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Release and Microbial Degradation of Dissolved Organic Carbon and Nitrogen from *Phragmites australis* and *Suaeda salsa* in the Wetland of the Yellow River Estuary

Yuanzhi Qi, Yuejun Xue and Xuchen Wang*

Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266100, China

Abstract

Release of dissolved organic matter (DOM) and chromophoric dissolved organic matter (CDOM) from two dominant plants *Phragmites australis* and *Suaeda salsa* in the Yellow River Estuarine wetlands was studied in laboratory incubation experiments. The release dynamic of dissolved organic carbon (DOC), dissolved nitrogen (DN) and CDOM from the plants was rapid process and hydrolysis played an important role in the initial leaching stage of the organic compounds from the plants. Bacterial activities enhanced the release processes of DOM during degradation of the plants and utilization of the released organic compounds. The fluorescence characteristics of the CDOM indicate that the protein-like organic substances are the major components released from *P. australis* and *S. salsa* in the initial stage and these compounds are labile and highly biodegradable. Our study suggests that leaching of DOM from *P. australis* and *S. salsa* provide not only major sources of DOC, DN, and CDOM that affect many biogeochemical processes, but also as important food sources supporting microbial communities in the Yellow River wetlands and adjacent coastal waters.

Keywords: Dissolved organic matter; Dissolved nitrogen; Wetland; Wetland plants; Yellow river estuary

Introduction

Wetlands and Salt marshes are highly diverse and productive ecosystems. They are widely distributed along the coasts worldwide, especially in estuarine systems [1,2]. These coastal ecosystems play important roles not only as buffer zones protecting the coastal lines from tidal erosion but also as essential habitats for coastal wildlife such as fish and seabirds [3,4]. More importantly, from geochemical viewpoints, wetlands and salt marshes are significant sinks for the atmospheric CO_2 due to the high rates of annual carbon sequestration in vegetation in these ecosystems. On the other hand, salt marshes also export large amount of dissolved organic matter (DOM) and nutrients, thus influence the primary production and biogeochemical processes in the coastal waters as well [5-8].

Salt marshes and wetlands are typically characterized by dense vegetation including rushes, sedges, and grasses which are the dominant primary producer and represent a large component of living biomass in these ecosystems [9-11]. Export of dissolved organic carbon (DOC) and dissolved nitrogen (DN) from salt marsh and wetland to coastal waters has been considered an important biogeochemical link between these coastal ecosystems and the ocean [5,12-16]. Contribution of DOC from marshes and wetlands is largely through the leaching and decomposition of plant biomass, especially during the late fall "dumping" period when plants began to die [8,17,18]. Previous studies have shown that DOC output from Southeastern U.S. salt marshes alone could contribute an equal amount of DOC as river inputs to the continental shelf [19,20]. In their recent field study, Schiebel et al. estimated that approximately 46% of the annual export of DOC from the Neponset Salt Marsh in Massachusetts, USA was contributed from the release of three dominant marsh plants *Spartina patens*, *Spartina alterniflora* and *Phragmites australis* [18].

Studies have shown that salt marsh and wetland plants are important sources contributing chromophoric dissolved organic matter (CDOM) to estuarine and coastal waters [8,17,18,21]. CDOM is the optically measureable component of DOM and is important for most photochemically-mediated processes in coastal waters [22,23]. It plays important roles in carbon cycle and biogeochemical processes in estuarine and coastal water [24,25]. Despite a large amount of DOC is produced from salt marsh and wetland plants, the chemical composition and bioavailability of DOC and CDOM produced from different plant species have not been well studied and there is limited knowledge on exactly how these DOC and CDOM pools are recycled in coastal waters.

The Yellow River Delta (YRD) wetland is located in the northeast coast of China (37°40'~38°10'N; 118°41'~119°16'E) with a total area of 1530 km^2. The wetland is one of the largest coastal wetlands in the northeast region of China [26-28]. Although the wetland is relatively young formed in the last few decades when the Yellow River Estuary changed its flow path, it has played major roles protecting and sustaining the coastal ecosystem and wildlife. In the wetland, *Phragmites australis* and *Suaeda salsa* are the two dominant plants with *P. australis* widely distributed in the upper wetlands and *S. salsa* mainly concentrated in the lower tidal zone of the wetlands. It is estimated that the biomass of *S. salsa* alone could account for about 47.8% of the total average biomass of the wetland, followed by *P. australis* [26]. It is not clear how these different wetland plants could contribute DOC while still acting as major carbon sequestration reservoirs. Based on our field studies, we measured relatively high concentrations of DOC (146 ± 6 μM) and DN (74 ± 7 μM) in the Yellow River Estuarine waters [29,30].

***Corresponding author:** Wang X, Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266100, China, Tel: +86-532-66782831, +86-532-66782871; E-mail: xuchenwang@ouc.edu.cn

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We expect that the high level DOC and DN in the coastal waters could be derived from the wetland plants. In this paper, we report the results from laboratory incubation studies to examine the leaching dynamics of DOC and DN, as well as CDOM from *P. australis* and *S. salsa*, and their bioavailability to microbial degradation. These results offer an insights into how these wetland plants play important roles as important sources of DOM to the biogeochemical cycle of carbon and nitrogen and food sources for microbial communities in the Yellow River estuarine and coastal waters.

Materials and Method

Sample collection

Fresh plants *Phragmites australis* and *Suaeda salsa* used for the incubation experiments were collected from the Yellow River Estuary wetland on October 11, 2015. These two plants were collected in their early senescing stage and only the aboveground fraction of stems and leaves was collected. After collection, samples were washed with river water and kept in clean plastic bags and brought back to the laboratory for the incubation experiments. Coastal seawater was collected in Jiaozhou Bay few kilometers from the campus and filtered using 0.7 μm GF/F filters (pre-combusted at 550°C for 5 hours) to remove particles for the incubation experiments.

Plant leaching experiments

Two sets of DOM leaching incubations were conducted: (1) bacteria-inhibited (poisoned) and (2) bacteria-active (non-poisoned), to quantify and compare the chemical leaching and bacteria-influenced leaching processes. For the bacteria-active leaching incubation, 10 g of fresh *P. australis* or *S. salsa* (half stem and half leaf) were added to each of 1.0 l glass bottles containing 1.0 liter of filtered seawater. For bacteria-inhibited leaching experiments, 2.0 ml saturated $HgCl_2$ solution was first added to each of the glass bottles containing 1.0 liter filtered seawater. Then the same amount of plant material was added as the bacteria-active incubation. Duplicates were conducted for each incubation. The chemical composition of plant materials and seawater used for the incubation experiments are given in Table 1. All bottles were incubated at room temperature (~25°C) in the dark for 25 days. The incubation bottles were bubbled using O_2 each day to keep the water going anoxic. At selected times (0, 1, 2, 3, 5, 7, 10, 15 and 25 days), water samples were collected from each bottle and filtered for DOC, TN, and CDOM fluorescence analyses. All glassware used in the sample collection and experimental process were acid-washed, Milli-Q water rinsed and pre-combusted at 550°C for 5 hours.

Chemical and fluorescence measurements

Concentrations of DOC and total dissolved nitrogen (DN) were analyzed by the high temperature combustion (HTC) method using a Shimadzu TOC-L analyzer equipped with an ASI-V auto-sampler. The concentrations of DOC and DN were calibrated using a 5-point calibration curve generated from DOC standard prepared using potassium hydrogen phthalate (KHP) and DN standard using potassium nitrate (KNO_3) and UV-oxidized Milli-Q water. Instrument blank and standard validation for both DOC and DN were checked against reference low carbon water and deep seawater reference materials (provided by Dr. Hansell at University of Miami, Rosenstiel School of Marine and Atmospheric Sciences). Blank subtraction was carried out using Milli-Q high purity water. Average blanks associated with DOC and DN measurements were about 5 μM and 4 μM and the analytic precisions on triplicate injections were ± 3% and ± 5%, respectively. All samples were analyzed in duplicate and average values were reported. The total organic carbon (TOC) and total nitrogen (TN) contents of the plant solid phases were measured using a Thermo Flash 2000 CHN elemental analyzer.

CDOM fluorescence measurements were conducted using an Edinburgh FS5 spectrofluorometer. Single fluorescence emission scans from 300 to 650 nm were collected for an excitation wavelength of 350 nm. The fluorescence of Milli-Q water served as a blank and was subtracted from sample spectra prior to integration. Peak areas were integrated and converted to quinine sulfate units (QSU) where 1 QSU is equivalent to the fluorescence emission of 1 μg/l quinine sulfate solution (pH 2) integrated from the same excitation wavelength.

The excitation-emission matrix spectroscopy (EEMs) was measured to characterize the chemical composition of CDOM leached from the plants. Excitation wavelengths ranged from 250 to 500 nm in 5 nm increments. Emission wavelength data were collected from 260 to 600 nm in 5 nm increments. Milli-Q water EEMs was measured as a blank. All samples were measured using a 1 cm quartz fluorescence cell at room temperature. The EEMs data were analyzed by parallel factor analysis (PARAFAC) using a MATLAB based toolbox [31].

UV–visible absorption spectra of the water samples were collected at wavelengths ranging from 200 to 900 nm in 1-cm quartz UV–visible cell at room temperature, using a UV-5200 UV–visible spectrophotometer (Shanghai Metash Instruments Co). Milli-Q water was measured as blank baseline and subtracted from the sample absorption spectra. We calculated the specific UV absorbance (SUVA) which has been shown to be a good indicator of the composition of DOC in natural water [32]. SUVA at 254 nm (SUVA254) is considered as an "average" absorptivity of all molecules in DOC and it provides a quantitative estimate for DOC aromaticity. A strong positive correlation between SUVA254 values and the content of aromatic organic matter has been reported [32,33]. The value of SUVA254 is calculated using the equation:

$$SUVA254=A \times L\text{-}1 \times C\text{-}1 \quad (1)$$

where A is the measured UV absorbance at 254 nm; L is the cuvette pathlength (m), and C is the DOC concentration (mg/l), respectively [32].

Results and Discussions

Leaching of DOC and DN

Leaching production of both DOC and DN from *P. australis* and *S. salsa* were very rapid processes, and large differences were found between the bacteria-inhibited and bacteria-active incubations (Figure 1). In the bacteria-inhibited incubations (Figure 1a), the concentrations of DOC released from *P. australis* and *S. salsa* all increased rapidly from the initial seawater DOC level (127 μM) to ~ 3500 μM/gdw (per gram of dry weight) after 3 days. The release of DOC from *P. australis* slowed down and remained relatively constant after 3 days but the release of

Sample name		TOC% (w/w)	TN% (w/w)	C/N ratio (molar)
phragmites australis	stem	45.32	1.22	43.2
	leaf	45.86	2.31	23.2
Suaeda salsa	stem	38.87	0.65	69.6
	leaf	30.16	1.21	29.0
Seawater				
DOC (μM)		127.4 ± 0.7		
DN (μM)		6.5 ± 0.9		
CDOM (QSU)		2.1 ± 0.2		

Table 1: Chemical composition of plants and seawater used in the incubation experiments.

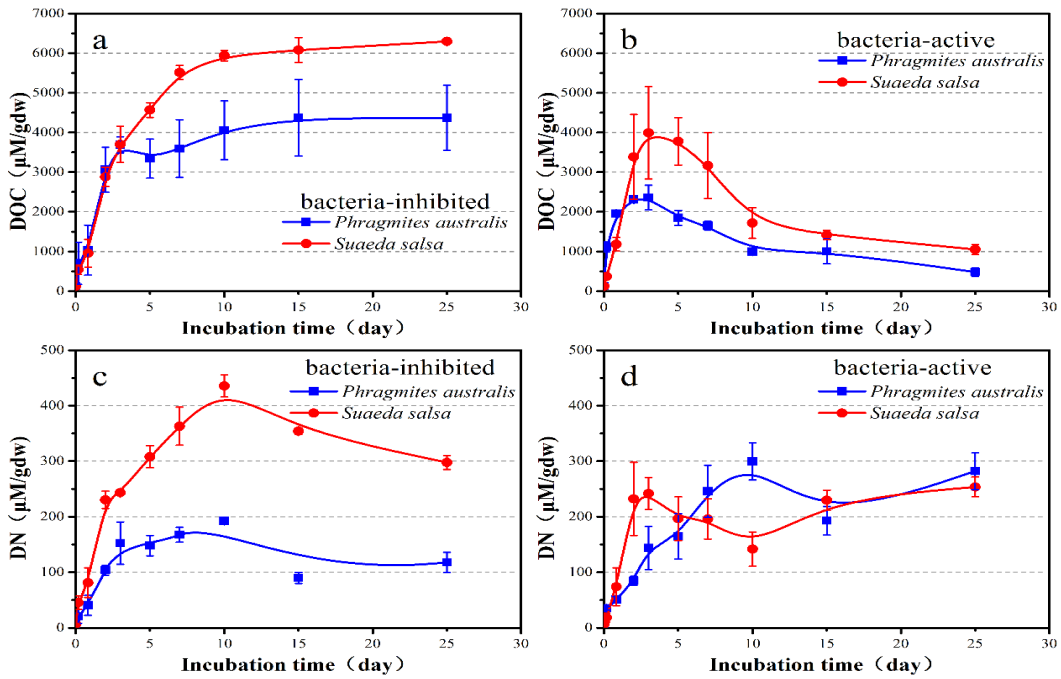

Figure 1: Leaching production of DOC and DN from *Phragmites australis* and *Suaeda salsa* with incubation time. DOC leaching in (a) bacteria-inhibited and (b) bacteria-active incubations; DN leaching in (c) bacteria-inhibited and (d) bacteria-active incubations.

DOC from *S. salsa* continuously increased to 10 days then remained no changes. At the end of the incubation (day 25), the concentrations of DOC leached from the plants reached to 4370 μM/gdw and 6300 μM/gdw from *P. australis* and *S. salsa*, with an overall rate of 175 μM/gdw/day and 252 μM/gdw/day, respectively. In contrast, the production of DOC in the bacteria-active incubations (Figure 1b) also showed a rapid accumulation from both *P. australis* and *S. salsa* in the first three days and then decreased rapidly with incubation times. At the end of the incubation, the concentrations of DOC were 480 μM/gdw and 1050 μM/gdw released from *P. australis* and *S. salsa*, only 11% and 17% of the DOC accumulation compared with that in the bacteria-inhibited incubations, respectively. In both incubations, more DOC was released from *S. salsa* than from *P. australis*.

The release of DN from *P. australis* and *S. salsa* also showed an initial phase of rapid increase and the concentrations reached to 160 μM/gdw on day 4 and 425 μM/gdw on day 10, with a similar rate of 40-42 μM/gdw/day in the bacteria-inhibited incubations (Figure 1c). After day 4 and 10, concentration of DN decreased slowly in both *S. salsa* and *P. australis* incubations. At the end of the incubation, 300 μM/gdw and 120 μM/gdw DN remained in solution. The release of DN in the bacteria-active incubations (Figure 1d) showed an initial rapid increase, reached to 300 μM/gdw on day 10 and 240 μM/gdw on day 3 from *P. australis* and *S. salsa* respectively. Concentration of DN decreased in both incubations after reaching the highest level then increased slowly again. At the end of the experiments, 280 μM/gdw and 250 μM/gdw DN accumulated in the solutions in the *P. australis* and *S. salsa* incubations.

These incubation results clearly suggest that both *P. australis* and *S. salsa* have great potential releasing large amount of DOC and DN when immerged in seawater. DOC and DN are likely leached out from the plant tissues through chemical hydrolysis initially, and the presence of bacteria enhanced the release processes by degradation and utilization of the released compounds. Early studies have demonstrated that the leaching and decomposition of DOM from salt marsh plants such as *Spartina alterniflora* occurred in three phases. The first phase is leaching of soluble organic matter within days, followed by the second stage of months with the biopolymers degraded and soluble organic substances released by the action of microorganisms. The third phase can take longer (years) than the first two phases, during which cellulose and lignin and other refractory organic compounds are remained [34-37]. Chen and Jiang have reported that bacteria activities played the most important roles in the late stage of plant degradation [38]. Our incubation results, however, only demonstrated the first two leaching stages of DOM from *P. australis* and *S. salsa* due to the limited incubation times.

By examining the correlations between DOC and DN released from *P. australis* and *S. salsa* as shown in Figure 2, a very good linear relationship between DOC and DN exists in the bacteria-inhibited incubations, with concentration of DN increased with increasing of DOC (for *P. australis*: R^2=0.96, p<0.001; for *S. salsa*: R^2=0.98, p<0.001). In the bacteria-active incubations, however, there is no such linear correlation. This indicates that N was proportionally released with C from *P. australis* and *S. salsa* and bacteria selectively utilized more N than C. In the bacteria-inhibited incubations, because all N released from the plants should be organic N, the C/N ratios of the released DOM (21.2 and 14.5, line slope) compared with their solid phase C/N ratio (~ 45 and 34, Table 1) suggest that more N-containing organic compounds, such as proteins were released from the plants in the early stage. This is also supported by the fluorescence results as discussed later.

The different release dynamics of DOC and DN in the bacteria-inhibited and bacteria-active incubations provide strong evidence that bacteria utilized the released organic compounds rapidly. Based on the different concentrations of DOC at the end of the incubations (Figures

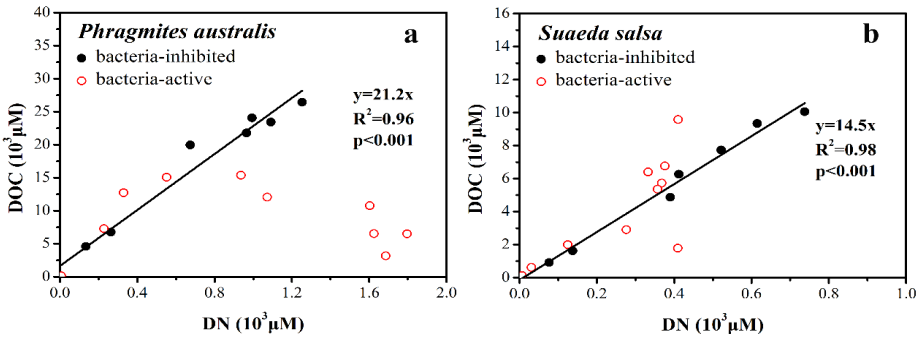

Figure 2: Plot of DOC *vs.* DN leached from (a) *Phragmites australis* and (b) *Suaeda salsa* in the bacteria-inhibited and bacteria-active incubations. The line is linear regression fitting of the bacteria-inhibited incubation data and the slope of each line represents C/N ratio of DOM leached from the plant.

1a and 1b), it is calculated that 89% and 83% of the DOC released from *P. australis* and *S. salsa* were consumed by bacteria. However, this calculation could not be applied to DN because in the bacteria-active incubations, the observed increase of DN in the later incubation was likely due to the conversion of organic N into inorganic N such as NH_4^+, NH_4^+ and NH_4^+ during DOM degradation [8,17,39-41]. Both labile organic substrates and useable forms of inorganic and organic N could promote bacterial production, thus complicates the release dynamics of DN. For the observed decrease of DN in the bacteria-inhibited incubations, we attributed this to the adsorption of N-containing organic compounds and inorganic N forms onto the surfaces of the plants and degraded particles in solution [42]. The adsorption effect was not significant for DOC because its concentration was 20-30 times higher than DN in the incubation.

Leaching and characterization of CDOM

As a major fraction of DOM in coastal waters, CDOM absorbs light over a wide range of both visible and UV wavelengths, and has a yellowish color and blue fluorescence when irradiated with UV light [22]. The fluorescence characteristics also vary with the chemical composition of CDOM. As shown in Figure 3, production of CDOM (as QSU) increased rapidly from *S. salsa* in the bacterial-inhibited incubations in the first 15 days, but much lower from *P. australis* (Figure 3a). In the bacteria-active case, after an initial rapid increase released from *S. salsa*, CDOM decreased on day 3 to the end of the incubation (Figure 3b). Production of CDOM from *P. australis* in the bacteria-active incubations was also lower and increased slowly with incubation times (Figure 3b).

The EEM spectra of CDOM leached from *P. australis* and *S. salsa* in both bacteria-inhibited and bacteria-active incubations are plotted in Figures 4 and 5. We compared the EEMs spectra on day 1, 7 and 25. For CDOM leached from *P. australis*, three peaks as P, H1 and H2 were identified which corresponding to the major components of CDOM based on their excitation and emission wavelength. According to Coble [24] and Stedmon and Markager [43], the P peak represents protein-associated (tyrosine-like and tryptophan-like) component; the H1 peak is humic-like CDOM and the H2 peak is terrestrial humic-like CDOM. Clearly, in the bacteria-inhibited incubations, the intensity of P and H2 peaks leached from *P. australis* increased from day 1 to day 7 and remained almost no changes on day 25 (Figure 4, left). In comparison, the intensity of P and H2 peaks were much higher on day 7 and decreased on day 25, especially the P peak in the bacterial-active incubations (Figure 4, right). For the EEM spectra of CDOM released from *S. salsa*, there was a H3 peak in addition to the P, H1 and H2 peaks (Figure 5). The H3 peak is also a humic-like component which could be blue-shifted [44,45]. It can be seen that in the bacterial-inhibited incubations, the intensity of the four CDOM peaks all increased from day 1 to day 7 and day 25 (Figure 5, left). In the bacteria-active incubations (Figure 5, right), the H3 peak was higher on day 1 and decreased on day 7 and 25. The intensity of P peak was also higher on day 1 and much higher on day 7, then disappeared almost on day 25.

The characteristics of the EEM spectra of CDOM released from both *P. australis* and *S. salsa* indicate that protein-associated CDOM (P peak) was a major organic component leached out from the plants and the present of bacteria not only enhanced the release processes of CDOM but alternated the fluorescent properties of CDOM as well. These results are consistent with the data reported by Wang et al. who examined the CDOM release from salt marsh plants and seagrasses. Bacteria utilized both protein-associated and humic-like CDOM released from *P. australis* and *S. salsa*, suggesting that these CDOM components are biologically labile for bacterial degradation in coastal waters [8,36,46-48]. These fluorescence results also support our DOC results as discussed above.

When analyzed the EEMs data using statistic PARAFAC model, three organic components, C1, C2 and C3 were identified and C1 represents a protein-like and C2 and C3 represent humic-like components (Table 2 and Figure 6), similar to the results of CDOM reported by other studies [24,43-45,49]. Component C1 is similar to a protein-like fluorescent compound that is analogous to free tyrosine dissolved in water derived mainly from terrestrial fluorescent materials [43]. The component C2 is identified as UVC humic-like fluorescent compound that often has a very high concentration in forest stream and wetlands [43]. The C3 component is similar to decomposed humic-like fluorescent compound which usually contains a large number of fulvic acid with high content of aromatic carbon, and large fraction of plant-derived and bacteria-derived humic substances [44,45]. These results indicate that CDOM released from *P. australis* and *S. salsa* represent a significant fraction of DOM and has similar fluorescent characteristic with CDOM derived from terrestrial plants found in many salt marsh and coastal waters.

To further examine the chemical composition of CDOM leached from *P. australis* and *S. salsa*, we plotted the UV-visible spectra $SUVA_{254}$ values with incubation time (Figure 7). The SUVA254 values decreased very quickly and then remained no changes after day 7 in the bacteria-inhibited incubations (Figure 7a). In comparison, the $SUVA_{254}$ values also decreased rapidly in the first few days in the bacteria-active incubations, but increased again after day 7 (Figure

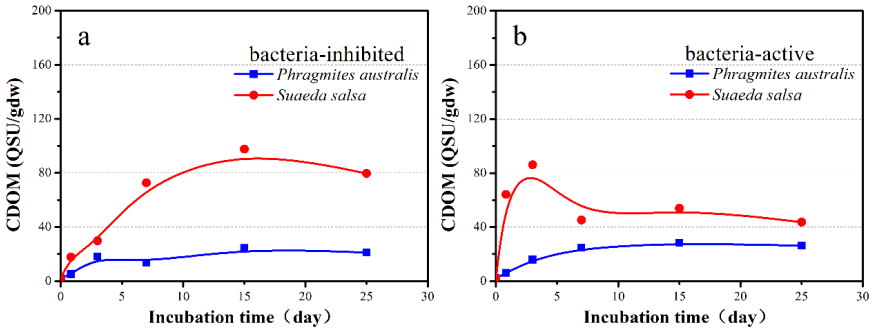

Figure 3: CDOM (as fluorescence) leached from *Phragmites australis* and *Suaeda salsa* in (a) bacteria-inhibited and (b) bacteria-active incubations.

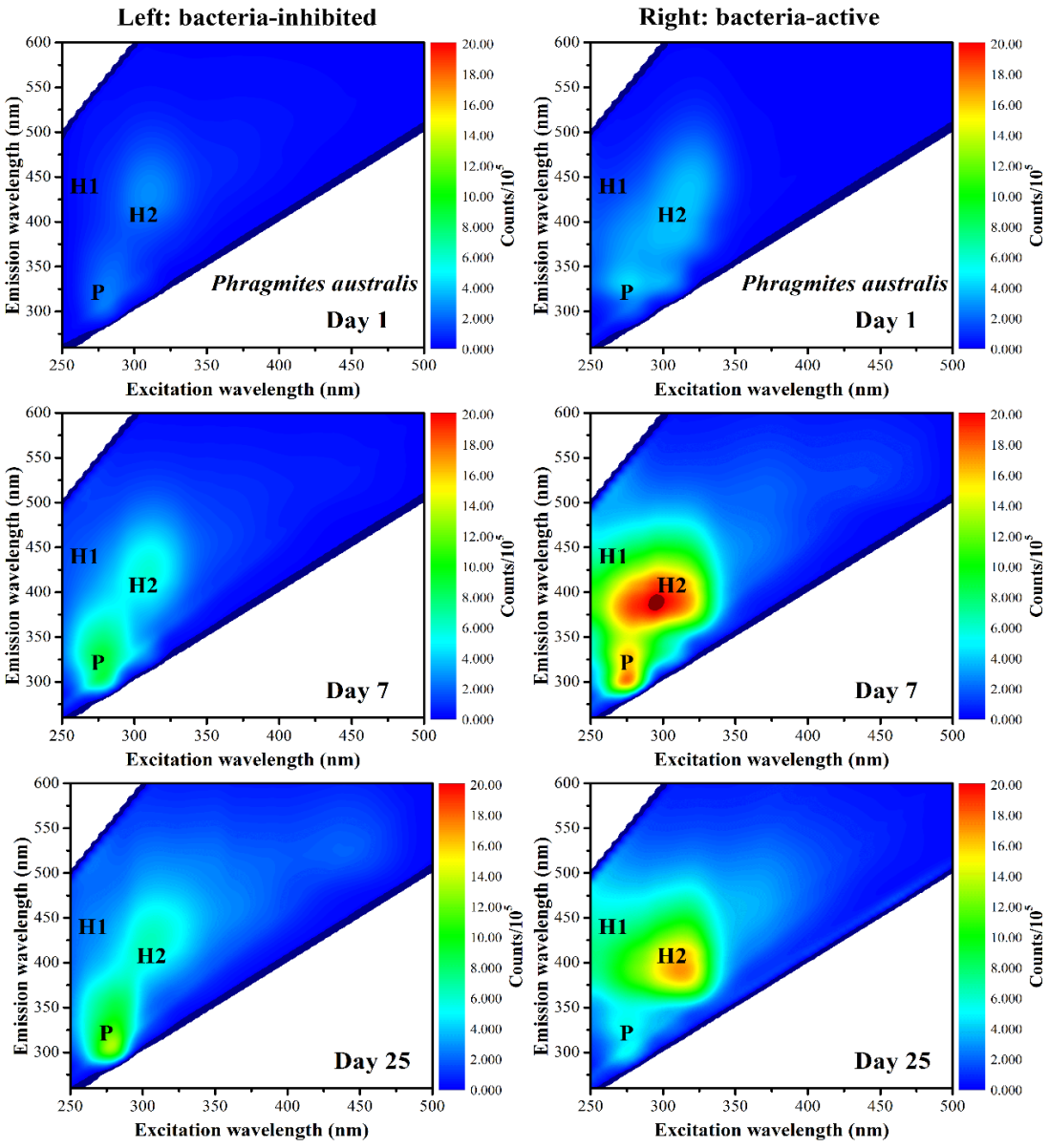

Figure 4: EEMs of CDOM leached from *Phragmites australis* in bacteria-inhibited (left) and bacteria-active (right) incubations.

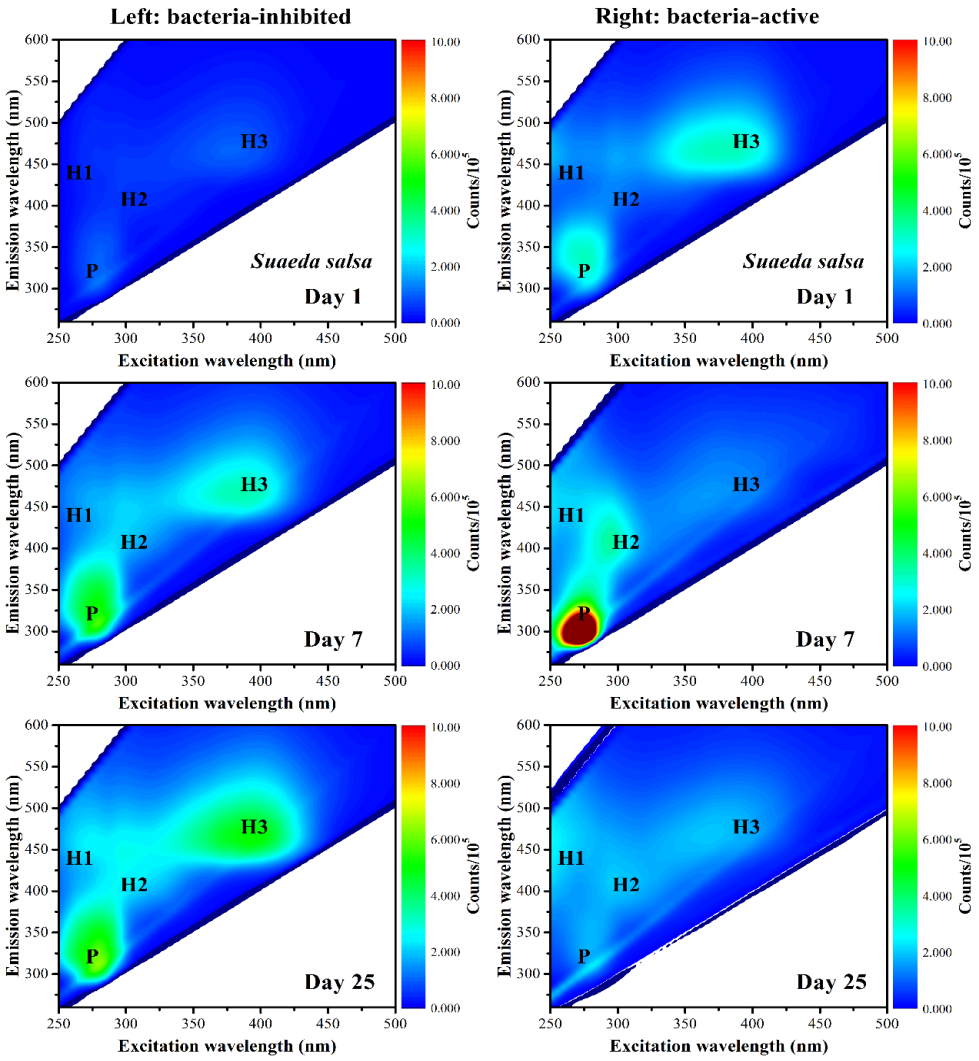

Figure 5: EEMs of CDOM leached from *Suaeda salsa* in bacteria-inhibited (left) and bacteria-active (right) incubations.

7b). Studies have shown that $SUVA_{254}$ is a good indicator of content of aromatic compounds of CDOM [32,33]. At $SUVA_{254}$ value of 3.0, CDOM could contain 23% of aromatic-structured compounds [32]. This suggests that in the initial leaching stage of CDOM from *P. australis* and *S. salsa*, more aromatic-structured compounds were leached out by hydrolysis. In general, humus contains relatively high aromaticity and protein-associated tyrosine and tryptophan both have aromatic structure [24]. With incubation time increase, more non-aromatic compounds were leached out which could decrease the observed SUVA254 values in the bacterial-inhibited incubations. When bacterial degradation of CDOM took place, non-aromatic compounds could be utilized fast and more aromatic structured humic-like CDOM remained in solution, resulted in an increased SUVA254 values in the bacteria-active incubations, consistent with the results of EEM spectra discussed above.

Conclusion

Results of laboratory incubation studies demonstrate that wetland plants *Phragmites australis* and *Suaeda salsa* are important sources of DOC, DN and CDOM in the wetlands. Large amount of DOC, DN and CDOM could be released rapidly from the plants when they submerged in seawater during their senesce stage. The fluorescence measurement indicate that protein-like substances are the main components of CDOM released from both *P. australis* and *S. salsa* in the initial leaching stage and these substances are highly labile and could be degraded rapidly by bacteria. Microbial activities played important roles not only enhanced the leaching processes of DOM, but changed the chemical composition and fluorescent characteristics of CDOM as well. Our results suggest that in the wetlands, *Phragmites australis* and *Suaeda salsa* could act as important sources of DOC, DN and CDOM to the Yellow River estuarine and coastal waters of Bohai Sea, thus not

No.	Ex_{max}(nm)	Em_{max}(nm)	Component	References
C1	275	320	protein-like	[24,43]
C2	310	410	UVC humic-like	[43,49]
C3	380/275	470	humic-like	[44,45]

Table 2: Special characteristics of excitation and emission maximum wavelengths and classification of three components identified by the PARAFAC model.

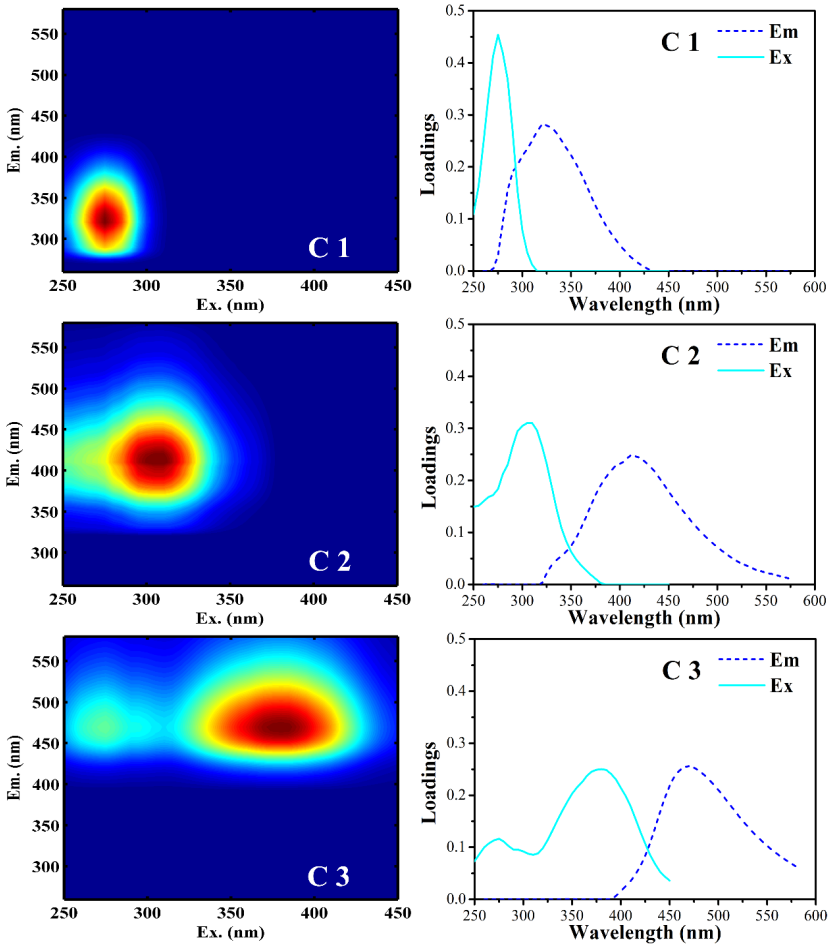

Figure 6: Validation of 3-component model and the spectral characteristics of each component identified by the PARAFAC model. Contour plots of each component are shown on the left. The solid and dotted lines show the excitation and the emission loadings, respectively.

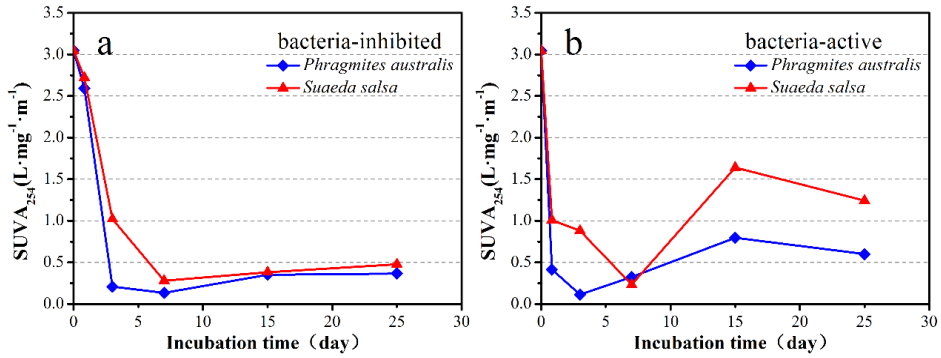

Figure 7: $SUVA_{254}$ values of CDOM leached from *Phragmites australis* and *Suaeda salsa* in (a) bacteria-inhibited and (b) bacteria-active incubations.

only influence the biogeochemical cycle of carbon and nitrogen, but also provide important food sources to microbial communities in the estuary and adjacent coastal waters.

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