

# Characterization of chromophoric dissolved organic matter and relationships among PARAFAC components and water quality parameters in Heilongjiang, China

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**Abstract** Chromophoric dissolved organic matter (CDOM) is an important optically active substance that can transport nutrients and pollutants from terrestrial to aquatic systems. Additionally, it is used as a measure of water quality. To investigate the source and composition of CDOM, we used chemical and fluorescent analyses to characterize CDOM in Heilongjiang. The composition of CDOM can be investigated by excitation-emission matrix (EEM) fluorescence and parallel factor analysis (PARAFAC). PARAFAC identified four individual components that were attributed to microbial humic-like (C1) and terrestrial humic-like (C2–4) in water samples collected from the Heilongjiang River. The relationships between the maximum fluorescence intensities of the four PARAFAC components and the water quality parameters indicate that the dynamic of the four components is related to nutrients in the Heilongjiang River. The relationships between the fluorescence component C3 and the biochemical oxygen demand (BOD<sub>5</sub>) indicates that component C3 makes a great contribution to BOD<sub>5</sub> and it can be used as a carbon source for microbes in the Heilongjiang River. Furthermore, the relationships between component C3, the particulate organic carbon (POC), and the chemical oxygen demand (COD<sub>Mn</sub>) show that

component C3 and POC make great contributions to BOD<sub>5</sub> and COD<sub>Mn</sub>. The use of these indexes along with PARAFAC results would be of help to characterize the co-variation between the CDOM and water quality parameters in the Heilongjiang River.

**Keywords** CDOM · EEM · PARAFAC · Water quality parameters · Heilongjiang River

## Introduction

Nature river ecosystems serve as important water resources and natural bioreactors for the degradation of pollution and are also a significant source of drinking water. Polluted river ecosystems may hamper local economic development (Qin et al. 2010; Wang et al. 2014). Dissolved organic matter (DOM) can be found in all natural aquatic environments, which represent one of the largest pools of organic carbon on Earth (Hedges, 1992). It is the main source of energy (carbon) for heterotrophic bacteria (Carlson, 2002). Chromophoric DOM (CDOM) is defined as the component of the total DOM that absorbs light in the ultraviolet and visible range of the electromagnetic spectrum (Coble, 2007). CDOM is a complex mixture of organic compounds that originates from a wide range of sources such as the decomposition of bacteria, algae, or plants and their continuous transformation through photochemical and microbial processes that plays an important role in the global carbon cycle (Coble, 2007; Kowalczyk et al. 2013). CDOM, present as humic-like components and protein-like components, represents a large fraction of the dissolved organic material that contributes to light absorption and fluorescence by non-chlorophyllous material in coastal regions (Fellman et al. 2010). CDOM can also contribute to global warming through the mineralization of

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dissolved organic carbon (DOC) (Bertisson and Tranvik, 2000; Zhang et al. 2011). The mineralization of carbon-containing organic compounds always leads to  $\text{CO}_2$  and CO. CDOM can be used to discriminate and trace water masses (Coble et al. 1998) and estimate the concentrations of DOC and specific constituents (e.g., amino acids and lignin) (Guo et al. 2011).

The fluorescent characteristics of CDOM have been extensively investigated owing to the technological advances in fluorescence spectroscopy, especially the development of excitation-emission matrixes (EEMs) (Coble, 1996), and successfully applied to identifying terrestrial, marine, and anthropogenic components (Baker 2011). EEM offers several major advantages over single-scan methodologies such as providing new information regarding the fluorescence CDOM composition and source of a sample (Coble et al. 1993). However, to conquer the difficulty in identifying individual fluorescence components in water, simple and multivariate data analysis techniques such as principal component analysis (PCA) (Boehme et al. 2004) and parallel factor analysis (PARAFAC) have been used (Stedmon et al. 2003). EEMs can capture miscellaneous fluorophores present in CDOM, which can also identify different characteristics of CDOM with various sources and biochemical pathways (Hudson et al. 2007). EEMs combined with PARAFAC have been applied to trace photochemical and microbial reactions with organic matter (Stedmon and Markager 2005a; Singh et al. 2010). PARAFAC analysis can decompose fluorescence EEMs into independent groups of fluorescence components (He et al. 2013) and provide a unique solution to the DOM EEM dataset, and it is regarded as an important analytical tool to characterize the CDOM in complex coastal regimes (Singh et al. 2010). Two primary fluorescent groups have been identified in CDOM in natural waters: humic- and protein-like. Humic-like substances are complex mixtures containing aromatic and aliphatic compounds derived from the decay of organic matter, whereas protein-like substances have to do with high biological activity (Harvey and Boran 1985; Stedmon et al. 2003). The number of studies on CDOM using EEM-PARAFAC in estuarine environments is increasing (He et al., 2013; Guo et al., 2011). In the environment, the variable concentration and composition of CDOM have substantial effects on the health of ecosystems through processes such as nutrient availability (Boavida and Wetzel, 1998) and light attenuation (Markager and Vincent, 2000). In the Heilongjiang River, the large quantity of humic substances confers the black color to stream water and has a significant impact on the quality of the water and carbon cycling. It is therefore of paramount relevance to improve our understanding of fate of CDOM at the high concentration of humic substances river water.

Eutrophication owing to high concentrations of phosphorus and nitrogen is a serious environment problem in estuarine

systems in China. Previous studies have shown that the turnover of DOM is proposed to occur with dissolved phosphorus and nitrogen indexes (Hopkinson et al. 2002; Kragh and SØndergaard, 2004). Qualls and Haines (1991) considered that the DOM plays an important role in terrestrial and stream ecosystems owing to its being a major mode of export of phosphorus and nitrogen. Therefore, characterizing the relationships between DOM and nutrients may provide an effective management of water quality and control of eutrophication. Heilongjiang is a large complex estuarine system located in the northeast of China that directly flows into the west bank of the Pacific. There are large quantities of humics in the water of the Heilongjiang River. In the southwest of the Heilongjiang River, there are many mountains from which runoff flows into Heilongjiang carrying a large amount of DOM. Therefore, the Heilongjiang receives substantial terrestrial deliveries of DOM. The CDOM fraction of the terrestrial deliveries confers a brown color to the water, often referred to as “browning,” and it plays a central role in the carbon cycle in Heilongjiang. Furthermore, the organic pollution indicators of biochemical oxygen demand ( $\text{BOD}_5$ ) and chemical oxygen demand ( $\text{COD}_{\text{Mn}}$ ) are at a high level in some areas of Heilongjiang. Therefore, there is a need to characterize the properties of DOM to identify the influencing indicators of water quality in the Heilongjiang River. However, to our knowledge, there have not been any detailed studies characterizing the concentration, composition, source, and properties of CDOM, despite the fact that it is an important biogeochemical factor for the carbon cycle in the Heilongjiang River. Therefore, further work is needed to characterize the CDOM in the Heilongjiang River.

In the present study, we used EEM fluorescence measurements to characterize the distribution of the CDOM concentration, composition, and sources in the Heilongjiang River. Moreover, examining the relationships between different fluorescence components and water quality parameters, we sought to understand (1) the characterization of CDOM in the Heilongjiang River, (2) the influencing factors of  $\text{BOD}_5$  and  $\text{COD}_{\text{Mn}}$ , and (3) the co-variation between CDOM and nutrients.

## Methods

### Sampling strategy

The Heilongjiang originates from the Hailar River and its stream length is up to 4370 km. It flows through China, Russia, and Mongolia, with its watershed area in China accounting for approximately 48 % of the whole basin area. The Greater Khingan Mountains, Lesser Khingan Mountains, and Changbai Mountains are along the Heilongjiang from northeast to southwest. Surface water samples were collected from

eight tributaries of the Heilongjiang River located in northeast China. The sampling areas are as follows: EER (121° 28' 59.8" E, 53°19' 55.1" N); LGR (121° 35' 24.2" E, 53° 21' 08.2" N); HMSR (126° 39' 59.1" E, 51° 44' 07.5" N); HMR (126° 36' 27.2" E, 51° 39' 54" N); HR (127° 31' 23.8" E; 50° 05' 28.7" N); MR (131° 03' 09" E, 47° 41' 12.6" N); TJR (132° 39' 51.9" E, 47° 57' 18.7" N); WSR (134° 40' 2.8" E, 48° 13' 55.8" N) (Fig. 1).

Water samples were collected using acid-cleaned Niskin bottles in June 2014 throughout these eight areas. We chose 20 stations to collect samples. After the sample collections, they were held on ice in the field (without being frozen) (Fellman et al. 2008) and immediately sent to the laboratory in darkness. Some water samples were filtered using pre-rinsed 0.45- $\mu\text{m}$  nucleopore membrane filters for DOC, phosphorus, nitrogen, chlorophyll (Chl-a), and fluorescence analyses. The remaining water samples were used for the measurement of  $\text{COD}_{\text{Mn}}$ ,  $\text{BOD}_5$ , and particulate organic carbon (POC). The water samples were stored in a refrigerator at 4 °C, and all of the measurements were made in the laboratory within 3 days after collection.

## Physico-chemical analyses

### Analysis of DOC

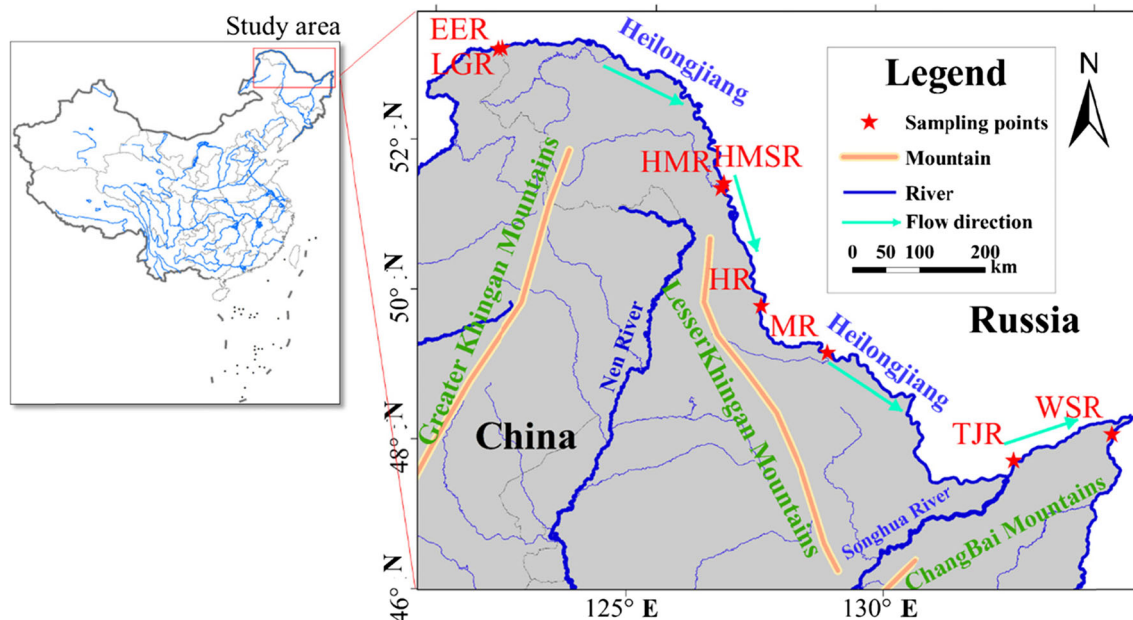
DOC was analyzed by using a SHIMADZU TOC-Vcph analyzer. First, the machine gives the total amount of carbon (TC) in the sample by burning it and the resulting  $\text{CO}_2$  is detected by a gas analyzer. Phosphoric acid is then added into the sample to obtain the inorganic carbon (IC). Finally, the subtraction of the IC from TC produces the DOC.

### Analysis of $\text{COD}_{\text{Mn}}$ and $\text{BOD}_5$

Titration with acidic potassium permanganate was used for the determination of  $\text{COD}_{\text{Mn}}$ , which is the equivalent amount of oxygen needed to decompose organic substances present in a sample when treated with acidic potassium permanganate. The measurement of  $\text{COD}_{\text{Mn}}$  was all based on the procedures from "Monitoring and Analytical Method of Water and Waste Water" (State Environment Protection Administration of China 2002). The samples were carried without attenuation to test  $\text{BOD}_5$  using a HACH BODTrak analyzer.

### Analysis of total dissolved phosphorus, dissolved reactive phosphorus, ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen

Total dissolved phosphorus (TDP) was determined by the method of phosphorus persulfate digestion. The analytical determination of dissolved reactive phosphorus (DRP) was based on the phosphomolybdic acid methodology, as modified by Murphy and Riley (1962). The method of Nessler's reagent colorimetry was used to measure the ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ) water quality (MEP China Ministry of Environmental Protection of the People's Republic of China 2009). Ultraviolet spectrophotometric method was used for determining nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ), and nitrite nitrogen ( $\text{NO}_2^-\text{-N}$ ) was measured by the method of N-(1-naphthyl) ethylenediamine spectrophotometry. These analytical methods were based on the procedures from "Monitoring and Analytical Method of Water and Waste Water" (State Environment Protection Administration of China 2002).



**Fig. 1** Location of the Heilongjiang River in China and the distribution of sampling sites

*Analysis of POC and Chl-a*

The filters for particulate organic carbon (POC) were dried for 24 h at 50 °C and stored in a vacuum dryer until analysis. All of the glassware was washed with 1 M HCl and ultrapure water and combusted at 450 °C for 6 h. All the filtration samples were washed with 1 M HCl to remove carbonate. POC was analyzed by a carbon/sulfur analyzer. To measure

Chl-a, the water samples were filtered using pre-rinsed 0.45-µm nucleopore membrane filters, and the filters were dried at a low temperature. After that, the filters were thoroughly dissolved in 90 % acetone solution. The extractions were centrifuged at a speed of 3000 r/min. Finally, the absorbance of the supernatant was measured at wavelengths of 750, 663, 645, and 630 nm. The formula for Chl-a was as follows:

$$\text{Chl-a}(\text{mg/m}^3) = \frac{[11.64 \times (D_{663}-D_{750})-2.16 \times (D_{645}-D_{745}) + 0.10 \times (D_{630}-D_{750}) \times V_1]}{V \times \delta}$$

where *V* is the volume of the water sample (L), *D* is the absorbance value, *V*<sub>1</sub> is the volume of the supernatant (mL), and *δ* is the path length of the cuvette (cm). The method of measuring Chl-a is based on the procedures for “Monitoring and Analytical Method of Water and Waste Water” (State Environment Protection Administration of China 2002).

**Fluorescence spectroscopy**

The EEMs were measured using a PerkinElmer LS50B fluorescence spectrophotometer with a clear quartz cuvette at room temperature. Filtered water samples were irradiated with excitation wavelengths from 200 to 490 nm at 5-nm intervals, emission wavelengths were recorded from 250 to 550 nm at 2-nm intervals, and the scan speed was adjusted to 1200 nm min<sup>-1</sup>. The methods of correcting and standardizing the EEMs were as follows (Stedmon and Bro, 2008; McKnight et al., 2001; Murphy, et al., 2013): (1) The instrument was corrected according to the manufacturer’s instructions before analyzing the samples. (2) To eliminate the inner-filter effect, the EEMs were corrected for absorbance by the multiplication of each value in the EEMs with a correction factor, based on the premise that the average path length of the absorption of excitation and emission light was one half of the cuvette length. The correction is expressed mathematically as  $F_{\text{IFE}} = F_{\text{Corr}} \times 10^{(A_{\text{Ex}} + A_{\text{Em}})/2}$ , where *F*<sub>IFE</sub> and *F*<sub>Corr</sub> are the corrected and uncorrected fluorescence intensities, respectively, and *A*<sub>Ex</sub> and *A*<sub>Em</sub> are the absorbance values at the current emission wavelengths. (3) Subsequently, Milli-Q water blank EEMs were subtracted from the sample EEMs to eliminate the Raman peaks, and then the EEMs were normalized to the daily determined water Raman-integrated area maximum fluorescence intensity (350<sub>Ex</sub>/397<sub>Em</sub>). (4) The Rayleigh and Raman scattering peaks of each scan centered on the respective scatter peaks were removed and then the excised data

were replaced with interpolated data using the Delaunay triangulation method by FLToolbox developed by Wade Sheldon (University of Georgia) for MATLAB. The fluorescence intensities measured were reported in Raman units (R.U.) in this study.

**PARAFAC analysis**

A total of 160 EEMs of water samples from the Heilongjiang River were analyzed by PARAFAC. The PARAFAC analysis was carried out in MATLAB with the DOM Fluor toolbox, following the procedure described by Stedmon and Bro (2008). For the PARAFAC modeling, excitation wave-lengths from 200 to 220 nm were deleted from each EEM because of random data fluctuations. PARAFAC statistically decomposes the DOM three-way data into individual fluorescence components with regard to their spectral shape or number (Stedmon et al. 2003). The model was run with non-negativity constraints applied to each dimension (Murphy, et al. 2013; Stedmon and Bro, 2008). An initial exploratory analysis was performed in which outliers were identified and removed from the dataset. No samples were considered outliers. Two to seven components were computed for the EEMs. The determination of the number of components was primarily based on split-half validation and the analysis of the residuals of residual loadings (Stedmon and Bro, 2008). The concentration scores of the PARAFAC components were expressed as maximum fluorescence intensities (*F*<sub>max</sub>) (R.U.) for each modeled component.

**Statistical analysis**

Correlation analysis and *t* test were conducted using SPSS version 22. Origin Pro 9.1 was also used to analyze the relationships between fluorescence components.

## Results

### PARAFAC model results

The combined data set was split into two random halves called “Half1” and “Half2” to perform the split-half validation procedure. PARAFAC models were run for each of the Half1 and Half2 data groups. The 4-component model was successfully validated in three independent data sets to ensure the correctness of the modeled CDOM components by the PARAFAC model using the split-half validation procedure. The EEM spectra and excitation and emission loadings of the four components are shown in Table 1. Table 2 provides the excitation and emission characteristics of the CDOM components with identified and examples of matching components identified by other researchers who modeled CDOM EEMs in various marine, oceanic, and estuarine environments. Although four individual components were determined for this dataset using the PARAFAC model (Table 1), our results do not suggest that only four types of fluorophores were present in all samples or that all four components were present in the majority of the transect samples (Table 1). Other fluorescent groups are certainly present, but their influence is so weak that they cannot be distinguished from the noise. Therefore, these four fluorescence components could explain most of the variation. All components contained multiple peaks, except for component C3. Generally, these four components had single emission maxima.

Component C1 is composed of two peaks with excitation maxima at 240 and 320 nm with one emission peak centered at 390 nm (Table 1). These fluorescence characteristics could be categorized as the previously defined humic-like peak M (Coble, 1996). The spectral features are similar to other reported microbial humic-like components and have a lower molecular weight (Stedmon et al. 2005b; Williams et al. 2010; Yamashita et al. 2008).

Component C2 is also composed of two peaks with excitation maxima at 240 and 340 nm, and emission maxima were observed in the EEMs at 436 nm, although they were red-shifted compared to C1 (Table 1). This component was characterized as a mixture of the traditional terrestrial humic-like peaks A and C according to Coble (1996). Previous studies have demonstrated that this component is derived from terrestrial plant or soil organic compounds (Fellman et al. 2010).

Component C3 exhibits a single excitation/emission wavelength pair of <240/448 nm (Table 1) and it is typically categorized as a terrestrial humic-like component (Yamashita et al. 2008; Kowalczyk et al. 2009; Mueller et al. 2012). This component has shown to be a dominant DOM exported from the catchments during the warmer months of the year and presents its highest concentrations in forest stream and wetlands (Stedmon et al. 2003; Stedmon and Markager, 2005a).

Component C4 is composed of two peaks with excitation maxima at 280 and 400 nm and one emission peak centered at 480 nm (Table 1), which could be categorized as the previously defined peaks A and C (Coble, 1996). This component represents fluorophores that have the longest excitation wavelength and broadest excitation band, as well as the longest emission wavelength associated with a broad emission band. Such excitation and emission characteristics are associated with terrestrial organic matter that is composed of large molecular weight and aromatic organic compounds (Stedmon and Markager 2005b; Kowalczyk et al. 2013). In the Heilongjiang River, we did not detect protein-like fluorescence, and the contributions of components C1–4 were all characterized as humic-like substances.

### Distribution of PARAFAC components

The spatial variations of the percentage distribution of the four PARAFAC components of each study area are shown in Fig. 2. The percent distributions of C–4 in the Heilongjiang River range from 41.8 to 53.0, 16.9 to 24.1, 11.1 to 23.1, and 14.0 to 18.5 %, respectively (Fig. 2). Component C1 generally had the highest relative distribution in the Heilongjiang River at each sample site. The high intensity of C1 indicates the impact of this type of organic matter on the Heilongjiang River. The spatial trends of the relative distribution of %C1 and %C4 were generally similar, with the highest values in HR and the lowest values in WSR. There were no significant differences in the relative contribution of C2 in most of the study areas. As for the terrestrial component C3, its spatial trend of the relative distribution was opposite from that of C1, with the highest values in WSR and the lowest values in HR (Fig. 2). Figure 3 shows the plots of components against each other for the data set and the variability in the  $F_{\max}$  of the fluorescence components. C1, C2, and C4 were almost perfectly linearly correlated ( $p < 0.001$ ), but C3 was not correlated with the other three components.

### Spatial distribution of water quality parameters

The mean values of the physico-chemical parameters in each study area are presented in Fig. 4. The mean values of DOC and  $\text{COD}_{\text{Mn}}$  ranged from a minimum of  $16.64 \pm 8.83$  and  $7.10 \pm 1.17$  mg/L in HMR to a maximum of  $33.73 \pm 8.76$  and  $27.60 \pm 6.80$  mg/L in HR, respectively. The mean values of the POC concentration ranged from a minimum of  $5.45 \pm 1.91$  mg/L in HMSR to a maximum of  $40.70 \pm 16.64$  mg/L in WSR. The mean  $\text{BOD}_5$  concentrations ranged from a minimum of  $0.157 \pm 0.124$  mg/L in EER to a maximum of  $2.390 \pm 0.457$  mg/L in MR. The spatial trends of  $\text{BOD}_5$  were similar to those of  $\text{COD}_{\text{Mn}}$ , with high values in HR and downstream (MR, TJR, and WSR) and low values in HMR and upstream (EER, LGR, and HMSR). The mean values of TDP ranged

**Table 1** PARAFAC model output showing fluorescence signatures of the four fluorescence components. The contour plots above present the spectral shapes of the excitation and emission of the four components. The line plots present split-half validation results of the four components;

excitation (left) and emission (right) spectra were estimated from the two independent halves of the dataset (red and green lines) and the complete dataset (black lines)

Component Label (this study)	Approximate EEM location	EEM	Spectral Loadings
C1	Ex: 240, 320 Em:390		
C2	Ex: 240, 340 Em:435		
C3	Ex: <240 Em:450		
C4	Ex: 280, 400 Em:480		

**Table 2** Spectral characteristics of excitation and emission maxima of the four components identified by PARAFAC modeling for the whole EEMs data set collected in the Heilongjiang watershed compared to previously identified sources

Fluorescent component	Peak position $\lambda_{Ex}/\lambda_{Em}$ (nm)	Description and probable source	Reference
C1	240, 320/390	Microbial humic-like substances	Microbial component 3 = 295/398 <sup>c</sup> Component 6 = <260 (325)/385 <sup>d</sup> Component 1 = (240) 320/396 <sup>f</sup>
C2	240, 340/435	Terrestrial humic-like substances	Component 4 = <250 (360)/440 <sup>b</sup> Component 5 = 345/434 <sup>c</sup> Component 8 = <260 (355)/434 <sup>g</sup>
C3	<240/450	Terrestrial humic-like substances	Terrestrial component 1 = <240/436 <sup>a</sup> Component 1 = <260/458 <sup>d</sup> Component 2 = 250/420 <sup>e</sup>
C4	280, 400/480	Terrestrial humic-like substances	Component 3 = (270) 360/478 <sup>a</sup> Component 3 = (275) 390/479 <sup>d</sup> Component 4 = (270) 390/508 <sup>f</sup>

<sup>a</sup> Stedmon et al. (2003)

<sup>b</sup> Stedmon et al. (Stedmon and Markager 2005a)

<sup>c</sup> Stedmon et al. (Stedmon and Markager 2005b)

<sup>d</sup> Yamashita et al. (2008)

<sup>e</sup> Kowalczyk et al. (2009)

<sup>f</sup> Kowalczyk et al. (2013)

<sup>g</sup> Murphy et al. (2008)

from a minimum of  $0.056 \pm 0.016$  mg/L in LGR to a maximum of  $0.086 \pm 0.032$  mg/L in MR. The mean DRP concentrations ranged from a minimum of  $0.017 \pm 0.006$  mg/L in LGR to a maximum of  $0.031 \pm 0.008$  mg/L in TJR. The mean values of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  ranged from a minimum of  $0.134 \pm 0.151$  and  $0.063 \pm 0.078$  mg/L in HMSR to a maximum of  $0.562 \pm 0.249$  and  $0.563 \pm 0.58$  mg/L in TJR.  $\text{NO}_2^-\text{-N}$  ranged from  $0.004 \pm 0.004$  mg/L in MR to  $0.012 \pm 0.066$  mg/L in TJR.

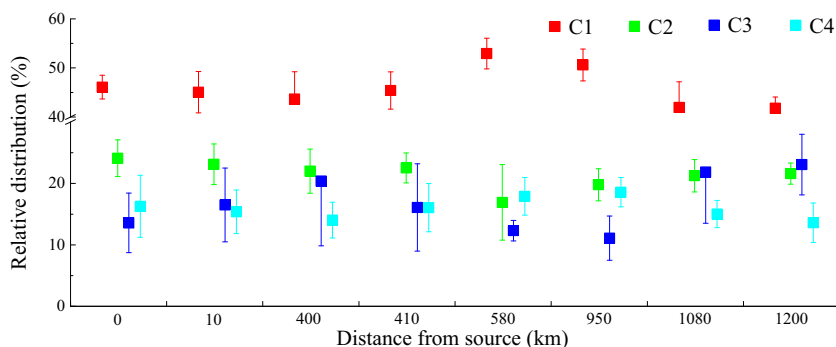
### Correlation among PARAFAC components and water quality parameters

$\text{COD}_{\text{Mn}}$  and  $\text{BOD}_5$  were used as basic water quality indexes to estimate the organic matter in the Heilongjiang River.  $\text{COD}_{\text{Mn}}$  was significantly correlated with POC, and fluorescence

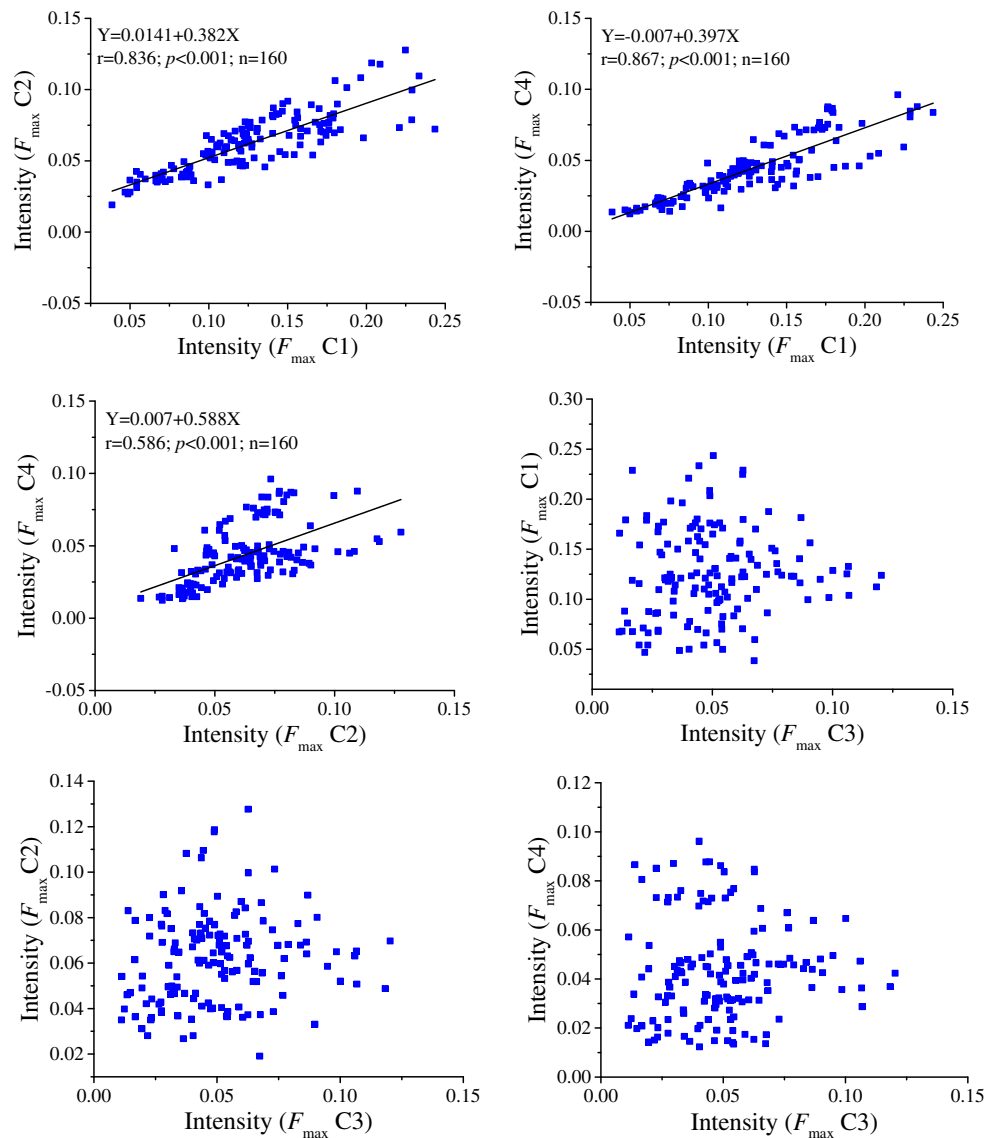
component C3, and the presence of  $\text{BOD}_5$  was complemented by the increased POC and fluorescence component C3 (Fig. 5), which suggests that POC and fluorescence component C3 made a great contribution to the  $\text{COD}_{\text{Mn}}$  in the Heilongjiang River.

As shown in Fig. 6, the fluorescence components were significantly correlated with the selected water quality parameters. Significant relationships were found between  $\text{NH}_4^+\text{-N}$ , DRP, Chl-a, and component C1 ( $r=0.273$ ,  $-0.375$ , and  $0.515$ ;  $p<0.001$ ,  $p<0.001$ , and  $p<0.001$ ;  $n=160$ ) (Fig. 6 a–c). Component C2 was highly correlated with DRP and Chl-a ( $r=0.290$  and  $0.431$ ;  $p<0.001$  and  $p<0.001$ ;  $n=160$ ) (Fig. 6d, e). Furthermore, significant linear relationships were found between POC,  $\text{NO}_2^-\text{-N}$ , TDP, Chl-a, and fluorescence component C3 ( $r=0.213$ ,  $0.200$ ,  $0.183$ , and  $-0.179$ ; and  $p<0.01$ ,  $p<0.05$ ,  $p<0.05$ , and  $p<0.01$ ;  $n=160$ )

**Fig. 2** Changes in the relative distributions (%) of the four PARAFAC-modeled CDOM components. Note: the sample sites from upstream to downstream are EER, LGR, HMSR, HMR, HR, MR, TGR, and WSR, respectively



**Fig. 3** Relationships between the four fluorescence components



(Fig. 5f–i), and component C4 was significantly correlated with  $\text{NH}_4^+\text{-N}$ , DRP, and Chl-a ( $r=0.289$ ,  $-0.322$ , and  $0.474$ ;  $p<0.001$ ,  $p<0.001$ , and  $p<0.001$ ;  $n=160$ ) (Fig. 6j–l).

**Discussion**

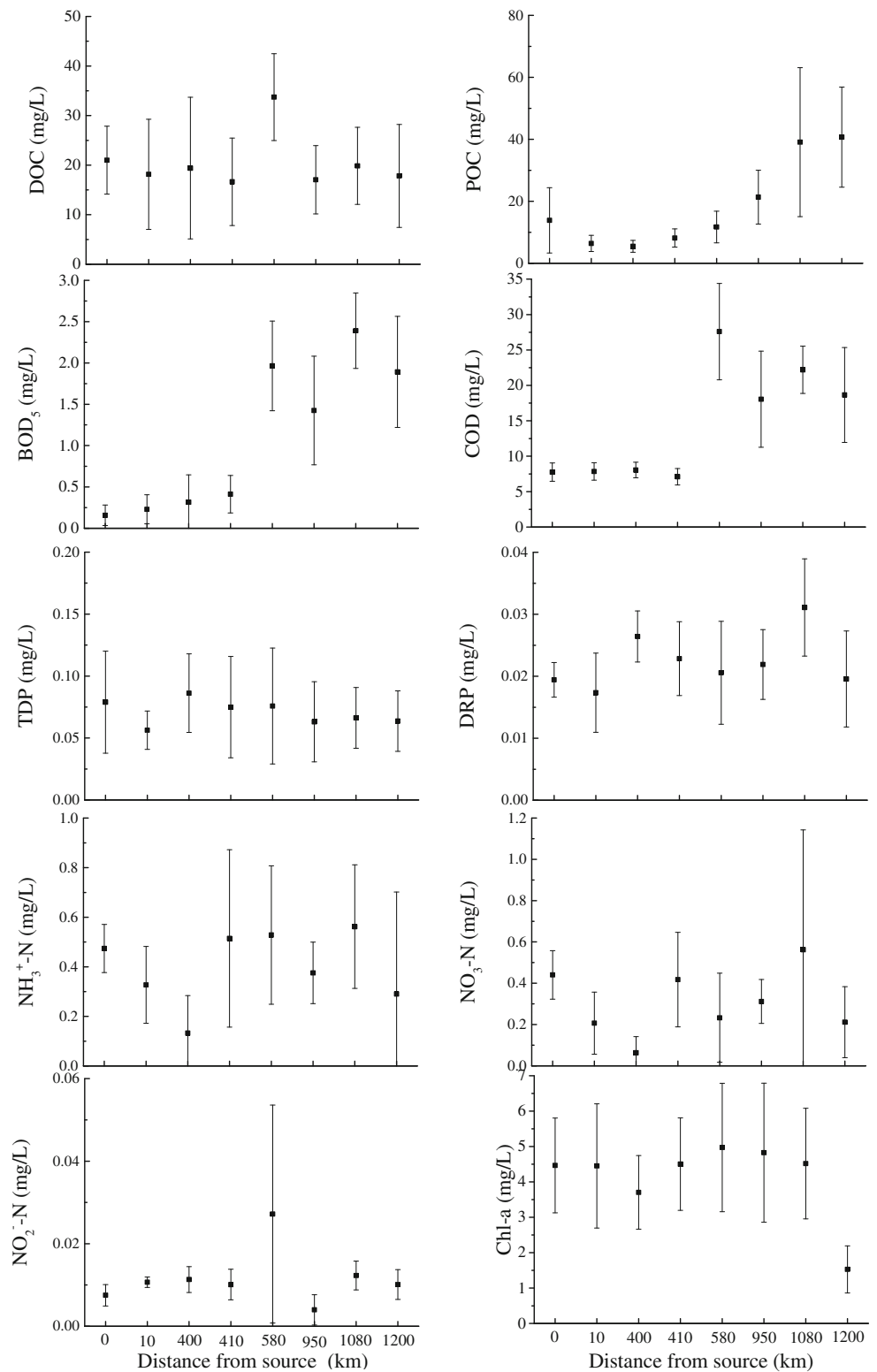
**Characteristic of CDOM in the Heilongjiang River**

In this study, all of the fluorescence components were assigned as humic-like material, but the spectral characteristics of the protein-like fluorophore were not detected in Heilongjiang (Table 2). The spectral characteristics of the excitation and emission of these four components presented in this study fit well with the components previously identified

by other authors and reported in the literature (Table 2). In some other inland water studies such as at White Clay Creek (Cory and Kaplan, 2012), Xinan River (Liu et al. 2014), Taihu Lake (Zhang et al. 2014), and Yangtze River (Wang et al. 2015), protein-like components were detected, but in the Heilongjiang River, protein-like components were not detected. The absence of protein-like components has seldom been noted in other lake and river water studies. The protein-like component is strongly associated with bioavailable and liable organic substrates as well as degraded peptide material (Hudson et al., 2008). Compared with humic-like components, protein-like fluorescence components are more likely used by microbes as an energy source. In a previous study, the protein-like component was used as an indicator of organic pollution (Baker and Inverarity 2004; Hur and Cho, 2012) derived from anthropogenic activities. Hence, the absence of



**Fig. 4** Spatial variations of DOC, POC, BOD<sub>5</sub>, COD<sub>Mn</sub>, TDP, DRP, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N, and Chl-a in different study regions. Note: the sample sites from upstream to downstream are EER, LGR, HMSR, HMR, HR, MR, TGR, and WSR, respectively

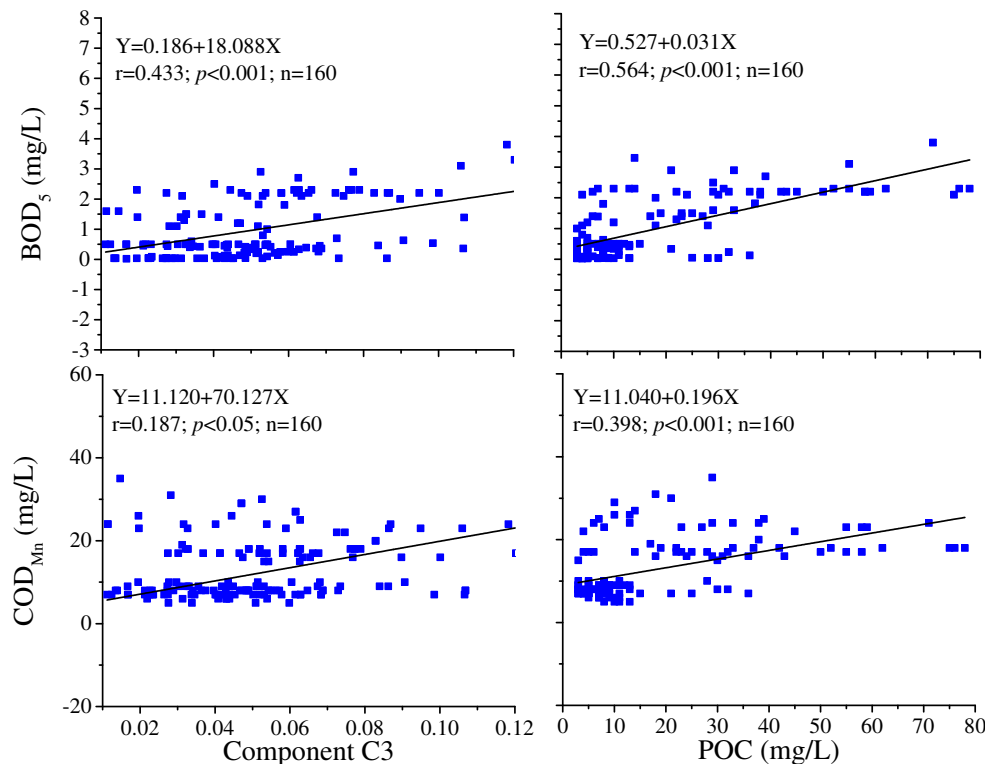


the protein-like fluorescence component in the Heilongjiang River indicates that the water quality of the Heilongjiang was not significantly influenced by anthropogenic activities.

Four components were identified by the PARAFAC modeling of the CDOM fluorescence spectroscopic data from the

Heilongjiang River. The microbial humic-like component C1 generally had the highest relative distribution (approximately 50 %), higher than those of terrestrial humic-like components C2–4 in each sample site. This result is similar to that in Tainmuhu Lake (Zhang et al. 2011), suggesting that microbial

**Fig. 5** Correlation between BOD<sub>5</sub>, COD<sub>Mn</sub>, and organic carbon (component C3, and POC). The unit of fluorescence intensity is calibrated and normalized in Raman Units (R.U.), and the sample numbers is 160. Note: the sample sites from upstream to downstream are EER, LGR, HMSR, HMR, HR, MR, TGR, and WSR, respectively

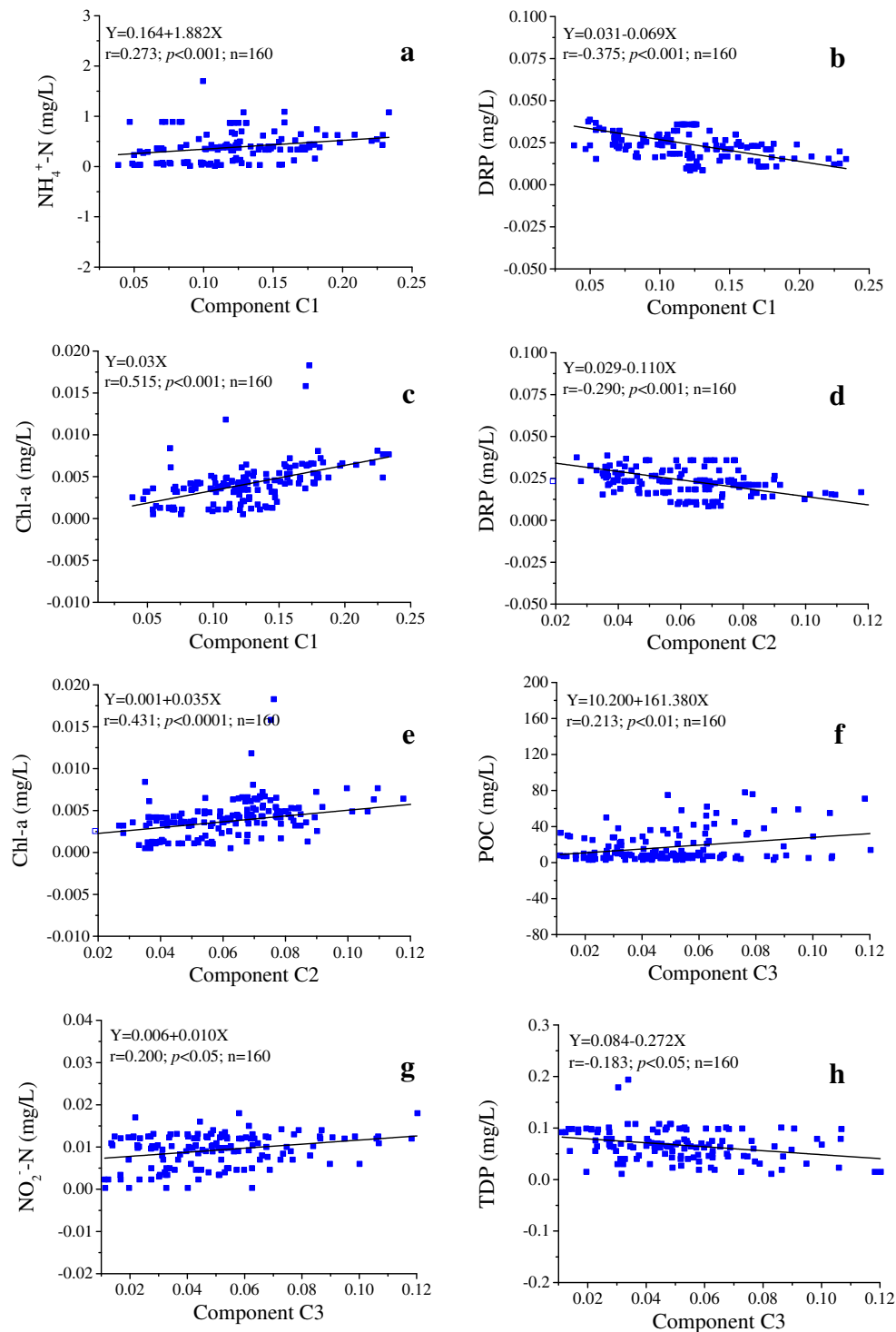


humic-like organic matter was an important source for these four components in the Heilongjiang River. The  $F_{max}$  of fluorescence components C1, C2, and C4 were almost perfectly linearly correlated ( $p<0.001$ ) (Fig. 3), indicating that they are controlled by the same factors (Liu et al. 2014). However, component C3 was not correlated with the other three components. This difference in the behavior of specific components from the general trend could be identified by the use of the PARAFAC technique, which helped identify the difference in C3 that was apparent in the single fluorescence excitation (Table 1). In addition, the total relative percentage (%) of terrestrial humic-like components (C2, C3, and C4) in each study site ranges from 47 to 58 %. This indicates that the concentration of fluorescence components at the sample site may be correlated to the terrestrial input in the Heilongjiang River. There are the Greater Khingan Mountains in the southwest of the upstream of Heilongjiang, the Lesser Khingan Mountains in the southwest of the midstream, and the Changbai mountains in the southwest of the downstream (Fig. 1). First, the inland tributaries would take partly terrestrial DOM into Heilongjiang. Second, the mountain runoff formed by the melting snow or rain carries a large amount of organic substances. As reported in the literature, the humic fraction was the largest soil organic carbon fraction of the black soil in the northeast region of China (Heilongjiang area) (Xing et al., 2005). The inland tributaries and mountains runoff are rich in organic matter. Therefore, the CDOM components in Heilongjiang are mainly influenced by the terrestrial

input organic matter. The delivery of the terrestrial DOM subsidy plays an important role in catchment carbon budgets influencing the ecology of surface waters. In addition, the inputs of allochthonous DOM into the watershed are tied to the carbon cycle and influence the environmental aspects of the Heilongjiang River.

**Relationships between BOD<sub>5</sub>, COD<sub>Mn</sub>, and fluorescence components in the Heilongjiang River**

The degree of organic pollution that occurs due to an excessive amount of organic matter has typically been monitored by measuring BOD<sub>5</sub> and COD<sub>Mn</sub> values in rivers (Hur and Cho, 2012). As previous studies reported, protein-like components make a great contribution to BOD<sub>5</sub> (Baker and Inverarity, 2004). In the Heilongjiang River, there were significant positive relationships between terrestrial humic-like component C3 ( $r=0.433$ ,  $p<0.001$ ;  $n=160$ ), POC ( $r=0.564$ ,  $p<0.001$ ;  $n=160$ ), and BOD<sub>5</sub> (Fig. 5). This result indicates that terrestrial humic-like component C3 may easily be used by microbes as a carbon source and the input of terrestrial organic matter would have an influence on the biomass in the Heilongjiang River. However, component C3 in the Heilongjiang River is not a major component (Fig. 2). These results indicate that the bioavailability of DOM in the Heilongjiang River is not high. The presence of COD<sub>Mn</sub> was complemented by the increased POC ( $r=0.398$ ,  $p<0.001$ ;  $n=160$ ) and component C3 ( $r=0.187$ ,  $p<0.05$ ;  $n=160$ )



**Fig. 6** Correlation among the four fluorescence components and general water quality properties. The unit of fluorescence intensity is calibrated and normalized in Raman Units (R.U.), and the sample number is 160.

Note: the sample sites from upstream to downstream are EER, LGR, HMSR, HMR, HR, MR, TGR, and WSR, respectively

(Fig. 5), which suggests that POC and component C3 make great contributions to  $\text{COD}_{\text{Mn}}$  in the Heilongjiang River. POC is a labile C pool in the forest soil system, which enters the ecosystem mainly from litter fall and leaves the ecosystem via soil respiration, lateral transport, and seepage (Sun and Wang,

2014). Therefore, the significant positive relationships between  $\text{BOD}_5$ ,  $\text{COD}_{\text{Mn}}$ , and POC indicate that POC transported from the forest system into water make a contribution to the carbon cycle in the Heilongjiang River. Furthermore, the fluorescence of terrestrial humic-like

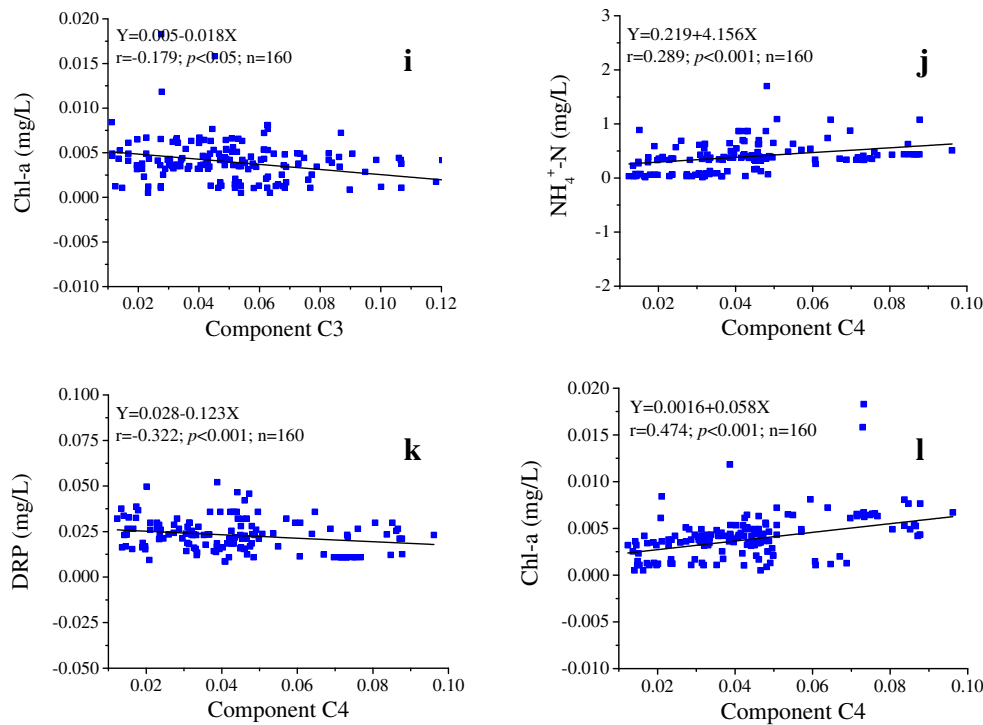


Fig. 6 continued.

component C3 shows significant positive relationships with BOD<sub>5</sub> and COD<sub>Mn</sub>. These results suggests that the water quality of the Heilongjiang River was mainly influenced (represented by COD<sub>Mn</sub> and BOD<sub>5</sub>) by terrestrial inputs organic material. A high level of BOD<sub>5</sub> deteriorates the river water quality by the rapid decomposition of biodegradable organic matter and the subsequent depletion of dissolved oxygen, while COD<sub>Mn</sub> traditionally represents the total organic matter. In Heilongjiang, high levels of BOD<sub>5</sub> and COD<sub>Mn</sub> occur at HR and downstream (Fig. 4), which can be attributed to the terrain. Many mountains are seated beside the HR and its downstream watershed, such as LKM, CBM (Fig. 1). Melting snow or runoff from the mountains that may carry a large amount of DOM would influence the quality of the water. Although there is also a GKM upstream of HR in Heilongjiang, owing to the geographical position of GKM, its melting snow or runoff would not enter the Heilongjiang River in a great amount and produce a great impact on the water (Fig. 1).

**Relationships between fluorescence components and water quality parameters**

Eutrophication poses severe threats to the quality, safety, and sustainability of water resources worldwide (Heisler et al. 2008; Qin et al. 2010). Phosphorus and nitrogen indexes that could evaluate the level of eutrophication were also tested in terms of the relationships with fluorescence components (Fig. 6). The indexes of dissolved phosphorus and dissolved

nitrogen can be attributed to the turnover of DOM (Qualls and Haines 1991; Hopkinson et al. 2002; Kragh and Søndergaard, 2004). Therefore, as an exporter of phosphorus and nitrogen, DOM plays an important role in terrestrial and stream ecosystems. Bacteria and algae in the water can use these nutrients for their growth. After algae die, their residues partly indirectly contribute to the content of DOM. Significant correlations between the fluorescence components and water quality parameters indicate a common controlling factor of the components and the selected parameters concentrations in the Heilongjiang River. In the Heilongjiang River, Chl-a showed significantly positive relationships with component C1 ( $r=0.264, p<0.001; n=160$ ), C2 ( $r=0.239, p<0.001; n=160$ ), and C4 ( $r=0.263, p<0.001; n=160$ ), but significantly negative relationships with component C3 ( $r=0.224, p<0.01; n=160$ ) (Fig. 6). Notably, the correlation coefficient between Chl-a and component C1 was higher than that between Chl-a and C2 or Chl-a and C4 (Fig. 6); this was partly attributed to higher relation of Chl-a and C1. The presence of C1 in our water samples was similar to microbial oxidized components (Cory and Mcknight, 2005) and phytoplankton degradation releases humic components due to microbial activity (Zhang et al., 2011). The appearance of component C1 in our river samples suggests a microbial humic component originating from the microbial transformation products. Previous studies have demonstrated that this component may be produced autochthonously in aqueous environments (Borisover et al. 2009, and Zhang et al. 2011). Chl-a is an important component in the cells of phytoplankton.

After phytoplankton dying, the Chl-a would be released from the phytoplankton cells to the water, while some organic matter of phytoplankton residue would also be transformed into C1 (Cory and McKnight, 2005; Zhang et al., 2011). Component C3 could be used as a carbon source for microbes, so some phosphorus and nitrogen would be released from the biodegradation of component C3. The released phosphorus and nitrogen could help phytoplankton growth. In addition, fluorescence components C1, C2, and C4 show significant negative relationships with DRP. The concentration of  $\text{NH}_4^+\text{-N}$  generally had negative relationships with fluorescence components C1 and C4. Fluorescence component C3, which has a negative relationship with Chl-a, shows significant relationship with  $\text{NO}_2^-\text{-N}$  and TDP, which may indicate that the dynamic of component C3 is different from those of components C1, C2, and C4 in the Heilongjiang River.

## Conclusions

In this study, four fluorescence components C1 (microbial humic-like component), C2–4 (terrestrial humic-like) were identified in the Heilongjiang River. The result of statistical analysis shows that terrestrial humic-like component C3 is easily used by microbes as a carbon source in the Heilongjiang River, and POC may make a great contribution to  $\text{COD}_{\text{Mn}}$ . The results of  $\text{BOD}_5$  and  $\text{COD}_{\text{Mn}}$  in HR and its downstream showed high values, indicating that melting snow or runoff from the mountains that may carry large amounts of DOM would influence the quality of the water. Relevant analysis results showed that the phytoplankton residue would also be turned over into C1 by microbial transformation and C3 could be potential nutrients for phytoplankton growth in the Heilongjiang River.

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## References

- Baker A (2011) Fluorescence excitation-emission matrix characterization of some sewage impacted rivers. *Environ Sci Technol* 35:948–953
- Baker A, Inverarity R (2004) Protein-like fluorescence intensity as a possible tool for determining river water quality. *Hydrol Process* 18:2927–2945
- Boavida MJ, Wetzel RG (1998) Inhibition of phosphatase activity by dissolved humic substances and hydrolytic reactivation by natural ultraviolet light. *Freshw Biol* 40(2):285–293
- Boehme J, Coble P, Conmy R, Stovall-Leonard A (2004) Examining CDOM fluorescence variability using principal component analysis: seasonal and regional modeling of three-dimensional fluorescence in the Gulf of Mexico. *Mar Chem* 89:3–14
- Borisover M, Laor Y, Parparov A, Bukhanovsky N, Lado M (2009) Spatial and seasonal patterns of fluorescent organic matter in Lake Kinneret (Sea of Galilee) and its catchment basin. *Water Res* 43(12):3104–3116
- Carlson CA (2002) *Biogeochemistry of marine dissolved organic matter*. Academic, New York, pp 91–152
- Coble PG (1996) Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Mar Chem* 51:325–346
- Coble PG (2007) Marine optical biogeochemistry: the chemistry of ocean color. *Chem Rev* 107:420–418
- Coble PG, Mopper K, Schultz CS (1993) Fluorescence contouring analysis of DOC intercalibration experiment samples: a comparison of techniques. *Mar Chem* 47:173–178
- Coble PG, Castillo CD, Avril B (1998) Distribution and optical properties of CDOM in the Arabian Sea during the 1995 southwest monsoon. *Deep-Sea Res* 45:2195–2223
- Cory RM, Kaplan LA (2012) Biological lability of streamwater fluorescent dissolved organic matter. *Limnol Oceanogr* 57(5):1347–1360
- Cory RM, McKnight DM (2005) Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environ Sci Technol* 39(21):8421–8149
- Fellman JB, D'Amore DV, Hood E (2008) An evaluation of freezing as a preservation technique for analyzing dissolved organic C, N and P in surface water samples. *Sci Total Environ* 392:305–312
- Fellman JB, Hood E, Spencer RGM (2010) Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: a review. *Limnol Oceanogr* 55(6):2452–2462
- Guo WD, Yang LY, Hong HH, Stedmon CA, Wang FL, Xu J, Xie YY (2011) Assessing the dynamics of chromophoric dissolved organic matter in a subtropical estuary using parallel factor analysis. *Mar Chem* 124:125–133
- Harvey GR, Boran DA (1985) The geochemistry of humic substances in seawater. In: Aiken GR, McKnight DM, Wershaw RL, MacCarthy P, editors. *Humic substances in soil, sediment, and water*. Wiley-Interscience, pp 233–247
- He XS, Xi BD, Li X, Pan HW, Da A, Bai SG, Li D, Cui DY (2013) Fluorescence excitation-emission matrix spectra coupled with parallel factor and regional analysis to characterize organic matter humification. *Chemosphere* 93:2208–2215
- Hedges JI (1992) Global biogeochemical cycles: progress and problems. *Mar Chem* 39:67–93
- Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8:3–13
- Hopkinson CS, Vallino JJ, Nolin AL (2002) Decomposition of dissolved organic matter from the continental margin. *Deep-Sea Res Part II* 49:4461–4478
- Hudson N, Baker A, Reynolds D (2007) Fluorescence analysis of dissolved organic matter in nature, waste and polluted waters—a review. *River Res and Appl* 23:631–649
- Hudson N, Baker A, Ward D, Reynolds D, Brunson C, Carliell-Marquet C, Browning S (2008) Can fluorescence spectrometry be used as a surrogate for the biochemical oxygen demand (BOD) test in water quality assessment? An example from South West England. *Sci Total Environ* 391:149–158
- Hur J, Cho J (2012) Prediction of BOD, COD, and total nitrogen concentrations in a typical urban river using a fluorescence excitation-emission matrix with PARAFAC and UV absorption indices. *Sensors* 12:972–986

- Kowalczyk P, Durako MJ, Young H, Kahn AE, Cooper WJ, Gonsior M (2009) Characterization of dissolved organic matter fluorescence in the South Atlantic Bight with use of PARAFAC model: interannual variability. *Mar Chem* 113:182–196
- Kowalczyk P, Tilstone GH, Zablocka M, R ttgers R, Thomas R (2013) Composition of dissolved organic matter along an Atlantic meridional transect from fluorescence spectroscopy and parallel factor analysis. *Mar Chem* 157:170–184
- Kragh T, SØndergaard M (2004) The bioavailability and degradation of autochthonous DOC: effect of mesozooplankton. *Aquat Microb Ecol* 36:61–72
- Liu X, Zhang Y, Shi K, Zhu G, Xu H, Zhu M (2014) Absorption and fluorescence properties of chromophoric dissolved organic matter: implications for the monitoring of water quality in a large subtropical reservoir. *Environ Sci Pollut Research* 21:14078–14090
- Markager S, Vincent WF (2000) Spectral light attenuation and the absorption of UV and blue light in natural waters. *Limnol Oceanogr* 45(3):642–650
- McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Andersen DT (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol Oceanogr* 46(1):38–48
- MEP China (Ministry of Environmental Protection of the People's Republic of China) (2009) Water quality—determination of ammonia nitrogen—Nessler's reagent spectrophotometry. HJ 535, Beijing, China
- Mueller KK, Fortin C, Campbell PGC (2012) Spatial variation in the optical properties of dissolved organic matter (DOM) in lakes on the Canadian Precambrian Shield and links to watershed characteristics. *Aquat Geochem* 18:21–44
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
- Murphy KR, Stedmon CA, Waite TS, Ruiz GM (2008) Distinguishing between terrestrial and autochthonous organic matter sources in marine environments using fluorescence spectroscopy. *Mar Chem* 108:40–58
- Murphy KR, Stedmon CA, Graeber D, Bro R (2013) Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Anal Methods* 5:6557–6566
- Qin B, Zhu G, Zhang Y, Li W, Pearl HW, Carmichael WW (2010) A drinking water crisis in Lake Taihu, China: linkage to climatic variability and lake management. *Environ Manage* 45:105–112
- Qualls RG, Haines BL (1991) Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. *Soil Sci Soc Am J* 55:1112–1123
- Singh S, D'Sa EJ, Swenson EM (2010) Chromophoric dissolved organic matter (CDOM) variability in Barataria basin using excitation-emission matrix (EEM) fluorescence and parallel factor analysis (PARAFAC). *Sci Total Environ* 408:3211–3222
- State Environment Protection Administration of China (2002) Analysis of water quality monitoring standards of practice handbook, 4th edn. China Environment Science Press, Beijing, pp 210–234 (In Chinese with English abstract)
- Stedmon CA, Bro R (2008) Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnol Oceanogr Meth* 6:572–579
- Stedmon CA, Markager S (2005a) Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnol Oceanogr* 50(2):686–697
- Stedmon CA, Markager S (2005b) Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. *Limnol Oceanogr* 50(5):1415–1426
- Stedmon CA, Markager S, Bro R (2003) Tracing dissolved organic matter in aquatic environment using a new approach to fluorescence spectroscopy. *Mar Chem* 82:219–254
- Sun ZL, Wang CK (2014) Dissolved and particulate carbon fluxes in forest ecosystems. *Acta Ecologica Sinica* 34(15):4133–4141
- Wang Y, Zhang D, Shen Z, Chen J, Feng C (2014) Characterization and special distribution variability of chromophoric dissolved organic matter (CDOM) in the Yangtze Estuary. *Chem* 95:353–362
- Wang Y, Zhang D, Shen ZY, Feng CH, Zhang X (2015) Investigation of the interaction between As and Sb species and dissolved organic matter in the Yangtze Estuary, China, using excitation–emission matrices with parallel factor analysis. *Environ Sci Pollut Research* 22:1819–1830
- Williams C, Yamashita Y, Wilson HF, Jaffé R, Xenopoulos MA (2010) Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems. *Limnol Oceanogr* 55(3):2259–1171
- Xing BS, Liu JD, Liu XB, Han XZ (2005) Extraction and characterization of humic acids and humic fractions from a black soil of China. *Pedosphere* 15(1):1–8
- Yamashita Y, Jaffé R, Maie N, Tanoue E (2008) Assessing the dynamics of dissolved organic matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC). *Limnol Oceanogr* 53(5):1900–1908
- Zhang YL, Yin Y, Feng LQ, Zhu GW, Shi ZQ, Liu XH, Zhang YZ (2011) Characterizing chromophoric dissolved organic matter in Lake Tianmuhu and its catchment basin using excitation-emission matrix fluorescence and parallel factor analysis. *Water Res* 45:5110–5122
- Zhang YL, Gao G, Shi K, Niu C, Zhou Y, Qin B, Liu X (2014) Absorption and fluorescence characteristics of rainwater CDOM and contribution to Lake Taihu, China. *Atmos Environ* 98:483–491