68]
Humic-like FDOM		
Bohai Sea	0.20 (Ex/Em: 250(215)/418 nm)	This study
Black Sea	0.066 (Ex/Em: 310/413 nm)	Margolin et al., 2018 [12]
Global Ocean	0.035 (Ex/Em: 250(340)/435 nm)	Jørgensen et al., 2011 [69]
Global Ocean	0.027 (Ex/Em: 250(320)/395 nm)	Jørgensen et al., 2011 [69]

4.3. Significance to Carbon Cycling in the Bohai Sea

Primary production in the Bohai Sea during summer ranges from 200 to 620 mg C m⁻² d⁻¹ [70,71]. The depth-integrated DOC production rate in the BL (10 m thickness, [34]) during deoxygenation is calculated to be 13.2 mg C m⁻² d⁻¹, using the water volume-based rate of 0.11 $\mu\text{mol L}^{-1} \text{d}^{-1}$ obtained above. Therefore, the accumulation of DOC in the BL is equivalent to 2–6% of organic carbon produced during photosynthesis in the upper euphotic zone. If this DOC accumulation rate applies to the entire bottom hypoxic zone in the Bohai Sea, the total DOC accumulation in the hypoxic waters (DO < 3 mg L⁻¹) during deoxygenation (~ 60 days) is estimated as 0.6×10^9 – 3.3×10^9 g C, based on the reported area (756–4200 km²) and thickness (10 m) of the hypoxic waters [34,72,73]. The annual riverine input of DOC to the Bohai Sea has

been estimated to be 550×10^9 – 1118×10^9 g C [53,54]. Hence, the DOC accumulation in the hypoxic waters is <1% of the riverine input. However, ~70% of the riverine DOC is biolabile [54], which can be remineralized during its seaward transport. The accumulated DOC in hypoxic waters over two months is thus equivalent up to 2% of the refractory riverine DOC input to the Bohai Sea. Notably, the bottom oxygen deficiency in the Bohai Sea, in terms of both the spatial distribution and intensity, has significant interannual differences [74]. Furthermore, the Bohai Sea is also subject to significant interannual physical and biochemical variabilities [75]. Both of the above environmental factors may regulate the amount and composition of DOM during deoxygenation and hence influence the organic carbon budget and carbon cycling in the Bohai Sea.

The accumulation of CDOM and humic-like FDOM suggests this fraction of DOM is microbially refractory [76]. Therefore, a large part of the accumulated DOM during deoxygenation in coastal seas could reside in the bottom water for relatively longer time and could be upwelled to the euphotic zone during ventilation where it can be rapidly photodegraded due to its enrichment in CDOM and FDOM [39]. Alternatively, it can be transported into deep open oceans and preserved as microbially refractory DOM.

5. Conclusions

In the BL, DOC, CDOM and humic-like FDOM accumulated during deoxygenation from mid-June to early August, with net rates of $0.11 \pm 0.04 \mu\text{mol L}^{-1} \text{d}^{-1}$, $0.002 \pm 0.0002 \text{m}^{-1} \text{d}^{-1}$ and $0.0004 \pm 0.0001 \text{R.U. d}^{-1}$, respectively. Further analysis reveals that the newly accumulated DOM in the bottom water was enriched with CDOM and humic-like FDOM, which are often microbially refractory. The newly accumulated DOC in bottom water during deoxygenation is ~2–6% of primary production in the Bohai Sea in summer and up to 2% of the refractory fraction of the annual riverine DOC input to the Bohai Sea. Unlike deeper systems (>40 m), the shallow Bohai Sea exhibits faster surface–bottom coupling, resulting in markedly higher accumulation efficiencies of DOC and humic-like FDOM.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w18131579/s1>. Table S1: The coordinates, water depths and sampling dates of the stations. Numbers in the parentheses are the corresponding day of year (DOY); Table S2: Transect-mean DOC, $a_{\text{CDOM}}(330)$ and C1 within the pycnocline and the depth range of 16–20 m on DOY 162, 200 and 220; Figure S1: The sectional distributions of temperature (a–d), salinity (e–h) and Chl *a* (i–l) during each cruise; Figure S2: The transect-mean Chl *a* concentration ($[\text{Chl } a]_{\text{T-M}}$) in the surface (≤ 16 m) and bottom layers (>16 m) during each cruise (b). The error bars are one standard deviations; Figure S3: The sectional distributions of DOC (a–d), $a_{\text{CDOM}}(330)$ (e–h), C1 (i–l) and Cp (m–p) during each cruise; Figure S4: The sectional distributions of $a^*_{\text{CDOM}}(254)$ (a–d), $S_{275-295}$ (e–h), HIX (i–l) and BIX(m–p) during each cruise.

Author Contributions: G.S. and H.X. designed the research plan, L.Z. organized the cruises, G.S. and L.Z. led the sampling. G.S. analyzed the samples. W.Z. performed the PARAFAC modeling. G.S. and H.Z. processed the raw data. G.S. wrote the manuscript, and H.X. extensively revised it. All authors have read and agreed to the published version of the manuscript.

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