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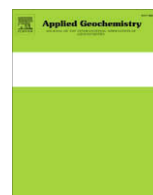
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## Comparative study of dissolved organic matter from groundwater and surface water in the Florida coastal Everglades using multi-dimensional spectrofluorometry combined with multivariate statistics

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### ABSTRACT

Dissolved organic matter (DOM) in groundwater and surface water samples from the Florida coastal Everglades were studied using excitation–emission matrix fluorescence modeled through parallel factor analysis (EEM-PARAFAC). DOM in both surface and groundwater from the eastern Everglades S332 basin reflected a terrestrial-derived fingerprint through dominantly higher abundances of humic-like PARAFAC components. In contrast, surface water DOM from northeastern Florida Bay featured a microbial-derived DOM signature based on the higher abundance of microbial humic-like and protein-like components consistent with its marine source. Surprisingly, groundwater DOM from northeastern Florida Bay reflected a terrestrial-derived source except for samples from central Florida Bay well, which mirrored a combination of terrestrial and marine end-member origin. Furthermore, surface water and groundwater displayed effects of different degradation pathways such as photodegradation and biodegradation as exemplified by two PARAFAC components seemingly indicative of such degradation processes. Finally, Principal Component Analysis of the EEM-PARAFAC data was able to distinguish and classify most of the samples according to DOM origins and degradation processes experienced, except for a small overlap of S332 surface water and groundwater, implying rather active surface-to-ground water interaction in some sites particularly during the rainy season. This study highlights that EEM-PARAFAC could be used successfully to trace and differentiate DOM from diverse sources across both horizontal and vertical flow profiles, and as such could be a convenient and useful tool for the better understanding of hydrological interactions and carbon biogeochemical cycling.

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### 1. Introduction

Dissolved organic matter (DOM) is an assemblage of heterogeneous organic molecules that is ubiquitous in aquatic ecosystems and plays diverse biogeochemical and ecological roles (Findlay and Sinsabaugh, 2003). As such, DOM has been shown to be a driver in microbial loop dynamics and a controlling factor in light availability to aquatic organisms, it can negatively impact water treatment processes and serve as a media for the transport of trace metals and organic pollutants, among others (Lu and Jaffé, 2001;

Cai et al., 2000; Findlay and Sinsabaugh, 2003; Yamashita and Jaffé, 2008).

DOM is derived primarily from decaying organisms such as plants or algae, and is often classified into humic substances and biochemically defined non-humic substances such as proteins, carbohydrates and lipids. Generally speaking, DOM from marine and aquatic sources is more enriched in aliphatic structures while DOM from terrestrial/higher plant sources features more conjugated structure and higher aromaticity (e.g., Maie et al., 2005). While aliphatic structures in DOM tend to affect fluorescence spectra through a blue-shift, highly conjugated structures tend to be characterized by more red-shifted spectra (Coble, 1996). These characteristics have successfully been used to differentiate terrestrial/higher plant derived DOM from marine/microbial sources (e.g., Coble, 1996; McKnight et al., 2001).

Diagenetic processes, such as photodegradation and biodegradation, can also alter DOM structure and composition (Obenosterer and Benner, 2004; Zhang et al., 2009). Humic substances in DOM

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can be directly or indirectly involved in microorganism-catalyzed redox reactions, either as electron shuttles or in some cases as terminal electron acceptors (Scott et al., 1998; Peretyazhko and Spósito, 2006; Ratasuk and Nanny, 2007; Jiang and Kappler, 2008). Cory and McKnight (2005) reported a reduced form of quinone and several partly reduced semiquinone fluorophores in Antarctic lake environments. They also found that the increase of a reduced form of quinone was concurrent with a decrease of an oxidized counterpart below the oxycline. Such studies have been possible thanks to the application of excitation–emission matrix with parallel factor analysis (EEM-PARAFAC) modeling. As such, this technique has ample potential to aid in the better understanding of DOM dynamics in groundwater and associated surface-to-groundwater exchange. However, only few reports have appeared in the literature regarding the use of fluorescent DOM as a natural tracer of groundwater sources, flow, and effects of pollution or land use (Baker and Genty, 1999; Baker, 2001, 2002; Lapworth et al., 2008). The main objective of this study was to apply EEM-PARAFAC to the assessment of DOM dynamics in groundwater from the Florida coastal Everglades (FCE) ecosystem.

The Everglades is one of the largest wetlands in the world and is undergoing an unprecedented ecological restoration aiming at restoring its historic quality, quantity, and timing of water flow (see <http://www.evergladesplan.org/>). A salinity gradient extends along FCE from freshwater marshes in the north, across a mangrove fringe along the coast, to the estuarine environment of Florida Bay and the Gulf of Mexico to the south. Environmental deterioration of this ecosystem due to changes in water quality and water delivery poses increasing urgency for biogeochemical and hydrological research. It is important to point out that most of the N and P in the Everglades are in an organic form (Boyer et al., 1997; Boyer, 2006), and thus associated with DOM. The objectives of the Comprehensive Everglades Restoration Plan (CERP) are to store large amounts of water underground (see <http://www.evergladesplan.org/>). Yet little information is available regarding DOM dynamics in groundwaters of the greater Everglades ecosystem, although active recharge–discharge interactions have been shown to exist (Corbett et al., 1999, 2000a; Price and Swart, 2006; Price et al., 2006; Harvey et al., 2006).

The sources of DOM to surface water in the FCE include: (1) autochthonous production by organisms such as emergent, floating, and submerged vegetation and periphyton; (2) oxidation of soil organic matter; (3) precipitation; (4) exchange with underlying ground water; and (5) canal inputs. Possible inputs of DOM to groundwater include: (1) recharge from the overlying surface water; (2) groundwater flow from upstream of the FCE; (3) desorption from the soils/sediments en route of percolating waters to the aquifer; (4) upward diffusion from deeper aquifers (Reese and Cunningham, 2000); and (5) possible input from the ocean side due to sea water intrusion. To better assess these surface-to-ground water dynamics for DOM, EEM-PARAFAC was applied in an attempt to discriminate DOM sources and quality among surface water and groundwater from different locations in the FCE, and in the process, trace the hydrological interactions between surface and groundwater. In the upland regions of the Everglades, where both the groundwater and surface water are fresh, it is hypothesized that a rapid exchange of surface water and groundwater would be seen given the karst nature of the limestone aquifer leading to a similar freshwater DOM signature in both water bodies. In the region of Florida Bay, the groundwater beneath the bay is salty to hypersaline with brackish water intruding into the aquifer beneath the mainland. It is hypothesized that DOM with a seawater signature similar to that found in the surface water of Florida Bay would be found in the underlying groundwater as well as in the brackish groundwater intruding beneath the mainland.

## 2. Experimental methods

### 2.1. Sites description

The eastern border of Everglades National Park and the NE section of Florida Bay were selected as the study sites for this project (Fig. 1). The region is immediately underlain by the Biscayne Aquifer. The Biscayne Aquifer is one of the most permeable karst aquifers in the world (Parker et al., 1955). The aquifer extends from Palm Beach County in the north to under Florida Bay and the Florida Keys in the south, and forms a wedge-shape with a thin edge along its western boarder and thickening in a SE direction to over

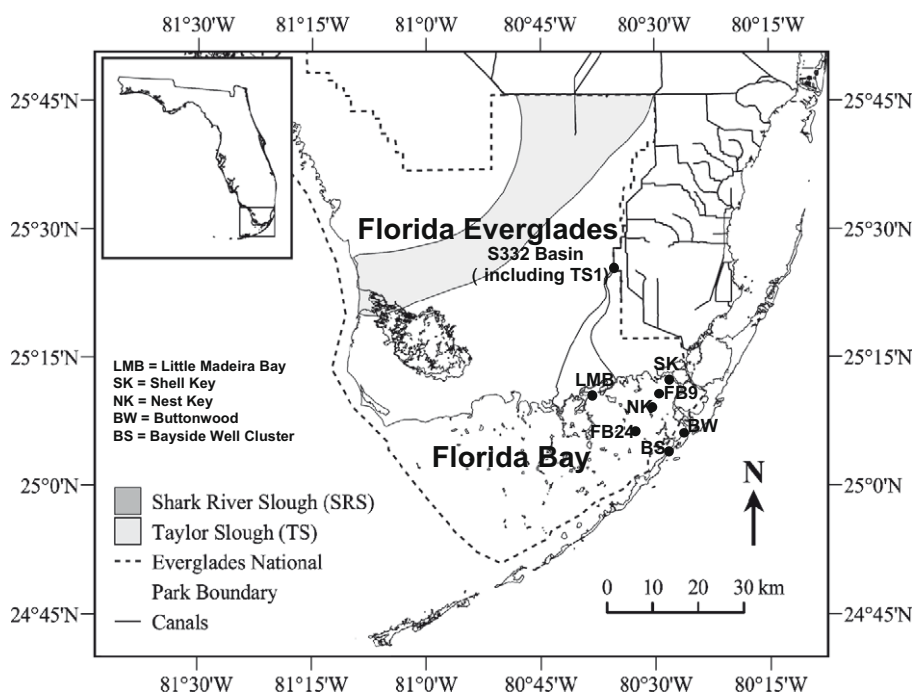


Fig. 1. Sampling sites in the Florida coastal Everglades (FCE). Groundwater and surface water were collected from the S332 basin area and Northeastern Florida Bay.

65 m along the coastline (Fish and Stewart, 1991; Bradner et al., 2005; Parker et al., 1955). The geology of the Biscayne aquifer is dominantly marine and freshwater carbonates deposited during the Pleistocene, overlain by recent deposits of peat and marl (Cunningham et al., 2006). The inorganic water chemistry of the Biscayne Aquifer beneath the FCE is dominantly Ca–HCO<sub>3</sub>, typical for groundwater in contact with limestone, except for along the coastline where seawater intrudes into the aquifer and shifts the groundwater chemistry to a Na–Cl type of water (Harvey and McCormick, 2009; Price and Swart, 2006). The shallow groundwater (<30 m) in the Biscayne Aquifer has been dated using <sup>3</sup>H/He age dating techniques to be less than 30 a (Price et al., 2003; Harvey et al., 2006). In contrast, the <sup>14</sup>C age of humic material isolated from groundwater from the Biscayne aquifer was found to be on average 683(±50) a (Thurman, 1985).

The study sites include the S332 (25.422°N, 80.590°W) basin area of Everglades National Park (ENP) in the north and Florida Bay in the south. The S332 basin area was historically a part of the natural wetlands of the Everglades, but was farmed for a time, and has since been incorporated into a water treatment basin. Most surface water flow in the basin is controlled through pumps that transfer water from the adjacent L31 canal. This water is allowed to drain slowly from the basin into the headwaters of Taylor Slough, the natural surface water flow-way in the region, or to infiltrate into the groundwater table. The purpose of the basin is to retain nutrients (P and N) that may be in the canal water prior to its release to Taylor Slough.

Florida Bay is a coastal lagoon lying between the southern tip of the Florida mainland and the Florida Keys (Fourqurean and Robblee, 1999). Here several sites were studied including Little Madeira Bay (LMB; adjacent to the outflow of the Taylor River) and Shell Key (SK), located on the coastal side of Florida Bay, where fringe mangrove forests are the dominant vegetation. Offshore sites include Nest Key (NK), FB9, and FB24, sites covered by a thin layer of calcitic marl underlying a blanket of seagrass. Two sites were located along the Florida Keys, namely the Buttonwood (BW) and Bay Side (BS) sites, both are in proximity to mangrove and urban environments.

## 2.2. Sampling

Groundwater from 21 wells in S332 basin was collected quarterly between September 2006 and September 2007. In addition, groundwater from 12 wells at 5 sites, scattered throughout north-eastern Florida Bay (Fig. 1), were collected in September 2007. At 4 of the sites, groundwater samples were pumped from both shallow (depth = 4.6–6.1 m) and deep (depth = 8.2–19.8 m) wells (LMB, SK, BS, and BW) while at NK groundwater was collected from a deep (depth = 11 m) well only. Prior to sampling of each well, they were purged of at least three well volumes using a high flow pump. During purging, specific water quality data, such as salinity, pH, and dissolved O<sub>2</sub>, was monitored until stable readings using an Orion meter (Table 1). Samples were pumped using a low flow peristaltic pump and filtered through a 0.45 μm filter (HDPE, MilliPore

**Table 1**  
Summary of water chemistry and optical parameters (mean ± SD).

Items	Unit	Sites											
		S332 surface (n = 38)	S332 ground (n = 42)	Florida Bay surface (n = 63)	Florida Bay ground								
					LMB		SK		NK	BS		BW	
			Shallow	Deep	Shallow	Deep	Deep	Shallow	Deep	Shallow	Deep		
Depth	(m)	0	4.9 ± 1.4	0	5.2	8.2	4.6	12.5	11	6.1	13.7	4.6	19.8
Salinity		Fresh water	Fresh water	37.7 ± 7.8	42.5	42.7	38	35.3	39.9	33.7	39.1	35.8	38
pH		N/A	7.3 ± 0.2	8.2 ± 0.2	7.2	7.2	6.9	7.2	7.3	7	7	7.3	7.4
DO <sup>a</sup>	(mg/L)	N/A	0.4 ± 0.1	6.3 ± 1.9	0.3	0.6	0.3	0.3	0.4	0.5	0.1	0.6	0.5
DOC <sup>b</sup> and TOC <sup>c</sup>	(mg/L)	9.9 ± 6.3 DOC	8.6 ± 2.3 DOC	7.7 ± 2.3 DOC	5 TOC	4.9 TOC	4.9 TOC	6 TOC	3.8 TOC	3.9 TOC	4.4 TOC	5 TOC	6.6 TOC
α(254) <sup>d</sup>	(m <sup>-1</sup> mg <sup>-1</sup> L)	6.3 ± 1.4	4.3 ± 2.0	3.0 ± 0.7	3.5	3.8	7.0	4.5	4.8	9.7	5.7	7.8	8.7
α(254) <sup>e</sup>	(m <sup>-1</sup> )	54.8 ± 10.8	39.5 ± 7.3	22.1 ± 4.6	17.7	18.5	34.2	27.2	18.3	37.8	25	38.9	57.2
S <sub>R</sub> <sup>f</sup>		1.0 ± 0.0	1.0 ± 0.1	1.3 ± 0.2	1.2	1.2	1.1	1.1	1.1	1.5	1.6	1.1	1.1
FI <sup>g</sup>		1.4 ± 0.0	1.5 ± 0.0	1.5 ± 0.1	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.5	1.5
C1	(%) <sup>h</sup>	31.9 ± 2.8	35.0 ± 1.8	22.3 ± 2.2	36.7	38.8	41.8	41.0	29.5	26.5	28.8	37.0	39.5
	(QSU) <sup>i</sup>	82.7 ± 21.4	95.1 ± 16.1	13.3 ± 4.8	48.9	50.6	96.8	80.1	47.5	38.4	42.9	97.8	122.9
C2	(%)	5.8 ± 2.3	5.0 ± 1.7	14.8 ± 2.6	0.0	0.0	0.0	0.4	0.0	0.5	0.0	2.1	0.8
	(QSU)	15.5 ± 8.6	14.2 ± 6.3	8.4 ± 2.2	0.0	0.0	0.0	0.8	0.0	0.8	0.0	5.6	2.5
C3	(%)	15.6 ± 5.0	7.6 ± 1.8	3.8 ± 2.0	0.0	0.0	1.2	0.6	0.0	0.1	0.0	2.1	5.2
	(QSU)	38.6 ± 9.9	20.9 ± 6.8	2.4 ± 1.8	0.0	0.0	2.8	1.2	0.0	0.1	0.0	5.4	15.9
C4	(%)	12.7 ± 0.6	13.5 ± 0.5	16.0 ± 1.2	16.5	16.5	14.3	14.9	13.7	11.8	12.3	13.6	12.5
	(QSU)	32.8 ± 7.3	36.8 ± 7.3	9.3 ± 2.7	22.0	21.5	33.1	29.1	22.1	17.1	18.4	36.0	39.0
C5	(%)	14.0 ± 0.7	13.8 ± 0.6	9.6 ± 0.7	13.1	13.6	15.4	14.2	12.6	21.0	12.7	16.2	17.5
	(QSU)	36.0 ± 8.5	37.7 ± 6.8	5.6 ± 1.7	17.4	17.7	35.6	27.8	20.3	30.5	19.0	42.9	54.6
C6	(%)	8.4 ± 3.9	14.2 ± 1.1	10.2 ± 1.2	21.1	21.8	20.5	21.0	17.2	17.9	16.2	17.2	15.5
	(QSU)	22.8 ± 12.4	38.6 ± 6.4	5.9 ± 1.8	28.1	28.4	47.6	41.1	27.6	26.0	24.2	45.3	48.3
C7	(%)	7.0 ± 2.5	7.0 ± 2.4	15.1 ± 3.5	8.3	5.1	3.6	4.4	12.3	13.2	11.9	8.8	6.6
	(QSU)	17.4 ± 6.0	19.1 ± 7.6	8.6 ± 2.4	11.1	6.7	8.4	8.6	19.7	19.3	17.9	23.4	20.8
C8	(%)	4.6 ± 1.2	3.9 ± 0.6	8.3 ± 2.1	4.3	4.2	3.2	3.4	14.7	9.0	18.0	2.9	2.5
	(QSU)	11.6 ± 2.5	10.7 ± 2.1	4.9 ± 2.2	5.7	5.4	7.3	6.7	23.6	13.0	30.6	7.8	7.7

N/A: not measured.

<sup>a</sup> DO: dissolved oxygen.

<sup>b</sup> DOC: dissolved organic carbon.

<sup>c</sup> TOC: total organic carbon. Most TOC is DOC in Florida Bay surface water.

<sup>d</sup> α(254 nm): specific α (254 nm) normalized to DOC.

<sup>e</sup> α(254 nm): absorption coefficient = UV absorbance at 254 nm times 2.303.

<sup>f</sup> S<sub>R</sub>: slope ratio of UV absorbance (275–295 nm to 350–400 nm).

<sup>g</sup> FI: Fluorescence Index – fluorescence emission intensity ratio of 470–520 nm at excitation wavelength of 370 nm.

<sup>h</sup> (%): relative abundance = absolute fluorescence intensity of each component divided by the sum of all the eight components' absolute values then times 100%.

<sup>i</sup> (QSU): absolute fluorescence intensity of EEM-PARAFAC components in quinine sulphate unit.

groundwater sampling capsule) in situ. A syringe was attached to the end of the filter, and water samples were collected in glass Vacutainer tubes which were first pre-cleaned in dilute (10%) HCl, flushed with He and then evacuated with a vacuum pump in an attempt to remove O<sub>2</sub>. Collected samples were stored in a cooler with ice, transported to the lab on the same day, and then stored in a refrigerator for measurements within 3 days.

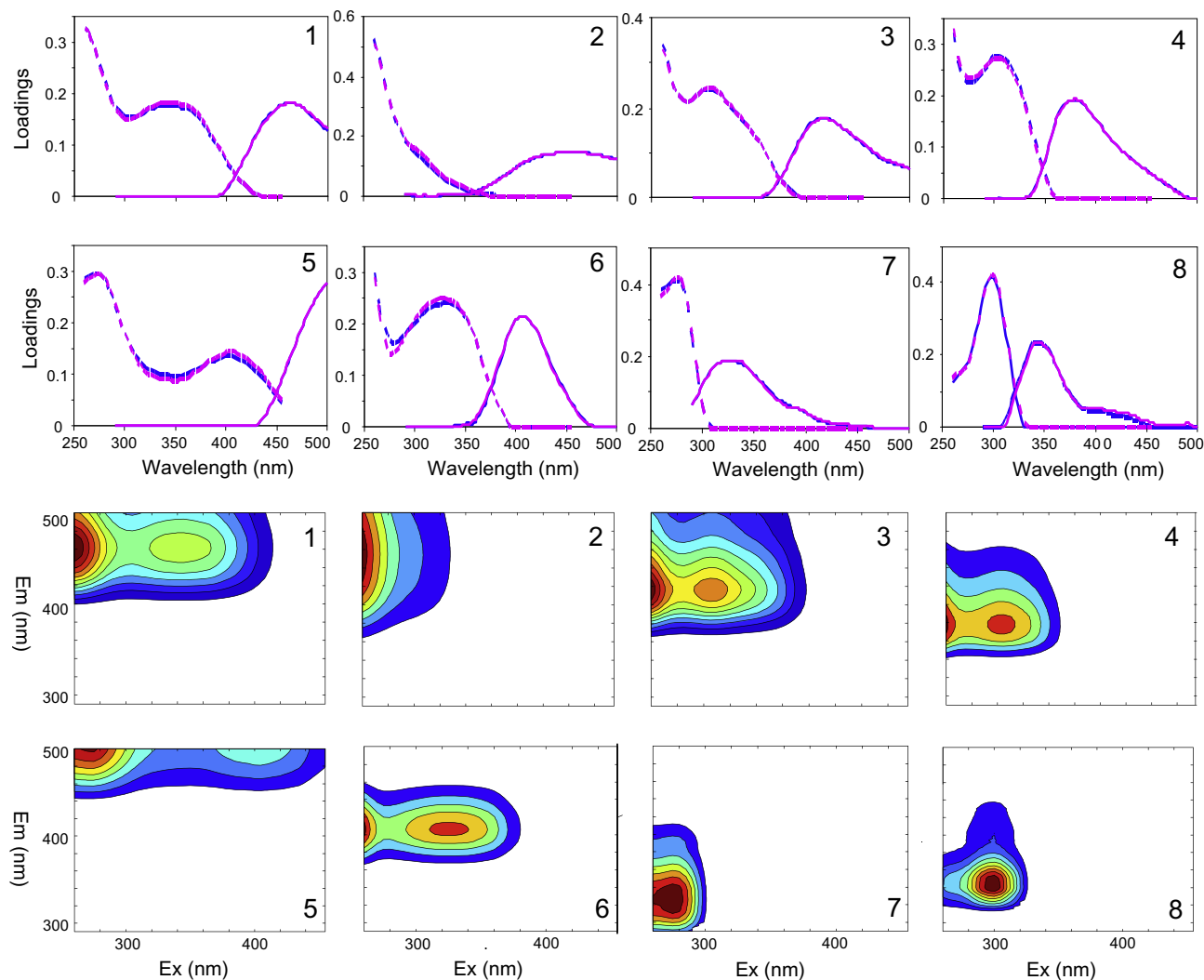
Surface water samples were collected monthly between October 2004 and September 2008 for TS1 (in S332 basin) and FB9 sites and from October 2004 to September 2006 for the FB24 site. The TS1 site became dry during the dry season (typically between January and May) so samples were primarily collected during the wet season. The FB9 and FB24 sites were selected since they are close to the groundwater sites and representative of marine surface water. Samples were collected in pre-cleaned, acid-washed, brown high density polyethylene bottles (Nalgene). Containers were rinsed three times before sample collection. All surface water samples were filtered in the lab with pre-combusted 0.7 µm GF/F filters and stored in a refrigerator until analyses within one week of collection.

### 2.3. Analytical measurements

DOC concentrations were measured using the high-temperature catalytic combustion method with a Shimadzu TOC-V total or-

ganic carbon analyzer. Samples were acidified to pH < 2 with 3 N HCl and were purged with CO<sub>2</sub> free air to remove inorganic C before measurements. DOC data were used to calculate the specific ultraviolet absorbance,  $a(254)^*$ , which is an indicator of DOM aromaticity and defined as UV absorption coefficient  $a(254)$  in inverse meters normalized to DOC concentration in mg/L (Hansell and Carlson, 2002; Weishaar et al., 2003). Due to very low levels of dissolved Fe in South Florida waters  $a(254)^*$  values were not corrected for Fe interference (Weishaar et al., 2003). The  $a(254)$  was determined using a Varian Cary 50 bio spectrophotometer with a 1 cm quartz cuvette scanning from 240 nm to 800 nm. The UV-Vis spectra were also used for inner filter correction for the EEMs according to McKnight et al. (2001) and to determine the slope ratio ( $S_R$ ) of 275–295 nm to 350–400 nm, as a proxy for the molecular weight distribution of DOM (Helms et al., 2008). The  $S_R$  value is an indirect measure of the average molecular weight of DOM, where low values are indicative of higher molecular weight, while high values indicate lower molecular weight distributions.

EEMs were measured using a Horiba Jovin Yvon SPEX Fluoromax-3 spectrofluorometer equipped with a 150 W continuous output Xe arc lamp. Slits were set at 5.7 nm for excitation and 2 nm for emission. Forty-four emissions scans were acquired at excitation wavelength ( $\lambda_{ex}$ ) between 240 and 455 nm at 5 nm steps. The emission wavelengths were scanned from  $\lambda_{ex} + 10$  nm to  $\lambda_{ex} + 250$  nm (i.e., between 250 and 705 nm) in 2 nm steps (Maie



**Fig. 2.** Spectral characteristics and contour plots of 8-component model for FCE surface water dataset ( $n = 1394$ ). The Dotted lines show the excitation loadings and the solid lines show the emission loadings. The split-half validation data are shown.

et al., 2006b). The 44 individually scanned spectra were concatenated to form EEMs. All fluorescence signals were acquired in signal over reference ratio mode (S/R) to eliminate potential fluctuation of the Xe lamp. Several post-acquisition steps were also carried out for correction and standardization: (1) inner filter effect correction using UV–Visible data according to McKnight et al. (2001); (2) instruments biases correction were performed using specific excitation and emission correction files provided by the manufacturer; (3) blank subtraction using Milli-Q water was performed; (4) daily fluorescence intensity variations were corrected using the area under the Milli-Q water Raman peak at excitation 350 nm (Cory and McKnight, 2005); and (5) all fluorescence measurements were converted to quinine sulfate units (QSU). The Fluorescence Index (FI) was determined as the ratio of the emission intensity at a wavelength of 470 nm to that at 520 nm, obtained with an excitation of 370 nm. FI values for DOM commonly range between 1.1 for terrestrially dominated DOM sources to 1.8 for microbially dominated DOM sources (Jaffé et al., 2008).

For groundwater samples, caution was taken to avoid O<sub>2</sub> exposure and possible DOM oxidation before or during EEM measurements. As described above, the samples were collected in vacuumed tubes and transferred to a sealable cuvette in an inert Ar gas box for measurements.

#### 2.4. Parallel factor analysis (PARAFAC)

PARAFAC is a statistical tool used to decompose multi-way data into different components. It is based on an alternating least square (ALS) algorithm. Thus, PARAFAC can statistically decompose EEMs into fluorescent groups (components). There are two ways to apply PARAFAC modeling, i.e., by creating and validating the model using the complete dataset of EEMs (e.g., Stedmon et al., 2003; Ohno and Bro, 2006) or by fitting the EEMs to an already established PARAFAC model (e.g., Yamashita and Jaffé, 2008; Fellman et al., 2009). Here groundwater EEMs were fitted to an existing Everglades/Florida Bay surface water PARAFAC model which had been well established using 1394 surface water samples (Fig. 2). The analysis was carried out in MATLAB 7.0.4 (Mathworks, Natick, MA) with the DOMFluor toolbox (Stedmon and Bro, 2008). No obvious residues were found after fitting the groundwater EEMs to the established eight components model, indicating that this model was applicable to groundwater DOM studies and that the fluorophores were similar between groundwater and surface water in the collected samples. PARAFAC component spectral characteristics and split-half validation data are shown in Fig. 2.

### 3. Results and discussion

#### 3.1. Water chemistry and optical parameters

Both surface water and groundwater from the S332 area were fresh as indicated by low salinity values while both surface water and groundwater from FB had salinity >30 (Table 1). The pH measurements ranged from 6.9 to 8.4 for all samples. FB groundwater had generally lower pH values compared to FB surface water. Dissolved O<sub>2</sub> values for all the groundwater samples were less than 1 mg/L compared to 6.3 (±1.9) mg/L for surface samples, implying suboxic conditions in the studied wells.

Bulk DOC data for S332 surface water, S332 groundwater and FB surface water showed average concentrations of 9.9 ± 6.3, 8.6 ± 2.3 and 7.7 ± 2.3 mg/L (see Table 1). DOC values for FB groundwater were not determined due to sample volume limitations, and only TOC (unfiltered samples) data was available for the latter. TOC values were used as equivalent to DOC for comparison purpose. Under this assumption, FB groundwater had the lowest DOC values com-

pared to the other surface and groundwater samples, indicating possible removal or dilution processes such as absorption by sediments and/or aquifer materials, bio-degradation processes, or dilution by low DOC water from underneath. DOC for the S332 surface water showed a much larger range (9.9 ± 6.3 mg/L) than at the other sites, suggesting a bigger seasonal change for this site than groundwater and also FB surface water.

The *a*(254)<sup>\*</sup> data confirm previous reports (Maie et al., 2005, 2006a) that FB surface water DOM is characterized by low aromaticity, in accordance with its microbial DOM origin and potential degradation effects due to intense solar radiation on the shallow waters of the Bay. The S332 surface water DOM showed the highest absorption coefficient *a*(254) values and several FB groundwater sites such as the BW site also displayed comparably elevated values. BS groundwater samples were observed having the highest slope ratio *S<sub>R</sub>* value of 1.5 for the shallow well (6.1 m) and 1.6 for the deep well (13.7 m), as compared with 1.0–1.3 for all the other samples, indicative of smaller molecular weight for DOM at this site. The FI values ranged from 1.4 to 1.6, with S332 surface water samples having the lowest values, consistent with their terrestrial (higher plant/soil) origin.

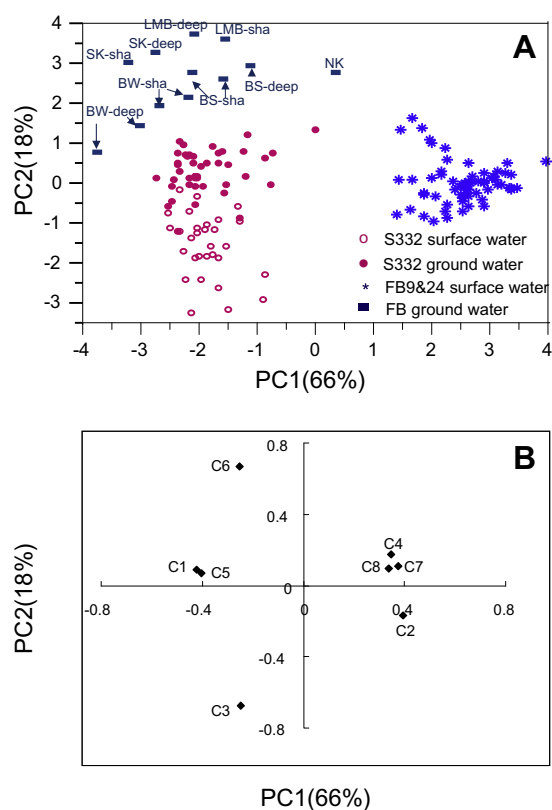
#### 3.2. Classification of water samples via PCA

EEM data were fitted to a previously established 8-component PARAFAC model for Florida Everglades surface water DOM (*n* = 1394). The advantage of using this model is that it can facilitate the direct comparison between FCE groundwater and surface water. No obvious residues were obtained from this model for this dataset. The spectral characteristics and contours of the modeled PARAFAC components are presented in Fig. 2. From the comparison of spectral characteristics of each component (Fig. 2) with those reported in previous studies (e.g., Stedmon and Markager, 2005; Cory and McKnight, 2005; Yamashita and Jaffé, 2008; Murphy et al., 2008; Santin et al., 2009), components 1, 2, 3, 5 and 6 were assigned as terrestrial or ubiquitous humic-like components, component 4 was assigned as a microbial (marine) humic-like component while C7 and C8 were protein-like components with the former tyrosine-like (and/or blue-shifted tryptophan-like) and the latter tryptophan-like. The trends in the absolute and relative abundance of EEM-PARAFAC data across a terrestrial to marine gradient will be discussed in detail later (see Fig. 4 below).

In an attempt to use the PARAFAC data as a fingerprinting tool for DOM source and quality, the relative abundance of all the EEM-PARAFAC components for all groundwater and surface water samples studied here (see Table 1 for definitions and calculations), were used for Principal Component Analysis (PCA). The PCA graph display (Fig. 3) produced different clusters, suggesting that samples could be classified according to DOM origins and different environmental conditions. Principal component 1 explained 66% of the variance, whereas principal component 2, accounted for a further 18% of the variance. The differences among these clusters are likely controlled by a variety of factors including DOM source strength, degree of diagenetic degradation, hydrological conditions and redox state.

##### 3.2.1. Effects of DOM sources and precursor types

Potential sources of DOM in groundwater and surface water in the FCE have been discussed earlier, and should play a central role in the PCA classification of the samples. From the loading plots of Fig. 3B, S332 surface water, S332 groundwater, and most FB groundwater (except for the NK site) seemed mostly controlled by the loadings of the terrestrial or ubiquitous humic-like components (C1 and C5), while Florida Bay surface water samples displayed more marine/microbial humic-like and protein-like signatures (C4, C7, and C8). The PC1 of the NK site from FB fell in



**Fig. 3.** Principal Component Analysis for EEM-PARAFAC results of all the surface and ground water samples. (A) Score plot. (B) Loading plot (sha = shallow).

the middle between the terrestrial and marine end-members, suggesting combined effects from both sources. Thus, PC1 seems to control the clustering based on DOM source. In contrast, for PC2 surface water samples seemed more controlled by the loadings of components C2 and C3 while the groundwater samples were more dependent on component C6. FB groundwater featured either extremely low levels or absence of components C2 or C3, with the exception of the BW site. Thus, PC2 seems dependent on both photo- and bio-degradation of DOM as described below.

Most sample types clustered separately on the PCA plot of the EEM-PARAFAC data (Fig. 3), and as such were distinguishable based on their fluorescence characteristics. The exceptions were several surface freshwater samples from June, July and August, and several groundwater samples from the S332 basin which were overlapping at the freshwater surface to groundwater clusters. This would not be surprising considering that the Biscayne aquifer is shallow and highly permeable, and the sources of groundwater recharge in the S332 basin are primarily from the overlying surface water in the wet season and from interactions with canals that penetrate the Biscayne Aquifer in the dry season (Price and Swart, 2006; Genereux and Slater, 1999). Harvey et al. (2006) found that surface water and groundwater 'actively exchanged' in the central Everglades, and estimated the storage depth of interactive groundwater as 3.1 m after adjustment for the porosity of different soil types. This was consistent with the study observations that the DOM samples from wells at depths of around 3.1 m were more similar in their optical properties to surface water than those of most of the deeper wells. Some deeper well samples also fell within this region, probably due to specific site characteristics such as sinkholes and conduits of higher porosity allowing for pathways of preferential flow (Cunningham et al., 2006). Geochemical evidence of surface water interactions with deeper wells in the vicin-

ity of the S332 basin was also reported by Price et al. (2003) and Wilcox et al. (2004), and explained by vertical and horizontal conduits in the karst limestone allowing for rapid infiltration of surface water or rainwater to deeper depths.

### 3.2.2. Diagenetic effects by photo-degradation and bio-degradation

As stated above, photo-induced transformations of biomass and soil-derived DOM from Florida coastal Everglades may be an important degradation pathway (Scully et al., 2004; Maie et al., 2008), and could be significant on a relatively short timescale. In contrast to the intense sunlight exposure to surface waters, especially in FB, light is not available in the subsurface. Hence, the groundwater DOM is expected to be less photodegraded than the surface water. This may explain why the relative abundance of C2, which has been considered to be a photoproduct or a photorefractory component (Chen unpublished data; Stedmon et al., 2007), was sparse in groundwater but more abundant in surface water as seen from Fig. 4. Therefore, the distribution of samples along PC2 may in part be controlled by the loadings of the photo-resistant component C2.

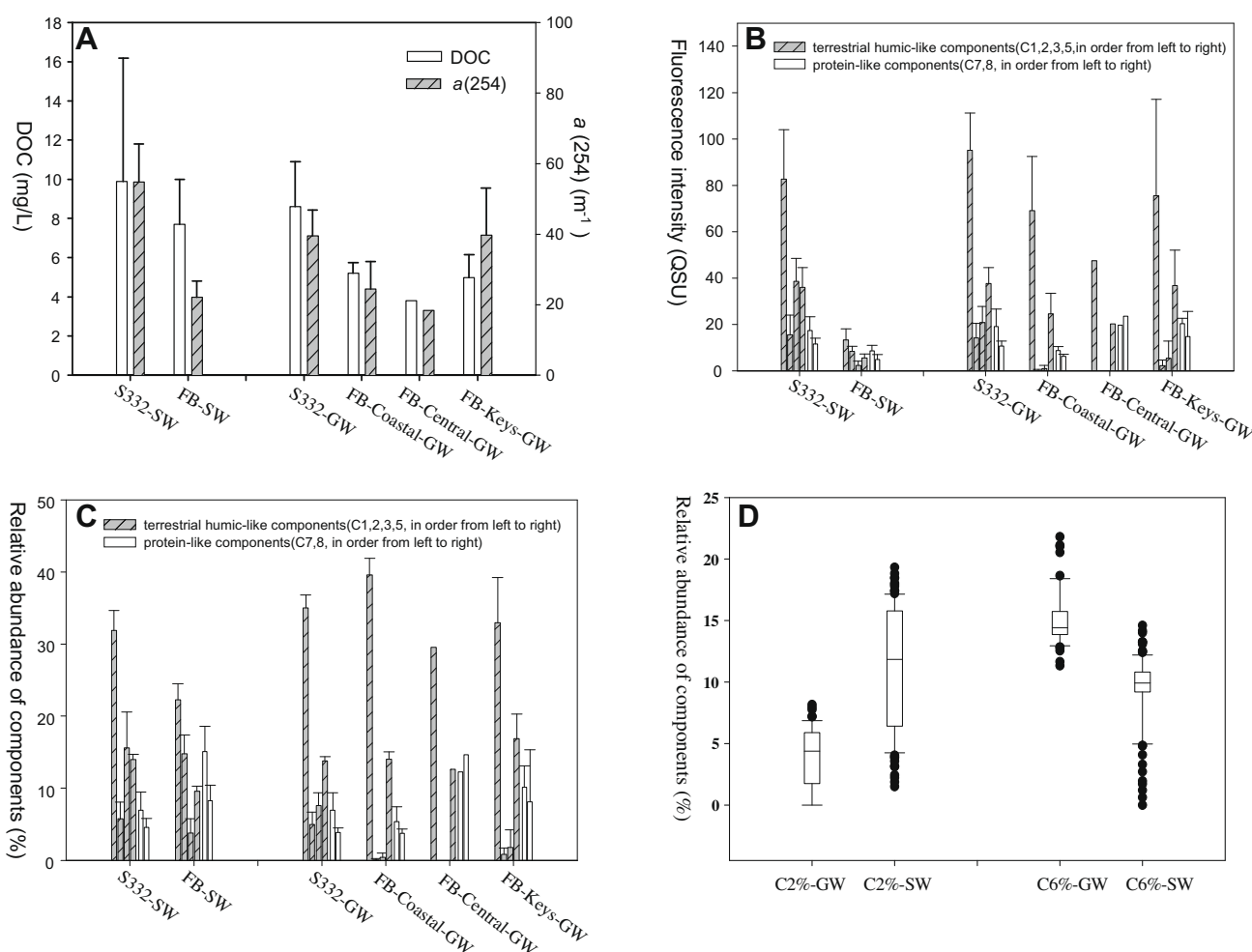
On the other hand, in addition to photo-exposure, DOM is also subjected to microbial degradation. Maie et al. (pers. comm.) found an inverse relationship between relative abundance of C3 and C6 ( $R = -0.73$ ) in FB suggesting that C6 might be a degradation product of C3. Based on the data shown in Fig. 3, and assuming that component C6 is in fact a microbial degradation product, it seems that groundwater DOM is more prone to bio-degradation than that of surface waters, and in fact Analysis of Variance (ANOVA) showed that the C6 was significantly higher in groundwater than in surface water ( $P < 0.0001$ , see box plot in Fig. 4). In addition, C6 has been recently suggested to be highly photo-reactive (Chet et al. unpublished), and could become enriched in GW. Consequently, the spread of the clusters as determined by PC2 seems to be controlled by DOM degradation/modification processes.

### 3.3. Trends of DOC, $a(254)$ , and EEM-PARAFAC components

Sample locations were grouped into six groups along the terrestrial to marine (N–S) transect. They are: S332 surface water (S332-SW), S332 groundwater (S332-GW), coastal FB groundwater sites LMB-GW and SK-GW (FB-Coastal-GW), Florida Bay surface water (FB-SW), central-east FB groundwater site NK (FB-Central-GW), and the Florida Keys groundwater sites BS-GW and BW-GW (FB-Keys-GW). The DOC,  $a(254)$ , and absolute and relative abundance of selected EEM-PARAFAC components were plotted and compared among these six groups (Fig. 4).

As seen in Fig. 4A, both DOC and  $a(254)$  values were significantly higher in the S332-SW compared to FB-SW, and initially decreased gradually across the N–S transect to increase again for FB-Keys-GW. This trend suggests a dilution of terrestrial DOM along the N–S transect, but additional DOM sources in groundwater adjacent to the Florida Keys. FB-SW had rather high DOC but low  $a(254)$ , suggesting that non-aromatic DOM such as carbohydrates make up an important fraction of the DOM in this region (Maie et al., 2005, 2006a). FB-SW had significantly higher levels of DOC compared to the samples from FB-Coastal-GW and FB-Central-GW, although the  $a(254)$  were quite similar.

Absolute and relative abundance of terrestrial or ubiquitous humic-like (C1, C2, C3, and C5) and protein-like (C7 and C8) EEM-PARAFAC components are shown in Fig. 4B and C, respectively, and mimic each other in their distribution. Marine humic-like component C4 and microbial-modified component C6 had different patterns as discussed below, and thus were not included for comparison here. Basically, fluorescence intensity values for individual components showed similar pattern as for DOC and  $a(254)$  although some components had higher fluorescence readings in



**Fig. 4.** Trends of UV absorption coefficient at 254 nm, DOC, and absolute (fluorescence intensity) and relative (% of total) abundance of EEM-PARAFAC terrestrial or ubiquitous humic-like (C1, C2, C3, and C5) and protein-like (C7 and C8) components. Box plot compared C2% and C6% between ground and surface water ( $P < 0.0001$ ). SW = surface water; GW = groundwater; FB = Florida Bay.

the GW samples compared to the SW samples. The four different terrestrial or ubiquitous humic-like components did not show the same behavior, with C1 and C5 being more enriched at all GW sites, whereas the abundance of humic-like components C2 and C3 were very much lower or even non-detectable in most of the Florida Bay GW. This suggests that the composition of SW and GW samples, particularly in FB are quite different possibly due to DOM source changes and to differences in DOM processing. While C2 has been suggested as a photo-product or photo-refractory component of DOM (Chen et al., unpublished data; Stedmon et al., 2007) and thus is more abundant in SW than GW, C3 may be a more biodegradable component, with higher absorption capacities to aquifer materials and/or precipitates out at high salinities and thus has a low tendency to accumulate in GW.

With respect to the protein-like components (C7 and C8), these were mostly enriched in FB samples, particularly FB-SW, and with C7 being more prominent compared to C8 in most samples except for FB-Central-GW and FB-Keys-GW. The tryptophan-like C8 has been reported to be more biodegradable, while the tyrosine-like (or blue-shifted tryptophan-like) C7 has been suggested as more refractory in natural aquatic environments (Yamashita and Tanoue, 2003, 2004; Maie et al., 2006b). However, it is possible that C8 preferentially accumulates as DOM in GW ages, and it may be more abundant at the FB-Keys-GW locations due to inputs from septic

tanks. The marine environment adjacent to the Florida Keys is affected by anthropogenic activities such as wastewater disposal and runoff, and most houses in the Florida Keys are still on septic tanks (Shinn et al., 1994; Corbett et al., 2000b; Cable et al., 2002). High EEM protein-like fluorescence intensities have previously been reported as typical of sewage impacted waters (Baker, 2001; Lamont-Black et al., 2005; Lapworth et al., 2008).

A box plot comparison of C2% and C6% between surface water and groundwater from both S332 and FB sites (Fig. 4D), shows that GW samples had a much lower relative abundance of C2 compared to SW samples, while the opposite was true for C6. This would make sense if the abovementioned suggestion that C2 is photo-resistant or a photo-degradation byproduct was correct. As for C6, another humic-like component that has previously been reported as being exported from catchments with high agricultural use (Stedmon and Markager, 2005), its enrichment in GW samples may be indicative of its high photo-reactivity or its nature as a microbial degradation product.

The combination of a terrestrial CDOM fingerprint and high salinity ( $38.6 \pm 3.7$ ) for FB-Coastal-GW and the Florida-Keys-GW samples suggests a possible combination of vertical surface water recharge, with horizontal GW exchange, where the later is particularly important for the FB coastal wells as a means of transport of a terrestrial enriched DOM fingerprint. Groundwater transport

and exchange from the freshwater Everglades to the central portion of Florida Bay is highly unlikely due to extensive seawater intrusion into the Biscayne aquifer along the entire coastline of the FCE (Price et al., 2006; Fitterman et al., 1999). However, significant exchange of groundwater from beneath the Florida Keys into Florida Bay has been documented due to differences in surface water levels between Florida Bay and the Atlantic Ocean over a tidal cycle (Shinn et al., 1994; Corbett et al., 1999; Chanton et al., 2003). The tidal cycling drives groundwater back and forth beneath the Keys between Florida Bay and the Atlantic Ocean. The resulting groundwater beneath the Keys and the submarine groundwater discharge into Florida Bay often has salinity close to 36 (Chanton et al., 2003). Thus, the dominant source of DOM for the FB coastal sites should be from a source that is both saline and contains high levels of terrestrial/higher plant (mangrove) derived DOM. The Florida Keys can provide this DOM source from mangrove soils and septic tank effluents. The contribution of soil-derived DOM from mangrove peat oxidation would agree with the 'old' DOM signature based on  $^{14}\text{C}$  dating (Thurman, 1985) previously reported in Biscayne aquifer DOM. Vertical recharge through sinkholes or vertical conduits often found in karst geology is another reasonable scenario for these cases since both are adjacent to fringe mangrove areas which serve as significant sources of DOM (Jaffé et al., 2004). However, even for the FB-central-GW (site NK) there was a significant difference in DOM fluorescence characteristics compared to FB-SW, suggesting horizontal recharge from oceanic waters underneath the Florida Keys and associated transport of mainly soil-derived DOM from mangrove peats from the Keys region.

#### 4. Conclusions and environmental implications

In summary, this study shows that the application of EEM fluorescence combined with PARAFAC modeling can provide insightful information regarding the sources, diagenetic status, and hydrological interactions of groundwater and surface water in coastal wetlands and estuaries. Subsequent PCA statistics of the PARAFAC data allowed for a plausible differentiation among the different DOM sources and their diagenetic state for the selected sample set based on both the source strength of terrestrial vs. marine sources and the susceptibility of the DOM to microbial and/or photochemical degradation. Furthermore, the combination of bulk DOC, UV-Vis and EEM-PARAFAC data provided clues regarding the influence of vertical and horizontal groundwater flow paths in the FCE-Florida Bay-Florida Keys area. For instance, the signature of the DOM in the freshwater Everglades (S332 region) indicates that surface water infiltrates relatively rapidly to the groundwater. Once in the groundwater, the DOM becomes diluted and possibly degraded as it flows towards the northern boundary of Florida Bay. Contrary to the initial hypothesis, the DOM signatures of the groundwater and surface water of Florida Bay suggest there is little interaction between those two water bodies. Instead, the DOM signature in the groundwater beneath Florida Bay suggests that a combination of fresh and saline groundwater flows from beneath the Florida Keys to at least the central portion of NE Florida Bay. This analytical approach may have wide applicability in other surface-to-ground water studies in different aquatic environments.

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