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RESEARCH ARTICLE

The ultraviolet–visible absorbance and fluorescence characterization of dissolved organic matter derived from the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix cheilophila*, and *Populus tomentosa*

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Abstract

Dissolved organic matter (DOM) derived from leaf litter plays an important role in maintaining carbon (C) and nitrogen (N) circulation between soils and plants, energy flow, and signaling pathways for plant-microbe interactions of terrestrial ecosystem. In this study, four DOM samples extracted with a 40:1 (v/w) water to sample ratio from the leaf litter of *Populus simonii* (S1), *Artemisia desertorum* (S2), *Salix cheilophila* (S3), and *Populus tomentosa* (S4) were investigated using the technologies of ultraviolet–visible (UV–Vis) and excitation–emission matrix (EEM) fluorescence spectroscopy. Results showed that the electricity (EC) values of four DOM extracts were significantly different due to the different composition and salt content of each plant. The values of chemical oxygen demand (COD), dissolved organic carbon (DOC), and the sum of values of all peaks' intensities divided by DOC (FI) indicated the higher contents of organic matter in the acid DOM extracts from S1, S2, and S3 (sand-fixing plants) than the neutral DOM extracted from S4. The absorbance shoulder between 250 and 285 nm in the UV–Vis spectra and EEM fluorescence spectra of each sample suggested the presence of many different chromophores such as aromatic or phenolic compounds in plant DOM. According to fluorescence regional integration (FRI) and peak picking results, the content of protein-like materials was higher than that of humic-like substances in DOM from S1, S2, and S3 while the opposite phenomena occurred in DOM from S4. Hence, the physicochemical and fluorescence characterization of DOM extracted from the genus *Populus* of the family Salicaceae S1 and S4 growing under different edaphic and climatic conditions changed much. The findings would be of great significance to understand the origin, composition, dynamics, and biotransformation of DOM in soils formed in different climatic environments.

Highlights
1. The properties of DOM from leaf litter under different climates were obviously different.
2. A linear correlation between DOC and COD was strong for the studied DOM ($r^2 = 0.96$).
3. There was a positive relationship between the contents of acid OM and DOC.
4. The optical properties of plant DOM are related to edaphic and climatic conditions.

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Introduction

DOM is a complex heterogeneous mixture mainly composed of humic substances, proteinaceous material, and carbohydrates. It plays a crucial role in the biogeochemical cycle of carbon (C) on local to global scales; regulates microbial action by providing nutrients, energy, and protection from ultraviolet radiation; and links various ecological compartments including soils to water bodies (Quails and Haines 1991; Cuss and Guéguen 2013; He and Wu 2015; Zhang and He 2015; Bayarsaikhan et al. 2016; Sankar et al. 2019). With different molecular weights and complex components and functional groups, such as the carboxyl group, phenol group, and carbonyl group, DOM can affect the environmental acid–base characteristics and various physical, chemical, and biological reactions through aggregation, photochemical reactivity, biodegradation, and so on (Jones and Bryan 1998; Leenheer and Croue 2003; Hudson et al. 2007, 2008; Pelaez et al. 2009). Hence, it also plays an important role in the adsorption, migration, and transformation of pollutants, organic matter formation, and microbial activity (Cuevas and Lugo 1998; Sun et al. 2013; Yan et al. 2013; Soong et al. 2015; Guo et al. 2019).

The composition of DOM is spatially and temporally dependent on and associated with source material variations and environmental transformation processes. Besides anthropogenic inputs, DOM is mainly derived from terrestrial or aquatic sources such as animal decomposition residues, plant litters, and root exudates. As one of the major plant litters, leaf litter is the dominant source of DOM in forested headwater streams. It plays important roles in maintaining C and nitrogen (N) circulation between soils and plants, energy flow such as CO_2 efflux through the heterotrophic respiration of soil microorganisms and animals, and signaling pathways for plant-microbe interactions (Aerts 1997; Abelho 2001; Leenheer and Stedmon 2009; Monreal 2015; Krishna and Mohan 2017). To the best of our knowledge, the composition, structure, nutrient contents (C, N, P, K, Ca, and Mg), distribution of the molecular size, carbon and nitrogen dynamics, decomposition process, optical properties, and lability of DOM derived from some species of plants leaf litter, such as crop residues and tropical and sub-tropical trees, have been investigated extensively (Cuevas and Lugo 1998; Smith et al. 1998; Hunt et al. 2007; He et al. 2009; Kiikkilä et al. 2011; Cuss and Guéguen 2013, 2015; Bayarsaikhan et al. 2016; Zhao et al. 2017b). However, little is known about the DOM characterization of leaf litter from *Populus simonii* and sand-fixing plants such as *Artemisia desertorum* and *Salix cheilophila* growing under temperate semi-arid and semi-desert plateau continental climatic conditions. Moreover, minimal information is available about the DOM derived from the leaf litter of *Populus tomentosa*, which is widely used as a species of protection forests growing under warm temperate continental monsoon climatic conditions.

Up to now, the fluorescence spectroscopy and ultraviolet and visible (UV–Vis) absorbance spectra have been widely used to measure and analyze the source, composition, structural characteristics, migration, and transformation mechanism of DOM (Hudson et al. 2007; Liu et al. 2009; Guo et al. 2010; Wang et al. 2014; Vera et al. 2017). Specifically, the use of excitation–emission matrix (EEM) fluorescence spectroscopy technology could offer various possibilities of data interpretation, including simple peak picking, fluorescence regional integration (FRI), self-organizing maps, the humification index (HIX), fluorescence index (FI), the ratio of β/α, and even the complex parallel factor analysis (Bieroza et al. 2011; Hassouna et al. 2012; Coble 1996; Zhang and He 2015). As a common method of spectral analysis, peak picking technique can identify specific fluorophore by assessing the position and intensity of fluorescence peak directly. In order to quantitatively characterize the superimposed spectra of EEM fluorescence spectroscopy, the method of FRI was developed to fully interpret the fluorescence information carried by the spectrum (Guo et al. 2010; Zhou et al. 2013; He and Fan 2016). Consequently, EEM fluorescence spectroscopy combined with FRI has been widely employed to analyze the characterization, distribution, and dynamics of DOM in rivers, sewage, mine drainage, and other water bodies (He and Fan 2016; Zhao et al. 2017a; Liu et al. 2019). In addition, different chromophores of DOM may be effectively distinguished through the analysis of UV–Vis absorbance spectra (He et al. 2009; Zhang and He 2015). Thus, efforts should be made to explore the characterization of DOM derived from the leaf litter of *Artemisia desertorum* and *Salix cheilophila* (sand-fixing plants) by using these technologies, which would be helpful in understanding the N and C cycles between the soil and plants in the ecologically fragile area belonging to temperate semi-arid and semi-desert plateau continental climate zone (Cuss and Guéguen 2013; Tanikawa et al. 2018). On the other hand, there are few studies focused on the differences in the physical and chemical properties and spectral characteristics of DOM from plant leaves of *Populus simonii* and *Populus tomentosa*, both of which belong to the genus *Populus* of the family Salicaceae. The findings will be useful for discriminating the origin, composition, dynamics, and biotransformation of DOM in soil formed under different climatic conditions.

The current study aims to (1) compare the contents of organic matter and physical properties of DOM released from

the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix cheilophila*, and *Populus tomentosa* growing under different edaphic and climatic conditions through the determination of dissolved organic carbon (DOC), chemical oxygen demand (COD), UV_{254}, pH, and electricity (EC); (2) characterize the UV–Vis spectroscopy of DOM extracted from the four kinds of plant fallen leaves; (3) investigate the differences in the characterization of EEM fluorescence spectra of the obtained plant DOM by using the "peak picking" approach combined with FRI.

Materials and methods

Sample sites

Ulanmulun town (the first study area) is located in the southeast of Yinjinhuoluo County, Ordos City, Inner Mongolia Province, Western China. The altitude of the town is 1130~1260 m. The study area belongs to the temperate semi-arid and semi-desert plateau continental climate zone. The average temperature over the years is 6.2 °C. The annual precipitation is between 195 and 532 mm $year^{-1}$, with the average value being 357 mm $year^{-1}$. The evapotranspiration is between 2297 and 2834 mm $year^{-1}$, with the average value of 2457 mm $year^{-1}$ in the last 30 years. Thus, the evapotranspiration is 5–11 times the precipitation in the first study area (Zhao et al. 2019). The soil type for the leaf litters of *Populus simonii*, *Artemisia desertorum*, and *Salix cheilophila* collected from the first study area belongs to Arenosols (Jin et al. 2010; Xu et al. 2016). The main plants in this area are *Artemisia desertorum*, *Salix cheilophila*, *Caragana*, *Camphor pine*, and *Populus simonii*, which are widely used for sand fixing (Liu and Man 2008).

Xinmi County (the second study area) is located in the southwest of Zhengzhou City, Henan Province, Central China. It is located 841 km southeast from Ulanmulun town (the first study area). The altitude of the County is 85~135 m. The study area belongs to the warm temperate continental monsoon climate zone. The average temperature over the years is 14.7 °C. The annual precipitation is between 600 and 707 mm $year^{-1}$, with the average value being 637 mm $year^{-1}$. The annual average evapotranspiration is 446 mm^{-1}. The main soil type at the sampling site of *Populus tomentosa* is eolian sand soil (Jiao et al. 2015). *Populus tomentosa* is widely cultivated in the Central China (Di et al. 2019).

DOM preparation

The fresh leaf litter of *Populus simonii* (Salicaceae, *Populus*), *Artemisia desertorum* (Compositae, *Sagebrush*), and *Salix cheilophila* (Salicaceae, *Salix*) were collected from Ulanmulun town, Yinjinhuoluo County, Ordos City, Inner Mongolia Province, China (39° 17′ 36″ N, 110° 02′ 13″ E) in October, 2017. The fresh leaf litter of *Populus tomentosa* (Salicaceae, *Populus*) were collected from Xinmi County, China (34° 32′ 22″ N, 113° 23′ 28″ E) in November, 2017, in order to investigate the differences in UV–Vis and fluorescence characterization of DOM derived from the genus *Populus* of the family Salicaceae growing under different climatic condition in China. To make the description more convenient, S1, S2, S3, and S4 were used to represent the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix cheilophila*, and *Populus tomentosa* respectively. A great part of the leaves were yellow, but some were still green. Before use, all collected leaves of four plants were cleaned, air dried, crushed, and ground to pass through a 1-mm nylon screen for preservation in polyethylene plastic bags. The contents of N in S1, S2, S3, and S4 are individually 1.45%, 2.19%, 1.78%, and 1.44%, respectively, and the contents of C in S1, S2, S3, and S4 are individually 43.03%, 44.27%, 45.29%, and 40.18%, respectively, which are close to the reported values (44~50%) for the fallen leaves of red oak, willow, birth, etc. (Bayarsaikhan et al. 2016). According to the literature, biopolymers, humic substances, building blocks, acids, and neutrals were the main DOC fractions in the fallen leaves, and lignin cellulose and polyphenols could be the main constituents of organic carbon in the collected leaves (Smith et al. 1998).

The studied DOM was extracted with a 40:1 (*v*/*w*) deionized water to plant leaves sample ratio (200 mL/5.0 g) using a temperature-controlled shaker. The agitation speed and temperature of shaker were individually fixed at 120 rpm and 4 °C for 24 h for all DOM extracting processes (He et al. 2009). Then the suspensions were filtered through 0.45 μm filter membranes to obtain the studied DOM samples, which would be stored at 4 °C and prepared for the measurement of physiochemical parameters including COD, DOC, pH, EC, UV–Vis absorbance, and EEM fluorescence spectra in not more than 24 h after sampling.

Measurement and analysis methods

The obtained DOM samples were diluted 1/20 with deionized water for the analysis of COD and DOC. COD was measured by closed reflux method using potassium dichromate. A TOC-LCSH (Shimadzu Corporation, Japan) was used to measure the concentration of DOC.

The obtained DOM samples were diluted 1/100 with deionized water for the UV–Vis absorbance and EEM fluorescence spectra analysis. UV–Vis absorbance spectra were measured by using UV-1800 (Shimadzu Corporation, Japan). Absorbance (cm^{-1}) is expressed as the ratio between optical density and optical length (1.0 cm) (Birdwell and Engel 2010). Specific UV absorbance at 254 nm ($SUVA_{254}$) (L·

$mg^{-1}·cm^{-1}$) was calculated by normalizing the absorbance at this wavelength (UV_{254}) by the concentration of dissolved organic carbon ($mg·L^{-1}$) of the corresponding sample ($100 \times UV_{254}/DOC$). EEM fluorescence spectra were acquired in 1-cm path-length quartz cells using F-7000 (Hitachi, Japan) fluorescence spectrophotometer with a 150-W xenon lamp as the instrument light source. Excitation and emission slit widths were 10 nm, and the scan speed was 12,000 nm/min. The excitation and emission wavelengths were respectively scanned from 200 to 400 nm and 200 to 550 nm in 5-nm steps to generate a 3DEEM. The Raman peak of water at 348 nm was used to check for instrument stability prior to analysis.

pH and EC for the obtained DOM were immediately measured by using FG2-FK pH meter and FG3-FK conductivity meter (Mettler Toledo Corporation, Swiss), respectively.

All collected leaves of four plants were crushed by FW-200 high-speed universal grinder (Zhongxing weiye instrument Co. LTD, Beijing).

Deionized water was used for the blank sample, to make up reagents and clean the quartz cell between samples.

The mean values of all data in this study were obtained by lab duplicates (two DOM samples by separate extraction of a same plant collection). Statistical analyses of DOM data were made with one-way ANOVA and *t* test on SPSS Statistics 22.0 software.

Calculation method of fluorescence organic matter content

Based on different wavelength ranges of excitation/emission, fluorescence spectra of DOM could be divided into five regions representing specific components of DOM as follows: excitation/emission set to 200 to 250 nm/(250 to 330 nm) (region I, aromatic proteins I such as tyrosine-like compounds), 200 to 250 nm/(330 to 380 nm) (region II, aromatic proteins II such as tryptophan-like compounds), 200 to 250 nm/(380 to 550 nm) (region III, fulvic-like acids), 250 to 400 nm/(250 to 380 nm) (region IV, soluble microbial-like products), 250 to 400 nm/(380 to 550 nm) (region V, humic-like acids). The content of fluorescence organic matter belonging to specific fluorescence region could be quantified by using FRI technique (Chen et al. 2003). The volume (Φ_i) was used to represent the cumulative fluorescence response of DOM with similar properties in each region and could be calculated as Eq. (1):

$$\Phi_i = \sum_{ex}\sum_{em} I(\lambda_{ex}\lambda_{em})\Delta\lambda_{ex}\Delta\lambda_{em} \quad (1)$$

where $\Delta\lambda_{ex}$ is the excitation wavelength increment (taken as 5 nm), $\Delta\lambda_{em}$ is the emission wavelength increment (taken as 5 nm), and $I(\lambda_{ex}\lambda_{em})$ is the fluorescence intensity of each excitation–emission wavelength pair.

By normalizing the cumulative excitation–emission area volumes to relative regional areas, the normalized excitation–emission area volumes referring to value of region i ($\Phi_{i,n}$) and value of the entire region ($\Phi_{T,n}$) were calculated with Eqs. (2) and (3):

$$\Phi_{i,n} = MF_i\Phi_i \quad (2)$$

where MF_i is a multiplication factor, equal to the inverse of the fractional projected excitation–emission area.

$$\Phi_{T,n} = \sum_{i=1}^{5}\Phi_{i,n} \quad (3)$$

The percent fluorescence response ($P_{i,n}$, %) was calculated as Eq. (4):

$$P_{i,n} = \frac{\Phi_{i,n}}{\Phi_{T,n}} \times 100\% \quad (4)$$

Results and discussion

pH and EC

The pH and EC of DOM samples extracted from the leaf litter of four species of plants are summarized in Table 1 (mean ± SD). According to Table 1, the pH values of DOM samples from S1, S2, and S3 were respectively 5.49 ± 0.04, 5.49 ± 0.005, and 5.03 ± 0.00, suggesting the weak acidic property of the studied DOM. However, the pH values of DOM extracts from S4 were 7.07 ± 0.07, indicating the neutral characterization of the studied DOM. Therefore, there was a significant difference in pH values between S1 and S4 although

Table 1 The pH and EC of DOM samples from the leaf litter of four plants (mean ± SD)

Parameter	S1	S2	S3	S4
pH	5.49 ± 0.04b	5.49 ± 0.005b	5.03 ± 0.00c	7.07 ± 0.07a
EC/μS·cm^{-1}	887.35 ± 11.55d	1941.00 ± 1.00a	1404.50 ± 14.50b	1330.00 ± 2.00c

S1, S2, S3 and S4: the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix psammophila*, *and Populus tomentosa* respectively. Different letters (a, b, c, and d) in a row indicate significant differences in the values of pH and EC for the DOM samples from the leaf litter of four plants by one-way ANOVA ($p < 0.05$)

both belonged to the genus *Populus* of the family Salicaceae ($p < 0.05$), indicating that the physical characteristics of plants belonging to the same family may be affected by edaphic and climatic conditions in consistent with the results of Wang (2006).

As illustrated in Table 1, the EC values of the studied DOM samples ranged from 887.30 ± 11.55 to 1941.00 ± 1.00 μS cm^{-1}, and there were significant differences in EC values of DOM samples from the leaf litter of four plants ($p < 0.05$). The result could be relative to the intrinsic physical characteristic including compositions and salt contents of each species of plants (Cuss and Guéguen 2013; He et al. 2014). Moreover, the edaphic and climatic conditions for the growth of S1 and S4 might also affect the levels of EC for the extracted DOM.

DOC and COD

The values of DOC and COD of four DOM samples are shown in Fig. 1. As shown in Fig. 1, the DOC contents of four plant DOM extracts ranged from 1588 to 2544 mg/L, close to the value of that for the DOM extracted from crop residues (He et al. 2009). As all extractions were performed with the same deionized water to sample ratio, the DOC levels in the extracts reflected the abundance of DOC in these plant leaves. Meanwhile, the variation trend of COD was similar to that of DOC for the studied DOM samples. Thus, there is a comparatively high correlation ($r^2 = 0.96$) between the values of DOC and COD for the studied DOM as the flowing equation describes:

$$\mathrm{COD} = 2.84\mathrm{DOC} + 493.37, \quad r^2 = 0.96, n = 4 \tag{5}$$

where r is the correlation coefficient and n is the number of data points. According to the literature, the value of r in the current work was obviously higher than the reported values ranging from 0.57 to 0.87 for the correlations between COD and DOC in rivers and sewage (Zhang 2015; Lee et al. 2016). Therefore both DOC and COD could be used to represent the organic content of the studied plants leaf litter. In addition, the values of organic matter contents in the DOM released from S1, S2, and S3 collected from the first study area were significantly higher than that in the DOM sample derived from S4 collected from the second study area as described by Fig. 1.

Moreover, COD and DOC could be well expressed as the linear function of pH:

$$\mathrm{COD} = -1225\mathrm{pH} + 13350, r^2 = 0.93, n = 4 \tag{6}$$

$$\mathrm{DOC} = -394\mathrm{pH} + 4315, r^2 = 0.81, n = 4 \tag{7}$$

As shown in Eqs. (6) and (7), the pH value of the studied DOM samples had a linear negative correlation with COD or DOC, indicating the positive relationship between the content of organic carbon and the contents of acid organic matter such as humic acid and fulvic acid in the studied DOM, in accordance with the reported literatures (Ferrari 2000; Wu et al. 2007). Moreover, the later discussion in "EEM fluorescence spectroscopy" could further confirm the above results.

Fig. 2 shows the relationship between DOC and UV_{254} of four studied DOM samples. According to the figure, the variation trend of DOC with every species of plant was different from that of UV_{254} obviously, indicating that there is not an obvious linear correlation between UV_{254} and DOC in the current work. As shown in Fig. 2, the value of UV_{254} for the DOM from S2 was the highest, suggesting the highest content of aromatic compounds among the studied four DOM samples (Korshin et al. 2009; Matilainen et al. 2011). The values of UV_{254} in the other three DOM samples were similar (20.08~21.58 cm^{-1}) although they were extracted from the leaf litter of three plants in two sites with different climate characterization. Given the lowest content of DOC in DOM

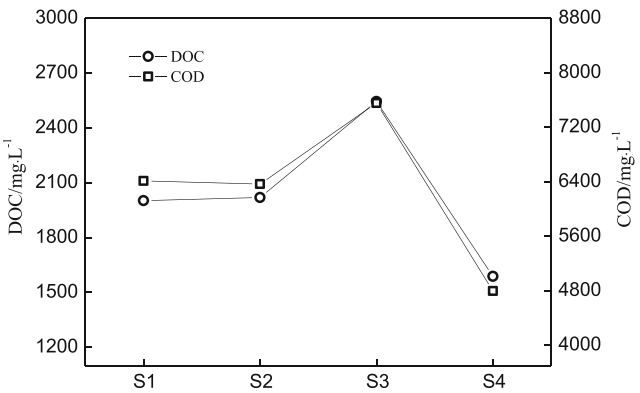

Fig. 1 The values of DOC and COD of DOM extracts from S1, S2, S3, and S4. S1, S2, S3, and S4 represent the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix psammophila*, and *Populus tomentosa* respectively

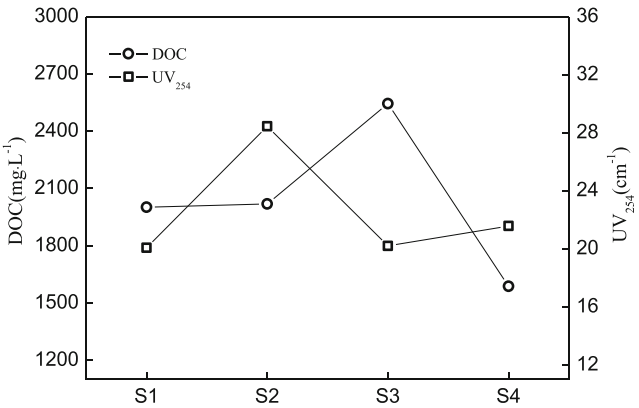

Fig. 2 The values of DOC and UV_{254} of DOM extracts from S1, S2, S3, and S4. S1, S2, S3, and S4 represent the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix psammophila*, and *Populus tomentosa* respectively

from S4, the value of $SUVA_{254}$ (1.36 $L \cdot mg^{-1} \cdot cm^{-1}$) was higher than that for the DOM extracts from S1 (1.01 $L \cdot mg^{-1} \cdot cm^{-1}$) and S3 (0.79 $L \cdot mg^{-1} \cdot cm^{-1}$).

UV–visible spectroscopy

The UV–Vis absorbance spectra of DOM samples extracted from S1, S2, S3, and S4 are presented in Fig. 3. As illustrated in Fig. 3, the UV absorbance spectra of the studied DOM samples all decreased monotonically with increasing wavelength because of the complex mixture of aliphatic and aromatic hydrocarbon structures in the samples. Thus the studied DOM samples did not exhibit an easily distinguishable spectrum, in accordance with the results frequently reported in the literature (He et al. 2009; Hassouna et al. 2012; Zhang and He 2015). However, it is worth noting that the UV–Vis spectra of each sample showed an absorbance shoulder between 250 and 285 nm, indicating the presence of many different chromophores in the studied DOM. These chromophores could be aromatic or phenolic compounds with carbonyl and conjugated carbonyl which have strong absorbance in the range of 200 to 300 nm as reported by Abbt-Braun et al. (2004), and the DOM exacts might contain amino acids, nucleic acids, and other phenolic compounds (Hunt and Ohno 2007). Thus, the content of aromatic compounds and chromophores might be the lowest in the extract from S4 because of the occurrence of the weakest absorbance shoulder for the obtained DOM exacted from S4 among four species of plants (Fig. 3).

EEM fluorescence spectroscopy

Fig. 4 shows the EEM spectra of DOM samples extracted from S1, S2, S3, and S4. The *Y*-axis is excitation wavelength ranging from 200 to 400 nm while the *X*-axis is the emission wavelength ranging from 250 to 550 nm. The contour lines are the distribution of fluorescence intensity of each excitation–emission wavelength pair. The inner contour lines suggest higher fluorescence intensity than the outer ones. The

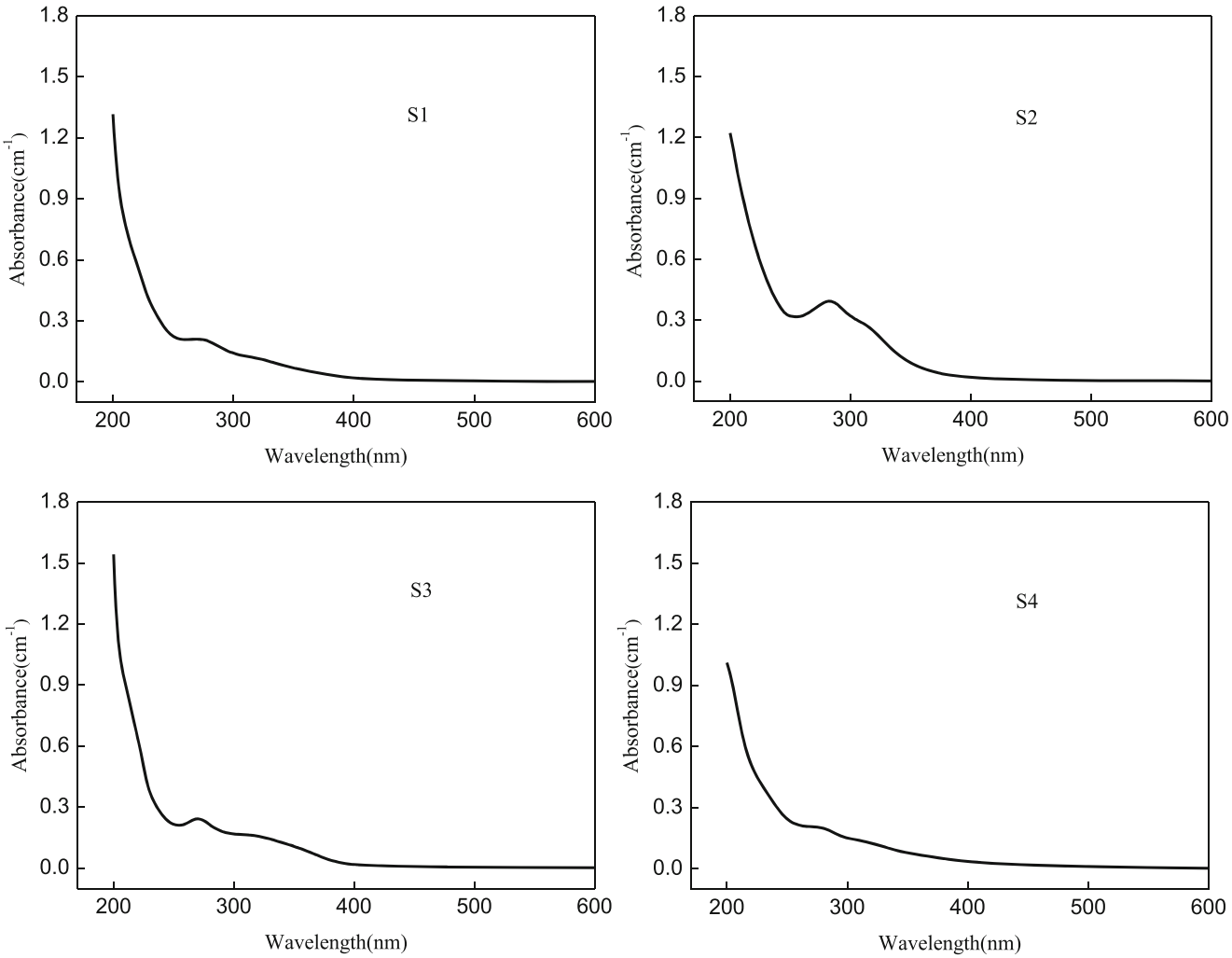

Fig. 3 UV–Vis spectra of DOM extracts from S1, S2, S3, and S4. S1, S2, S3, and S4 represent the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix psammophila*, and *Populus tomentosa* respectively

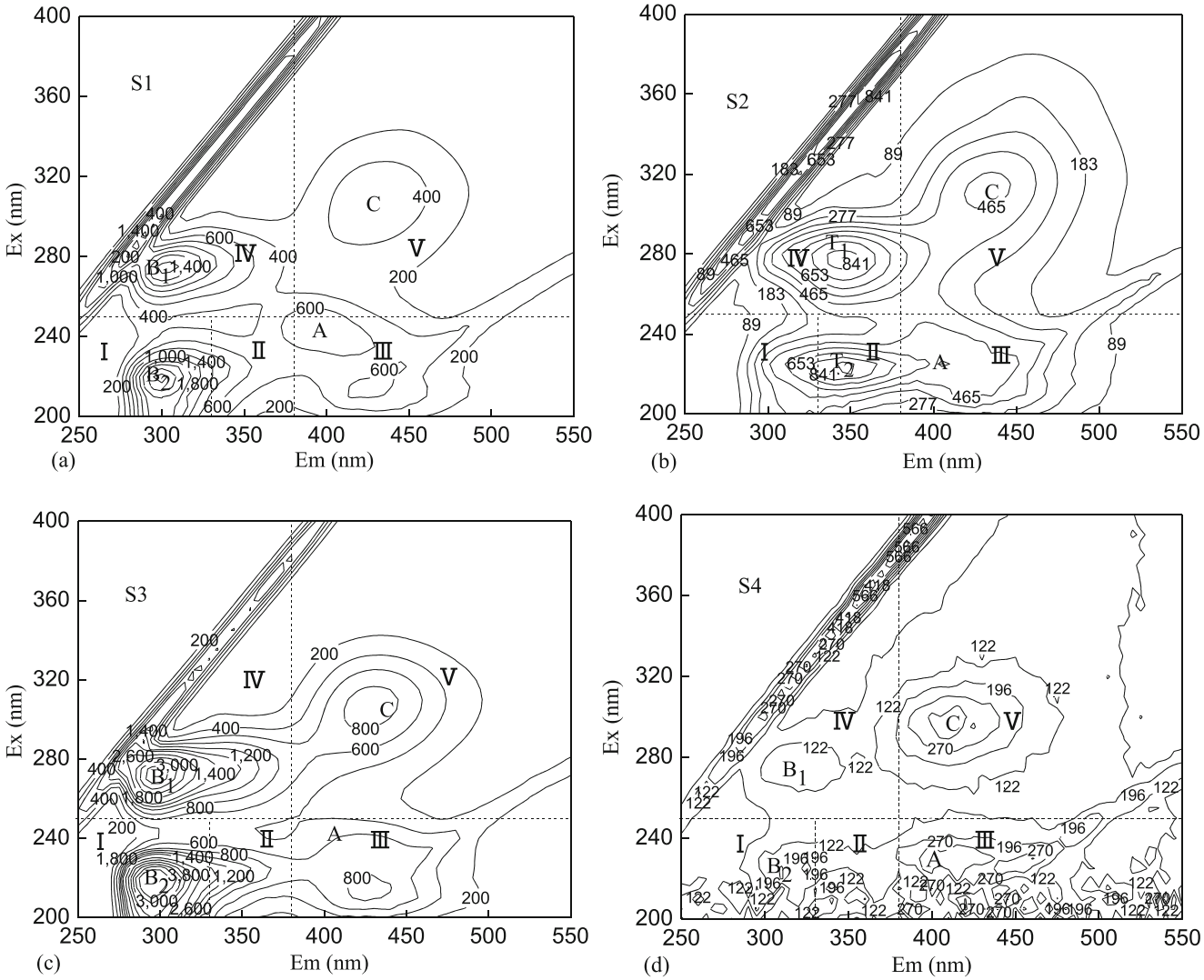

Fig. 4 EEM spectra of DOM extracts from S1, S2, S3, and S4. S1, S2, S3, and S4 represent the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix psammophila*, and *Populus tomentosa* respectively. I, II, III, IV, and V represent the excitation–emission area related to tyrosine-like compounds, tryptophan-like compounds, fulvic-like acids, soluble microbial-like products, and humic-like acids respectively

specific peaks in an EEM spectrum could be attributed to the occurrence of specific fluorophores produced by specific types of DOM (Chen et al. 2003; Hassouna et al. 2012; Coble 1996). As illustrated in Fig. 4, a fulvic acid-like peak (peak A, $\lambda_{ex}/\lambda_{em}$ = 210–245 nm/390–425 nm) and a humic acid-like peak (peak C, $\lambda_{ex}/\lambda_{em}$ = 295–315 nm/415–435 nm) were all detected in the four studied DOM samples, in accordance with previous studies (Hassouna et al. 2012; Xie et al. 2013; Wang et al. 2014; Cuss and Guéguen 2013, 2015). In addition, two protein-like peaks B_1 ($\lambda_{ex}/\lambda_{em}$ = 275–280 nm/300–320 nm) and B_2 ($\lambda_{ex}/\lambda_{em}$ = 220–225 nm/295–310 nm) were observed in three DOM samples from S1, S3, and S4 while two other protein-like peaks T_1 ($\lambda_{ex}/\lambda_{em}$ = 275 nm/345 nm) and T_2 ($\lambda_{ex}/\lambda_{em}$ = 225 nm/345 nm) were detected in the DOM from S2. Similar fluorescent components were also observed for the leachates of leaf litter from red maple, bur oak, sugar maple, and white spruce trees (Cuss and Guéguen 2013). However, only two protein-like peaks T_1 ($\lambda_{ex}/\lambda_{em}$ = 226 nm/335–350 nm) and T_2 ($\lambda_{ex}/\lambda_{em}$ = 277 nm/335–350 nm) were detected for the cottonseed protein fractions (He et al. 2014). According to the literatures (He et al. 2014; Yu et al. 2014; Carstea et al. 2016; Zhao et al. 2018), peaks B and T have been associated with living and dead cellular material and their exudates and could indicate microbial activity of fluorescence molecules. Specifically speaking, peaks B_1 and B_2 are associated with tyrosine-like materials as free molecules or as molecules bound to amino acids proteins, whereas peaks T_1 and T_2 are related to tryptophan-like compounds as free molecules or molecules bound to proteins, or humic structures (Hudson et al. 2008; Coble 1996; Chen et al. 2003; Leenheer and Croue 2003; Cuss and Guéguen 2013, 2015). In addition, fluorescence emitted by soluble microbial byproduct-like materials is commonly seen in region IV because the amount of protein-like fluorescence has been

considered an indicator of lability for the DOM of leaf leachate during biotransformation (Chen et al. 2003; Cuss and Guéguen 2013, 2015).

The major fluorescent components of the studied DOM extracted from S1, S2, S3, and S4 are reported in Table 2. According to Table 2, the sum of values of all peaks' intensities divided by DOC (FI) for the DOM from S1, S2, S3, and S4 were 2.58, 1.39, 3.79, and 0.70 respectively. The values of two protein-like peaks' intensities (divided by DOC) were all higher than those of peak A and peak C for all the DOM samples except the DOM from S4 as shown in Table 2. Moreover, the value of peak B_2's intensity(divided by DOC) for the DOM samples from S1, S3 and S4, and that of peak T_2's intensity(divided by DOC) for the DOM sample from S2 accounted for 40.70%, 47.76%, 18.84%, and 31.65% of the sum of values of all peak intensities divided by DOC (FI) respectively. Thus, the results suggested the higher content of aromatic protein compounds in the DOM of plant leaf litter growing under semi-arid and semi-desert climatic conditions than that in semi-humid climatic conditions. In addition, the value of FI (divided by DOC) for the DOM extracted from S4 was the lowest among the four samples, in accordance with the result of DOC as described in Section 3.2. Fig. 5 shows the FI/DOC values of four fluorescence peaks in DOM extracted from the genus *Populus* of the family Salicaceae (S1 and S4). According to the figure, there were significant differences in the values of FI_A/DOC, FI_{B1}/DOC, and FI_{B2}/DOC between S1 and S4 ($p < 0.05$) although they belonged to the same family. Moreover, the obvious differences in the values of pH, EC, DOC, and COD between S1 and S4 could also be observed as described in the earlier sections. Therefore, the physicochemical and fluorescence characterization of DOM extracted from the genus *Populus* of the family Salicaceae S1 and S4 growing under different edaphic and climatic conditions changed much.

Table 2 Major fluorescent components in DOM extracted from S1, S2, S3, and S4

Peak	Location	S1	S2	S3	S4
A	$\lambda_{ex/em}$	245/390	225/405	210/425	230/410
	FI_A/DOC	0.39	0.28	0.34	0.22
C	$\lambda_{ex/em}$	310/430	315/435	305/430	295/415
	FI_C/DOC	0.29	0.24	0.35	0.24
B_1	$\lambda_{ex/em}$	275/305	/	275/300	280/320
	FI_{B1}/DOC	0.85	/	1.29	0.11
B_2	$\lambda_{ex/em}$	220/300	/	220/295	225/310
	FI_{B2}/DOC	1.05	/	1.81	0.13
T_1	$\lambda_{ex/em}$	/	275/345	/	/
	FI_{T1}/DOC	/	0.43	/	/
T_2	$\lambda_{ex/em}$	/	225/345	/	/
	FI_{T2}/DOC	/	0.44	/	/

FI_A/DOC, FI_C/DOC, FI_{B1}/DOC, FI_{B2}/DOC, FI_{T1}/DOC, FI_{T2}/DOC: individual fluorescence intensity of peak A, C, B_1, B_2, T_1, and T_2 divided by DOC

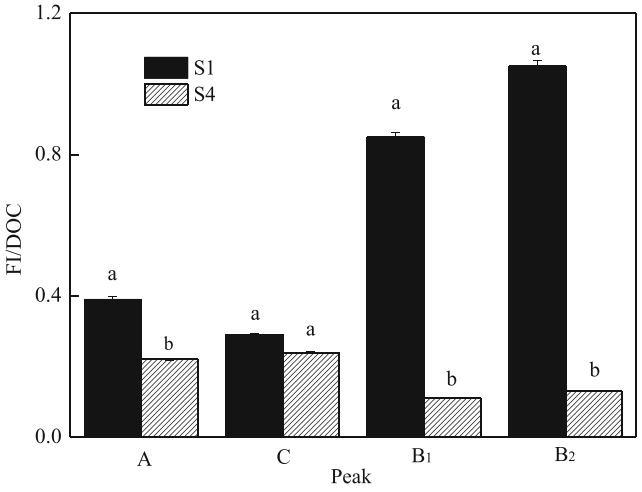

Fig. 5 The FI/DOC values of peaks A, C, B_1, and B_2 in DOM extracted from the genus *Populus* of the family Salicaceae S1 and S4. S1 and S4 represent the leaf litter of *Populus simonii* and *Populus tomentosa* respectively. Statistical significance of differences between S1 and S4 are marked with different letters (a and b) by *t* test ($p < 0.05$)

The correlations between fluorescence intensity of peak A divided by DOC (FI_A/DOC) and pH, fluorescence intensity of peak C divided by DOC (FI_C/DOC) and pH, and fluorescence intensity of all fluorescence peaks divided by DOC (FI) and DOC are listed in Table 3. As shown in the Table, the contents of humic acid-like and fulvic acid-like substances decreased with pH because of the linear negative correlations between FI_A/DOC and pH ($r^2 = -0.62$), FI_C/DOC, and pH ($r^2 = -0.51$). Moreover, there was a linear correlation between FI and DOC ($r^2 = 0.9$) as presented in Table 3, indicating that the value of FI could reflect the content of DOM extracted from the studied plant leaf litter to some extent. Thus, the high content of humic-like substances may induce the high level of DOM in accordance with the result of Section 3.2.

FRI analysis

Fig. 6 shows the FRI results for the DOM extracts from S1, S2, S4, and S4. As shown in Fig. 6, each EEM was delineated into five regions (regions I–V) according to the approach reported previously, and the volumes ($\Phi_{i,n}$) representing the cumulative fluorescence response of each region in the EEM spectra were calculated based on the FRI technique (Chen et al. 2003). The fluorescence intensities for the DOM extracted from S1, S2, and S3 were in the order of: region V > region II > region I > region IV > region III, while the values for the DOM extracted from S4 were in the order of: region V > region IV > region II > region III > region I. Thus, humic-like acids belonging to region V was the leading fluorescent component in the studied plant DOM.

Table 3 Correlations of fluorescence intensity with pH and DOC

Peak intensity of fluorescence	Linear equation	Correlation coefficient (r^2)	The number of data points (n)
FI_A/DOC	FI_A/DOC = − 0.07pH + 0.69	− 0.62	4
FI_C/DOC	FI_C/DOC = − 0.04pH + 0.53	− 0.51	4
FI	FI = 9DOC-13,657	0.90	4

FI_A/DOC: fluorescence intensity of peak A divided by DOC; FI_C/DOC: fluorescence intensity of peak C divided by DOC; FI: fluorescence intensity of all fluorescence peaks divided by DOC

Fig. 7 shows the distribution of $P_{i,n}$ in DOM extracted from S1, S2, S3, and S4. As shown in Fig. 7, the $P_{i,n}$ order in five regions (regions I–V) for the DOM extracted from S1, S2, S3, and S4 were the same as the above results ($\Phi_{i,n}$). On the other hand, the total value of $P_{i,n}$ in region I, II, and IV were individually 61.07, 57.42, and 56.67%, which were higher than that in region III and V for the DOM extracts from S1, S2, and S3. Hence, the content of protein-like materials was higher than that of humic-like substances despite the highest content of humic-like acids in DOM from S1, S2, and S3. Nevertheless, the total value of $P_{i,n}$ in region III and V (61.91%) was higher than that of region I, II, and IV (38.09%) for the DOM from S4, suggesting the higher content of humic-like substances in DOM from S4. Consequently, the specific optical properties may be linked to the species of plants growing under different edaphic and climatic conditions, although protein-like and humic-like substances could be usually observed in the leachates of leaf litter through the measurement of EEM spectra. The findings were in accordance with the results of Cuss and Guéguen (2013, 2015).

Conclusions

The characterization of DOM derived from four species of plant leaf litter growing under different edaphic and climatic conditions were compared by using the technologies of UV–Vis spectroscopy and EEM fluorescence spectroscopy combined with the test of DOC, COD, pH, and EC. In general, there were significant differences in the pH and EC values of DOM samples from the leaf litter of four plants, indicating the intrinsic characteristics of a plant species growing under specific edaphic and climatic conditions ($p < 0.05$). Considering the obvious linear correlation between COD and DOC, the pH value was negatively correlated with COD and DOC respectively, implying the positive relationship between the contents of acid organic matter and organic carbon in the studied DOM, which could be further confirmed by the results of EEMs. Moreover, the organic matter contents in the DOM from S1, S2, and S3 were much higher than that in the DOM from S4.

The UV–Vis spectra suggested that there were various chromophores such as aromatic or phenolic compounds in

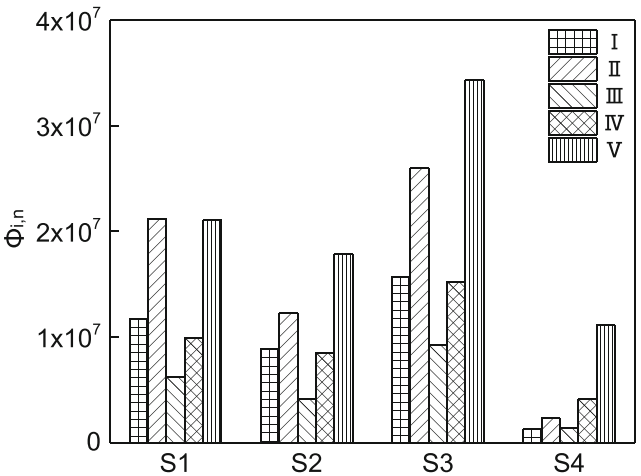

Fig. 6 FRI results for the DOM samples extracted from S1, S2, S3, and S4. S1, S2, S3, and S4 represent the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix psammophila*, and *Populus tomentosa* respectively. Regions I, II, III, IV, and V represent the values of the normalized excitation–emission area volumes related to tyrosine-like compounds, tryptophan-like compounds, fulvic-like acids, soluble microbial-like products, and humic-like acids respectively

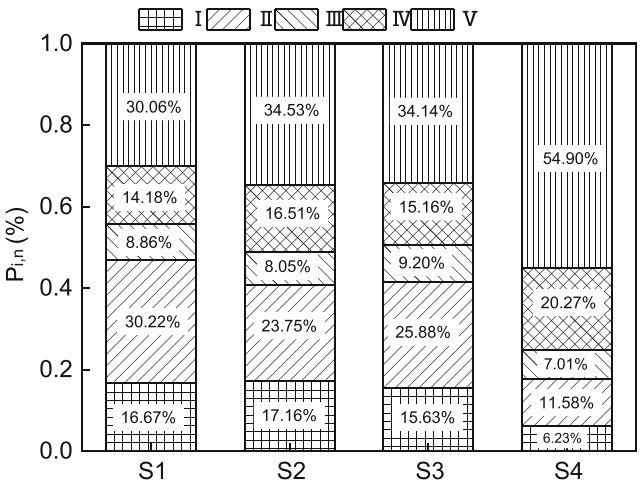

Fig. 7 Distribution of $P_{i,n}$ in the EEM spectra of DOM extracts from S1, S2, S3, and S4. S1, S2, S3 and S4 represent the leaf litter of *Populus simonii*, *Artemisia desertorum*, *Salix psammophila*, and *Populus tomentosa* respectively. Regions I, II, III, IV, and V represent the values of the normalized excitation–emission area volumes related to tyrosine-like compounds, tryptophan-like compounds, fulvic-like acids, soluble microbial-like products, and humic-like acids respectively

each DOM samples. Specifically, the highest value of UV_{254} for the DOM from S2 indicated the highest content of aromatic compounds while the weakest absorbance shoulder in the DOM from S4 indicated the lowest contents of chromophores. The measurement of EEM fluorescence spectroscopy indicated that a fulvic acid-like peak and a humic acid-like peak were all detected in the studied DOM. Meanwhile, two tyrosine-like peaks were observed in the DOM extracts from S1, S3, and S4 while two tryptophan-like peaks were detected in the DOM from S2. Notably, the content of protein-like fluorescent materials was higher than that of humic-like fluorescent substances in the DOM from S1, S2, and S3 whereas the opposite phenomena occurred in the DOM derived from S4 based on FRI analysis. Furthermore, the fluorescence characterization and the content of fluorescence molecules of DOM extracted from the genus *Populus* of the family Salicaceae S1 and S4 differed significantly ($p < 0.05$), given the different edaphic and climatic conditions for their growth.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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