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RESEARCH ARTICLE

Compositional and spectroscopic analysis of dissolved organic matter samples from Everglades periphyton and water

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Abstract
Periphyton is a ubiquitous niche in aquatic environments and can be a significant source of dissolved organic matter (DOM) production and leaching, especially in such environment as the Everglades, a slow-water flow wetland in Florida, USA. We employed an array of methods, including compositional analysis, 3-dimensional excitation emission matrix (3-D EEM) fluorescence spectroscopy, and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, to perform quantitative and qualitative analyses on the DOM produced by periphyton and DOM in surrounding surface water and periphyton overlying water for comparison purposes. Higher dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) contents in periphyton pore water than surface water and periphyton overlying water indicated the remarkable contribution from periphyton-produced DOM. Higher total protein, carbohydrate, and thiol contents in periphyton pore water than in surface water and periphyton overlying water underscored the possibility of periphyton pore water DOM leached from periphyton. These results agreed with 3-D EEM and ATR-FTIR analyses that showed the prevalence of possible microbial source of periphyton pore water DOM as indicated by higher fluorescence index (FI) than surface water and periphyton overlying water. Similarly, the size-fractionated DOM from surface water demonstrated terrestrial sources, and periphyton pore water demonstrated microbial sources regardless of their differences in size based on their FI values. The types of periphyton affect the production and composition of DOM, as evidenced by higher total protein, carbohydrate, and chlorophyll-a (Chl-a) contents in floating mat on the water surface than in epiphyton attached to submerged phytoplankton, probably because the former is photo-synthetically more productive than the latter due to different light availability. This study provided fundamental information on periphyton DOM that is essential for further investigating its role in carbon cycle and its biogeochemistry.

Keywords Dissolved organic matter · Periphyton · Total organic carbon · 3D excitation emission matrices fluorescence spectroscopy · Fourier transform infrared spectroscopy

Introduction

Dissolved organic matter (DOM), defined operationally by filtration through 0.2-, 0.45-, or, 0.7-μm filters (Dong et al. 2011; Liu et al. 2011; McKnight et al. 1985), is a complex mixture of organic substances that originate from a variety of sources including plants, soils, bacteria, and living organisms (McKnight et al. 1985). DOM can be classified based on its origin, structure of functional groups and reactivity, and presence of different fluorophores (Cory and McKnight 2005). Humic substances are usually refractory end products which are derived from terrestrial plant sources which may contain lignin and cellulose through microbial degradation (McKnight et al. 1985). Aquatic humic substances may also derive from soil leachates (Beck et al. 1974). Non-humic

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substances contain mainly carbohydrates, proteins, amino acids, peptides, fats, waxes, lipids, alkanes, etc. (Magdoff and Weil 2004). DOM can also be classified based on their ability to fluoresce, fluorescent DOM (FDOM), such as humic-like and protein-like DOM (Chen et al. 2010). Freshly produced DOM may originate from primary productivity of autotrophs (Lu et al. 2003), while the humic-like DOM are likely humified DOM after the labile portion degrades. Therefore, the non-humic-like or protein-like DOM can likely be an autochthonous source of DOM which can be derived from microbial source (Lu et al. 2003), while humic-like DOM can be an allochthonous source of DOM from decomposed plants, leaf litter; which can possibly be derived from terrestrial source. Humic-like DOM may also derive from microbial or marine sources (Chen et al. 2010).

The negatively charged oxygen, nitrogen, and sulfur-containing functional groups of DOM have potential to bind to metals (Skyllberg et al. 2000), which significantly controls metal speciation and cycling in the freshwater ecosystem. For example, DOM forms complexes with inorganic mercuric ion (Hg^{2+}) and methyl mercury (CH_3Hg^+), and especially the reduced sulfur functional groups of DOM have high affinity for mercury which results in strong complexation between Hg compounds and DOM (Jiang et al. 2018). When DOM is bound to Hg, it could retain Hg species in water and reduce the adsorption, precipitation, and sedimentation of Hg for the formation of Hg attached to solid matrices. On the other hand, low-molecular-weight (LMW) DOM and mercury complexes may be more bioavailable to the microbial community for methylation (Mangal et al. 2018). The LMW thiol contents also show direct relationship to total mercury contents (Leclerc et al. 2015) and indicate potential mercury bioavailability and methylation in periphyton (Leclerc et al. 2015).

Periphyton is an important source of DOM as it performs photosynthesis and produces fresh organic matter (OM) which can possibly be unique in its quality (Lu and Jaffe 2001). Periphyton community is composed of a complex mixture of green algae, cyanobacteria, microbes, diatoms, mineral particles, and detrital organic matter (Hagerthey et al. 2011), and its green algal component may function as an autotroph. Therefore, it may influence the aquatic ecosystem through autotrophic activity (Lodge 2010). It uses nutrients especially nitrogen (Wozniak et al. 2008) and phosphorus compounds for growth (McCormick et al. 1996; Gaiser 2009). Periphyton may exist in different types based on pH, nutrient availability, and location or preferred substrate and can be classified based on location where it is attached or submerged to. The epiphyton is attached to the plants, while the metaphyton floats on the surface water (Hagerthey et al. 2011).

The Everglades is a subtropical wetland located in South Florida, USA, and various periphytons are ubiquitously present throughout the ecosystem. Metaphyton can be of several types based on appearance, pH, and nutrient conditions in the Everglades, where the cyanobacteria dominated calcite mats are the most common which are known as floating mats (Lodge 2010). The floating mats look yellow to brown colored with spongy texture and float on the surface water and adapted to oligotrophic nutrient conditions (Lodge 2010). The presence of the periphyton community is influential for Everglades ecosystem as it may control its environmental conditions by its phototrophic and heterotrophic activities (Azim 2009). As periphyton is phototroph, it produces fresh OM and oxygen because of its primary productivity. These freshly leached organic matters from periphyton may go through microbial degradation to carbon dioxide (CO_2) by bacterial community of periphyton which indicates its heterotrophic activity (Azim 2009). The photosynthetic algal community of periphyton, on the other hand, may then use CO_2 and uptake nutrients (containing nitrogen and phosphorus) to perform its primary productivity in the presence of light which yields organic matters and oxygen (Azim 2009). As results of oxygen production and diffusion into the water column, aerobic metabolism of organic matters may occur (Azim 2009). The periphyton organic matter pool may contain some organic matters from its surrounding aquatic plants, such as sawgrass.

Trophic state dynamics in terms of nutrient enrichment is an important environmental factor in the Everglades; such as oligotrophic, mesotrophic, and eutrophic conditions. The trophic state dynamics are closely related to different pH levels, which may influence periphyton growth and biomass in terms of nutrient availability, and result in increased dissolved oxygen concentration (Vaithiyanathan and Richardson 1998). For example, Florida Everglades has an oligotrophic condition, especially in periphyton-dominated areas, since periphyton uptakes nutrients including dissolved phosphorus (P) as it is a limiting nutrient in freshwater ecosystem (Phelps and Osborne 2019) from the water column (Grimshaw et al. 1993). This may cause low nutrient–enriched conditions and also alkaline ($pH > 7$) conditions which may result in calcium carbonate ($CaCO_3$) precipitation (Lu and Jaffe 2001). As periphyton performs photosynthesis, carbon dioxide (CO_2) may outgas from the water column (Lu and Jaffe 2001), while dissolved oxygen and organic matter concentrations may increase in the water column. Nutrient uptake may cause algal biomass to increase in periphyton and it can also store P (Dodds 2003).

Previous studies have attempted to characterize DOM leached from periphyton using both optical property and mass spectrometry (MS) analyses. The DOM produced from periphyton in the Everglades are mostly aliphatic and unsaturated hydrocarbons (Hertkorn et al. 2016). The leached DOM from periphyton contains only 3% of aromaticity; the 1,2,4-trimethoxybenzene and 1,4-dimethoxybenzene are the most common aromatic compounds in periphyton, and greater than 63% of the DOM leachates are oxygen-alkyl carbon and greater than 14% of alkyl carbon (Hagerthey et al. 2011). The ultra-filtered DOM (UDOM > 1 kDa) in periphyton in the Everglades also contains neutralsaccharides, such as arabinose, ribose, xylose, rhamnose, fucose, mannose, galactose, and glucose (Maie et al. 2005). Extracellular polymeric substances (EPS) from periphyton contain low-molecular-weight thiol compounds, e.g., cysteine and glutathione (Leclerc et al. 2015). The concentration of these functional groups demonstrates a direct proportional relationship to chlorophyll-a content which suggests phototrophic activity by green algae (Leclerc et al. 2015) and, therefore, may indicate fresh OM produced by periphyton phototrophic activity.

However, detailed characterization of DOM from periphyton is needed, especially utilizing advanced spectroscopic techniques. Both optical property analysis and ATR-FTIR analysis are useful in obtaining qualitative data for DOM. Optical property can indicate possible sources of DOM, while ATR-FTIR can provide quick information on both functional groups and fingerprint regions. Using 3-dimensional excitation emission matrix (3-D EEM) fluorescence spectroscopy, qualitative information of fluorescent DOM (FDOM) associated with the type, source, and reactivity of DOM can be obtained based on the excitation and emission regions (Hertkorn et al. 2016). Fourier transform infrared (FTIR) spectroscopy coupled with attenuated total reflectance (ATR) could provide high-quality spectra which yields identification of functional groups and fingerprint regions of DOM, as has been used for OM from surface water (Yusuf and Audu 2017) and algal cells (Murdock and Wetzel 2009). The hypothesis of this study is that DOM produced by periphyton can be unique in its quantity and quality, which can further influence and control Everglades' environmental conditions. This study aimed to use an array of methods to perform quantitative and qualitative characterization of periphyton-originated DOM and water DOM for comparison purpose. We conducted quantitative analysis to yield information on dissolved organic carbon, total dissolved nitrogen, total dissolved phosphorus, total protein, total carbohydrate, total thiol, and chlorophyll-a contents. We performed qualitative analysis using 3-D EEM fluorescence spectroscopy for information on DOM types, sources, and reactivity and ATR-FTIR spectroscopy for functional groups and fingerprint regions of periphyton DOM at molecular level.

Materials and methods

Sample collection

Sample collection was performed in two different sites in the south part of the Everglades; one of them was rich in floating mats (site 1, Mahogany Hammock Trail; geographic coordinates, 25.323644, – 80.832124), and another was rich in epiphyton attached to sawgrass (site 2: Pa-Hay-Okee Trail; geographic coordinates, 25.440959, – 80.783826). These sites were selected because of their representation in two different types of periphyton in order to evaluate different periphyton-type-dominated ecosystems in Everglades. Floating mats were collected from the surface water; and epiphyton was collected by cutting the sawgrass, which had periphyton attached to it, using a knife. Along with different types of periphyton collection, surface water was collected at a depth of approximately 30 cm from above the sediment, which was located approximately 1 m away from the periphyton community. Periphyton overlying water, which is the water that covers the surface part of periphyton, was also collected from the top portion of the periphyton community (Xiang et al. 2021). The surface water and periphyton overlying water samples were collected in pre-acidified and rinsed Teflon bottles, and periphyton samples were collected in plastic zip-lock bags. The pH level was measured in the sampling sites using the Milwaukee pH meter. A pH level of 7.7 was obtained in both sites. A 2nd set of floating mat samples were collected at 3 different locations from only site 1 in order to measure floating mat periphyton autotrophic index. All samples were stored in ice inside a cooler until reaching the lab.

After arriving at the lab, the periphyton samples were centrifuged at 7409 RCF (relative centrifugal force) for 30 min to collect periphyton pore water, which is the water sample from the pore of the periphyton. Therefore, periphyton pore water DOM can be operationally defined as the DOM from the pore water of periphyton indicating leached DOM from periphyton, and periphyton overlying water DOM can be defined as the DOM covering the top surface of periphyton (Xiang et al. 2021). All water samples were filtered with pre-combusted GF/F filter of 0.7 µm to remove the particulates and the dissolved portion of OM was obtained.

Chemicals and reagents

The chemicals purchased in order to prepare stock standard solutions for functional groups and Chl-a measurements were bovine serum albumin, Coomassie brilliant blue G-250, methanol, reagent grade phosphoric acid, tris-(2-amino-2-(hydroxymethyl)-1,3-propanediol) buffer, reagent grade sulfuric acid, reagent grade phenol, D-glucose, DTNB (5,5-dithio-bis-(2-nitrobenzoic acid), sodium acetate trihydrate, acetyl cysteine (N-Acetyl-L-cysteine), and reagent grade acetone.

Elemental and composition analyses of DOM

Dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) were measured in the surface water, periphyton overlying water, and periphyton pore water samples in triplicate. The DOC content was measured by utilizing the Shimadzu total organic carbon (TOC) analyzer. The trace SN cube from Elementar was used for TDN analysis and the QuAAtro39 nutrient analyzer from Seal Analytical was used to measure the TDP contents in the surface, overlying, and periphyton pore water samples.

Total protein contents of surface water, periphyton overlying water, and pore water DOM were measured using Bradford protein assay (Bradford 1976; Stoscheck 1990). The standard assay procedure for sample with 5–100 µg ml^{-1} protein was used. Bovine serum albumin (BSA) standards were prepared using six dilutions. For the standards, Milli-Q water was used as a blank, while for the surface, overlying, and periphyton pore water samples, Tris buffer was used instead. They were incubated at room temperature for 5 min; and the absorbance at 595 nm was measured using Cary 100 UV–Vis spectrophotometer; and the concentrations were obtained using the standard curve.

Total carbohydrate contents were measured using the phenol sulfuric acid method (Dubois et al. 1956; Krishnaveni et al. 1984). At first, phenol (5%) solution was prepared and sulfuric acid reagent grade was obtained. Glucose standard stock solution was prepared by dissolving 0.1 g in 100 mL Milli-Q water; then, 10 mL stock was diluted up to 100 mL with Milli-Q water. After that, a series of dilution were made with the working standard in separate test tubes. One milliliter of water was obtained for blank. The absorbance was measured at 490 nm using the Cary 100 UV–Vis spectrophotometer, and the concentrations were obtained from the standard curve.

The chlorophyll-a (Chl-a) concentrations were measured for both floating mats and epiphyton. The ESS method 150.1 (EPA 1991) was used to determine the Chl-a concentrations; by extracting using 90% buffered acetone and by measuring using spectrophotometric method with UV spectroscopy (Rai 1973). Approximately 0.2 g of periphyton samples were obtained and extracted using 15 mL of 90% buffered acetone and were filtered using Whatman filter papers (90-mm diameter); and Chl-a solutions were measured using spectrophotometric method by using the Cary 100 UV–Vis spectrophotometer (Weber et al. 1986).

Optical property analysis of DOM

Optical properties of DOM were measured by running the Horiba Aqualog Fluorometer for 3-D excitation emission matrix (EEM) spectra in emission mode (Yamashita et al. 2010; Hertkorn et al. 2016). The range of the excitation and emission wavelengths was from 250 to 600 nm; and the EEM data were corrected for Rayleigh scattering of first and second orders and normalized to Raman units (RUs) (Stedmon et al. 2003). The obtained EEM data were fitted to an existing 8-component PARAFAC model for Florida Everglades region (Chen et al. 2010; Yamashita et al. 2010). The fluorescence indices, such as fluorescence index (FI), humification index (HIX), and biological index (BIX), were obtained as well (Hansen et al. 2016).

Optical analysis was also performed on size-fractionated high- and low-molecular-weight DOM samples. Size fractionation of the filtered surface water and periphyton pore water DOM was performed with samples from site 1 using Pall Macrosep Centrifugal filtration devices (molecular weight cutoff 1 kDa, 20 mL). DOM with molecular weight from higher than or equal to 1 kDa to 0.7 µm (previously filtered using GF/F filter) was defined as high-molecular-weight (HMW) DOM and less than 1 kDa was defined as low-molecular-weight (LMW) DOM in this method (Xu et al. 2018). A 20-mL volume was used for both surface and periphyton pore water DOM (Xu et al. 2018), and they were centrifuged for 30 min at 7409 RCF. After centrifugation, 5 mL concentrated DOM for surface water DOM and 8 mL concentrated DOM for pore water were obtained in the top portions of the devices. The remaining volume went down the bottom portion of the devices. The top concentrated portion had mostly HMW DOM, while the diluted bottom portion had 90% recovery of LMW DOM with water as a solvent.

Functional groups and fingerprint regions of surface water DOM and periphyton

The differences in functional groups between surface water DOM and periphyton were examined with samples from site 1. The analysis was performed only for site 1 samples because site 1 was enriched in the most abundant type of periphyton, floating mat. Surface water DOM and floating mat was analyzed using ATR-FTIR spectroscopy in order to identify different functional groups and fingerprint regions. For the surface water DOM, 10 mL of sample was analyzed. For the periphyton sample, approximately 5 g of floating mat was placed in the oven to dry at 80 °C for 24 h (Havens et al. 1996). Then, it was grinded using a mortar and pestle; and the solid powdered periphyton was analyzed using ATR-FTIR (Stehfest et al. 2005). A diamond-ATR crystal was used to hold the solid periphyton powder (Ferro et al. 2019).

Total thiol contents were also measured using Ellman's Test Protocol (Ellman 1959; Bulaj et al. 1998) to check the differences between surface water DOM and periphyton, considering the importance of thiols in metal biogeochemistry. At first, 2 mM DTNB stock solution was prepared in

50 mM sodium acetate solution. A 1.0-M Tris solution was also prepared. After that, the DTNB working solution was prepared by mixing 50 µL DTNB stock solution, 100 µL Tris solution, and 840 µL Milli-Q water, which was used as a blank. Then, 10 µL of sample was added, mixed, and incubated at room temperature for 5 min, and the absorbance was measured using the Cary 100 UV–Vis spectrophotometer.

Results and discussion

Elemental and compositional analysis of Everglades DOM

The surface water and periphyton overlying water had similar DOC, with 14.19 and 15.33 mg/L in site 1, and 10.18 and 10.20 mg/L in site 2, respectively (Table 1). The periphyton pore water, on the other hand, had higher DOC contents, 348.4 mg/L for floating mat, and 62.29 mg/L for epiphyton compared to respective surface and overlying waters ($p < 0.05$) (Table 1). Periphyton produces OM (phototrophic activity by its green algal community) and uptakes OM (heterotrophic activity by its bacterial community). The higher DOC content in periphyton pore water in comparison to the surface and overlying waters may attribute to the fact that large quantity of DOM may produce from periphyton. As both sampling sites have a negligible flow rate, the mixing rate should be lower in the field resulting in slower diffusion of OM from pore water to the surface water region (Jiang et al. 2018). This can be a reason for the pore water region to have higher DOC content than the surface water region. The floating mat, being present on the water surface, is exposed to more sunlight in comparison to epiphyton. Stronger sunlight can facilitate higher primary productivity and increase production of organic matters (Azim 2009). The epiphyton pore water showed lower DOC content because its primary productivity was possibly limited by light availability as epiphyton was attached to sawgrass and located below the surface in site 2. Floating mat and epiphyton may contain different algal contents which may also influence its difference in OM production as a study had been shown to explore algal biomass in periphyton using Chl-a as it has the ability to reemit absorbed light energy to fluorescence (Azim 2009).

The TDN contents of the surface water and periphyton overlying waters were similar at both locations with 1.11 and 1.22 mg/L in site 1, 0.78 and 0.74 mg/L in site 2, in surface and overlying water respectively. Periphyton pore water TDN contents were higher than the surface and overlying water of both types of periphyton (p <0.05). Floating mat pore water contained higher ($p < 0.05$) TDN content (36.85 mg/L) than epiphyton pore water (7.09 mg/L). The TDP contents were higher ($p < 0.05$) in the surface water of site 1 (31.10 µg/L) than the surface water of site 2 (18.27 µg/L). The TDP contents were the same for the pore water of both types of periphyton regardless of their types and location; 30.03 and 30.23 µg/L for sites 1 and 2, respectively. A study had shown that epipelon (attached to soil), epiphyton (attached to plants), and metaphyton (floats on surface water) differed in their nutrient contents due to their location in the Everglades (McCormick et al. 1997). Epipelon demonstrated to have total nitrogen (TN) and total phosphorus (TP) contents significantly larger than metaphyton; however, the carbon to nitrogen ratio (C:N) was lower. On the other hand, epiphyton, in wet season, demonstrated to have medial TN and TP contents; however, due to lack of presence, it was not assayed in dry season. This study also had shown that P is the limiting nutrient for periphyton under oligotrophic conditions in Everglades as their interior marsh sites' surface waters and periphyton demonstrated to have large nitrogen to phosphorus ratios (N:P) but low phosphorus (P) content (McCormick et al. 1997). Other studies had also demonstrated epiphyton to dominate P uptake from the water column (Wetzel 2001). Based on our results, it can be concluded that TDN and TDP are dissolved nitrogen and phosphorus contents that periphyton uptakes as nutrients for its growth. As phosphorus is usually a limiting nutrient for periphyton growth, a certain amount of TDP need to be available for the periphyton to grow which is present in periphyton pore water regardless of different types. As periphyton overlying water is the region that is closer to periphyton

Table 1 Dissolved organic carbon (DOC), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total protein, and total carbohydrate contents (mg/L, except for TDP in µg/L) in Everglades surface water, periphyton overlying water, and periphyton pore water from floating mats and epiphyton from Mahogany Hammock Trail (site 1) and Pa-Hay-Okee Trail (site 2); and Chl-a concentrations (µg/g) in floating mats and epiphyton periphyton

Location	Sample	DOC	TDN	TDP	Total protein	Total carbohydrate	Chl-a
Site 1	Surface water	14.19 ± 0.23	1.11 ± 0.05	31.10 ± 0.66	13.77 ± N/A	1.01 ± 0.03	
	Periphyton overlying water	15.33 ± 0.13	1.22 ± 0.03	15.70 ± 0.72	14.73 ± N/A	1.96 ± 0.46	
	Periphyton pore water	348.4 ± 3.28	36.85 ± 1.85	30.03 ± 2.77	21.09 ± 9.58	16.83 ± 12.05	129.92 ± 3.89
Site 2	Surface water	10.18 ± 0.12	0.78 ± 0.01	18.27 ± 3.29	22.42 ± 8.02	1.86 ± 0.03	
	Periphyton overlying water	10.20 ± 0.01	0.74 ± 0.02	11.40 ± 2.31	21.65 ± 10.94	2.12 ± 0.90	
	Periphyton pore water	62.29 ± 0.59	7.09 ± 0.08	30.23 ± 2.17	25.92 ± 9.84	2.34 ± 0.55	46.65 ± 2.93

community than surface water region, the TDP content there is lower for both types of periphyton because of the uptake of TDP from the overlying water region. Surface water at site 2 has lower TDP content than at site 1, probably because both epiphyton and the associated sawgrass need TDP for growth at site 2 while site 1 has only floating mat without presence of other phytoplankton.

The total protein concentration of floating mat pore water (21.09 mg/L) and epiphyton pore water (25.92 mg/L) showed no significant difference ($p > 0.6$). In site 1, the total protein concentrations were similar in surface water (13.77 mg/L) and overlying water (14.73 mg/L). The same trend was observed in site 2, in terms of the total protein concentrations in surface (22.42 mg/L) and overlying (21.65 mg/L) water. Similarly, the total carbohydrate concentration of floating mat pore water (16.83 mg/L) and epiphyton pore water (2.34 mg/L) showed no significant difference ($p > 0.3$). In site 1, the total carbohydrate concentrations were similar in surface water (1.01 mg/L) and overlying water (1.96 mg/L). The same trend was observed in site 2, in terms of the total carbohydrate concentrations in surface (1.86 mg/L) and overlying (2.12 mg/L) water. On the other hand, floating mat from site 1 demonstrated to have a Chl-a concentration of 129.92 µg/g, while epiphyton from site 2 contained 46.65 µg/g.

A study had shown that epiphyton and metaphyton had demonstrated to have significantly different productivity which is biomass-specific in between wet and dry seasons and in between oligotrophic and eutrophic conditions (McCormick et al. 1997). Other studies had also shown that periphyton biomass and productivity had demonstrated to be decreased due to shading from the macrophyte canopy in emergent macrophytes stands (Charlene et al. 1976; Murkin et al. 1992). The results from our study suggest that floating mats may be able to perform higher photosynthetic productivity than epiphyton as floating mats float on the surface in site 1 and are exposed to more light than epiphyton in site 2. On the other hand, epiphyton in site 2 is attached to plants and submerged below the surface; causing less light availability and thus less photosynthetic organic matter production. Overall, the results suggest that the pore water of periphyton contains more organic matter, including DOC, protein, and carbohydrate than its respective surface and overlying water, because periphyton is able to produce fresh organic matter and that floating mats are possibly photosynthetically more productive than epiphyton.

Optical properties of fluorescent DOM in surface water and periphyton

Based on the optical property analysis (Fig. 1) of DOM characterization using Everglades PARAFAC model (supplementary material section), the surface water and the periphyton overlying water from both sites had same types of DOM. They had mostly humic-like DOM which are derived from both ubiquitous and terrestrial origin. According to the obtained EEM PARAFAC components, they had prevalence of components C1 and C5, which indicate ubiquitous and terrestrial origin, respectively. They also had minor prevalence of components C8 and C4, which indicate tryptophan-like and microbial origin, respectively. On the other hand, the periphyton pore waters from both sites had different DOM types than the surface and overlying water. The DOM types from the pore waters were microbial humic-like and humic-like from ubiquitous and terrestrial sources. According to the obtained EEM PARAFAC components, floating mat pore water had prevalence of component C4, with minor contributions from C5 and C1. It is possible that different types of DOM were slightly mixed in between different DOM sources. For example, minor contribution of C4 in surface water and periphyton overlying water was likely a contribution from periphyton pore water DOM pool, while minor contribution of C5 and C1 in periphyton pore water was possibly a contribution from surface water DOM pool. There were differences between the abundance of each types of DOM depending on the periphyton types and locations. For example, the epiphyton pore water from site 2 contained mostly terrestrial humic-like (C5), followed by ubiquitous humic-like (C1), and then humic-like from microbial origin (C4) likely, while the floating mat pore water from site 1 contained mostly microbial humic-like (C4), followed by humic-like from possibly terrestrial (C5) and ubiquitous (C1) origin. It is possible that epiphyton pore water DOM has a significant contribution from surface water DOM pool (major presence of C5 and C1) as well as periphyton (C4). This difference may also be site or location specific because of surrounding sawgrass, which may indicate DOM from higher plants or terrestrial sources.

Fluorescence parameters can be obtained from the 3-D EEM fluorescence spectroscopy, which can give specific qualitative information on DOM. The ratio of the emission intensity at 470/520-nm wavelength at an excitation of 370-nm wavelength was determined to be the fluorescence index (FI). This index was obtained using corrected spectra in one study (Cory et al. 2010). This study had demonstrated the FI to be less than 1.4 for terrestrial source of DOM and higher than 1.4 for microbial source of DOM which was independent of correction factor application (Cory et al. 2010). A previous study had also obtained FI at the ratio of the emission intensity at 450/500 nm at 370-nm excitation using uncorrected spectra (McKnight et al. 2001). This study had demonstrated that an FI of approximately 1.9 corresponded to microbial source of DOM, while approximately 1.4 corresponded to terrestrial source of DOM (McKnight et al. 2001). Usually, an FI of approximately 1.8 or higher may correspond to

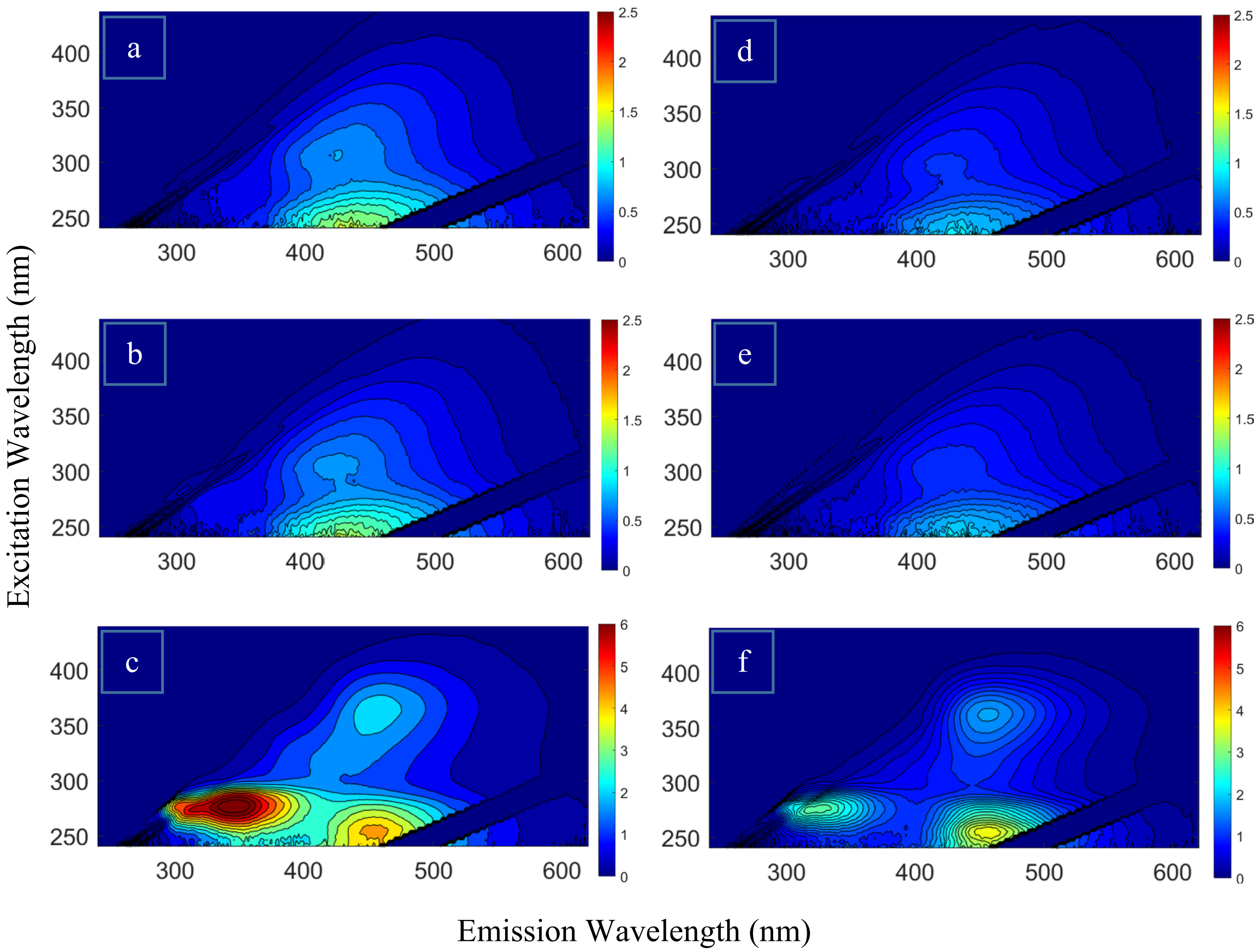

Fig. 1 3D EEM analysis of surface water, periphyton overlying water, and periphyton pore water dissolved organic matter (DOM) from site 1 (**a**, **b**, and **c** for surface, periphyton overlying, and periphyton pore water, respectively) and site 2 (**d**, **e**, and **f** for surface, periphyton overlying, and periphyton pore water, respectively) in the Everglades. Note the differences in the colorbar scales between surface/overlying water (**a**, **b**, **d**, **e**) and periphyton pore water (**c**, **f**), indicative of higher fluorescence intensities in periphyton pore water due to higher DOM concentrations

microbial DOM and approximately 1.1 or lower may correspond to terrestrial DOM (Jaffé et al. 2008). Our study had demonstrated the FI to be in a range from 1.4 to 1.6 approximately for surface water and periphyton overlying water, which may indicate presence of terrestrial DOM and the FI to be in a range from 2.2 to 2.5 approximately for periphyton pore water, which may indicate presence of microbial DOM (Table 2). The humification index (HIX) (supplementary material section) provides information on the humification of OM (Larsen et al. 2010) and corresponds to more humic-like DOM (Hansen et al. 2016). The biological index (BIX) (supplementary material section) provides information on more freshly produced OM (Hansen et al. 2016) and a value of greater than 1 corresponds to recently produced or autochthonous source of DOM (Hansen et al. 2016).

The fluorescence indices of these different types of DOM (Table 2) provided information on their sources more selectively. For example, surface water and periphyton overlying water DOM from both sites had DOM from terrestrial sources possibly as FI values were within a range from 1.4 to 1.6 approximately. Both periphyton pore waters contained FI values within a range from 2.2 to 2.5 approximately, which indicated microbial origin of DOM or OM input from microbial biomass possibly. HIX value was higher for the surface water and overlying water DOM than the pore water DOM from periphyton indicating that the surface and overlying water DOM had more degraded OM input from higher plants. These OM possibly got deposited into the soil and likely got degraded and contributed to the OM in the water column, while pore water contained less aged OM produced by periphyton. BIX value, on the other hand, was lower for

Table 2 Fluorescence indices for DOM from surface water, periphyton overlying water, and periphyton pore water from floating mat at Mahogany Hammock Trail (site 1) and from epiphyton at Pa-Hay-Okee Trail (site 2) and for size-fractionated high-molecular-weight (HMW) and low-molecular-weight (LMW) DOM from surface water and periphyton pore water from Mahogany Hammock Trail (site 1) in the Everglades

Sample name	Fluorescence index	Humification index	Biological index
Site 1 surface water	1.50 ± 0.01	5.03 ± 0.55	0.58 ± 0.01
Site 1 periphyton overlying water	1.49 ± 0.00	3.90 ± 0.34	0.62 ± 0.01
Site 1 periphyton pore water	2.23 ± 0.01	1.58 ± 0.02	0.76 ± 0.01
Site 2 surface water	1.49 ± 0.03	4.07 ± 0.33	0.64 ± 0.02
Site 2 periphyton overlying water	1.47 ± 0.03	6.02 ± 3.55	0.64 ± 0.01
Site 2 periphyton pore water	2.37 ± 0.01	2.77 ± 0.23	0.56 ± 0.01
Site 1 HMW surface water	1.56 ± N/A	N/A	0.52 ± N/A
Site 1 LMW surface water	1.38 ± N/A	N/A	0.49 ± N/A
Site 1 HMW periphyton pore water	2.50 ± N/A	N/A	0.83 ± N/A
Site 1 LMW periphyton pore water	2.21 ± N/A	N/A	0.69 ± N/A

the surface and overlying water than the pore water in site 1, indicating that the floating mat pore water had more freshly leached DOM input from floating mat primary productivity. A study had shown that periphyton, sawgrass, and mangrove leaves were demonstrated to have different fluorescent structures in their respective synchronous fluorescence spectra due to differences in sources. Only one of the fluorescent peak demonstrated to be common in all these samples due to DOM being freshly produced from these samples (Lu and Jaffe 2001). Our results suggest that the FDOM is possibly different in different types of periphyton community. The epiphyton pore water had higher HIX value than the floating mat likely due to its substrate plants contributing to its OM content. As BIX value was lower for the surface and overlying water than the pore water in site 1, it can be concluded that the floating mat pore water had more freshly leached DOM input from floating mats primary productivity. Epiphyton pore water BIX value was slightly lower because epiphyton had less light availability due to its attachment to the sawgrass in site 2; and possibly less photosynthetic activity was performed compared to floating mats. According to the obtained PARAFAC components results (supplementary material section), the epiphyton pore water had DOM likely from terrestrial and ubiquitous sources (C5 and C1 respectively) along with microbial source (C4).

The FDOM analysis implies that the DOM from the surface and overlying the periphyton are strongly influenced by inputs from terrestrially derived organic matters from the higher plants of the Everglades. On the other hand, FDOM in periphyton pore waters has different DOM types and the abundance of each types of DOM can vary depending on the periphyton types and locations. Both pore waters from these periphyton have sufficient inputs from possibly microbial source of DOM based on their FI values obtained (Table 2).

The HMW and LMW DOM fractions of surface water and periphyton pore water DOM from site 1 was analyzed qualitatively for their optical properties using 3-D EEM fluorescence spectroscopy. Based on the analysis (Fig. 2), the HMW surface water DOM contained humic-like DOM derived from terrestrial and ubiquitous sources. The LMW surface water DOM, similarly, contained DOM from the same sources. Based on the relative abundance (%) of EEMs PARAFAC components (supplementary material section), HMW surface water showed higher abundance of C1 component (15.03%) than LMW surface water (5.18%). For periphyton pore water DOM, the HMW fraction contained mostly microbial humic-like DOM (C4), followed by humic-like from terrestrial (C5) and ubiquitous (C1) origin, while the LMW contained mostly microbial humic-like (C4) and terrestrial humic-like (C5); ubiquitous humic-like (C1) was negligible. It is possible that the C1 (ubiquitous humic-like) component contains solely HMW DOM which is also a contribution from the surface water DOM pool. Based on the fluorescence indices (Table 2), both the HMW and LMW fractions of the surface water DOM contained DOM possibly from terrestrial sources as their FI values were within a range from 1.4 to 1.6 approximately. On the other hand, both fractions from the periphyton pore water DOM contained DOM from microbial sources possibly as indicated by their FI values within a range from 2.2 to 2.5 approximately, showing no differences in DOM sources between HMW and LMW fractions. The BIX values were higher in both fractions of periphyton pore water DOM than surface water DOM, again indicating more freshly leached DOM for the periphyton.

Functional groups and fingerprint regions of surface water and periphyton DOM

Everglades surface water DOM and floating mat functional group and fingerprint regions from site 1 were analyzed using ATR-FTIR spectroscopy along with their total thiol contents (Table 3). Based on the FTIR spectra, the surface water DOM (Fig. 3a) yielded the following information on its functional groups and fingerprint regions, the broad region with the strongest absorbance at 3280 cm^{-1} was assigned to the –OH stretching of carboxyl, phenol, and alcohols (Yusuf and Audu 2017). The strong signal at

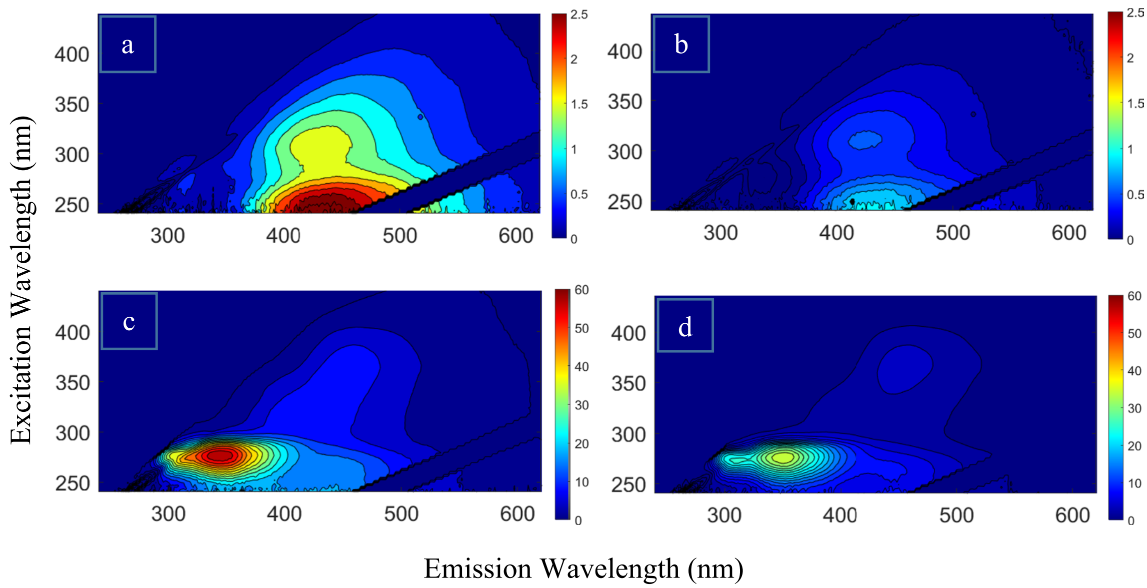

Fig. 2 DOM 3-D EEMs analysis of high-molecular-weight (HMW, left) and low-molecular-weight (LMW, right) dissolved organic matter (DOM) from surface water (**a** and **b**) and periphyton pore water (**c** and **d**) in the Everglades. Note the differences in the colorbar scales between surface (**a** and **b**) and periphyton pore water (**c** and **d**), indicative of higher fluorescence intensities in periphyton pore water due to higher DOM concentrations

Table 3 Total thiol (μM) in surface water, periphyton overlying water DOM, and periphyton pore water DOM from floating mat at Mahogany Hammock Trail (site 1) in the Everglades

Sample name	Total thiol
Surface water	36.40 ± 5.15
Periphyton overlying water	60.66 ± 9.73
Periphyton pore water	75.25 ± 15.35

1644 cm^{-1} was from C = O stretching of COO^{-}, ketonic C = O, and aromatic C = C. The signal at 1336 cm^{-1} was assigned to the polyhydroxylated phenols, and C-N stretching of aromatic amines; and the signal at 1061 cm^{-1} was from the C-O group of polysaccharides (Yusuf and Audu 2017). The peak at 2953 cm^{-1} was likely from the C-H asymmetric stretching in CH_2 and CH_3 of alkanes (Yusuf and Audu 2017).

Based on the FTIR spectra, the floating mat (Fig. 3b) yielded the following information on its functional groups and fingerprint regions, the strongest absorbance was obtained for amide II, from the 1418 cm^{-1} wavenumber region which was assigned to CH_3, CH_2 of proteins or, C-O of COO^{-} groups (Stehfest et al. 2005). The broad 1032 cm^{-1} region was assigned to C–O–C of saccharides, the sharp 875 cm^{-1} and 712 cm^{-1} regions were assigned to calcite mats (Reig et al. 2002). There were some weak absorbance obtained for amide I, C = O of amides from proteins, (approximately 1650 cm^{-1}), lipid (1794 cm^{-1}), and phosphor (approximately 1250 cm^{-1}) regions (Stehfest et al. 2005). A very weak absorbance of thiol (2507 cm^{-1}) was obtained as well.

The functional groups and fingerprint regions in periphyton were different than in the surface water DOM based on the results from ATR-FTIR spectroscopy. The surface water DOM functional groups and fingerprint regions demonstrated to be more aromatic compounds than the periphyton. For example, presence of carboxyl, phenols, aromatic amines, and aromatic C = C indicated presence of HMW humic-like substances which corresponded to their optical property analysis, as 3D EEM spectra of surface water DOM demonstrated to be humic-like from ubiquitous and terrestrial origin. On the other hand, the periphyton functional group and fingerprint regions demonstrated to be rich in protein, carbohydrate, thiols, calcites, and lipids, which were different than surface water DOM. These results correlated to their quantitative measurements of components and optical property. Periphyton pore water was shown to contain more total protein and carbohydrate than surface water DOM, and the 3D EEMs spectra of the periphyton pore water DOM also demonstrated its difference than surface water DOM, e.g., prevalence of microbial humic-like DOM. Periphyton pore water also demonstrated to contain higher total thiol content compared to corresponding surface water (Table 3). It is possible that the higher total thiol content in periphyton pore water is an input from cysteine residues of extracellular polymeric substances (EPS) of periphyton (Leclerc et al. 2015).

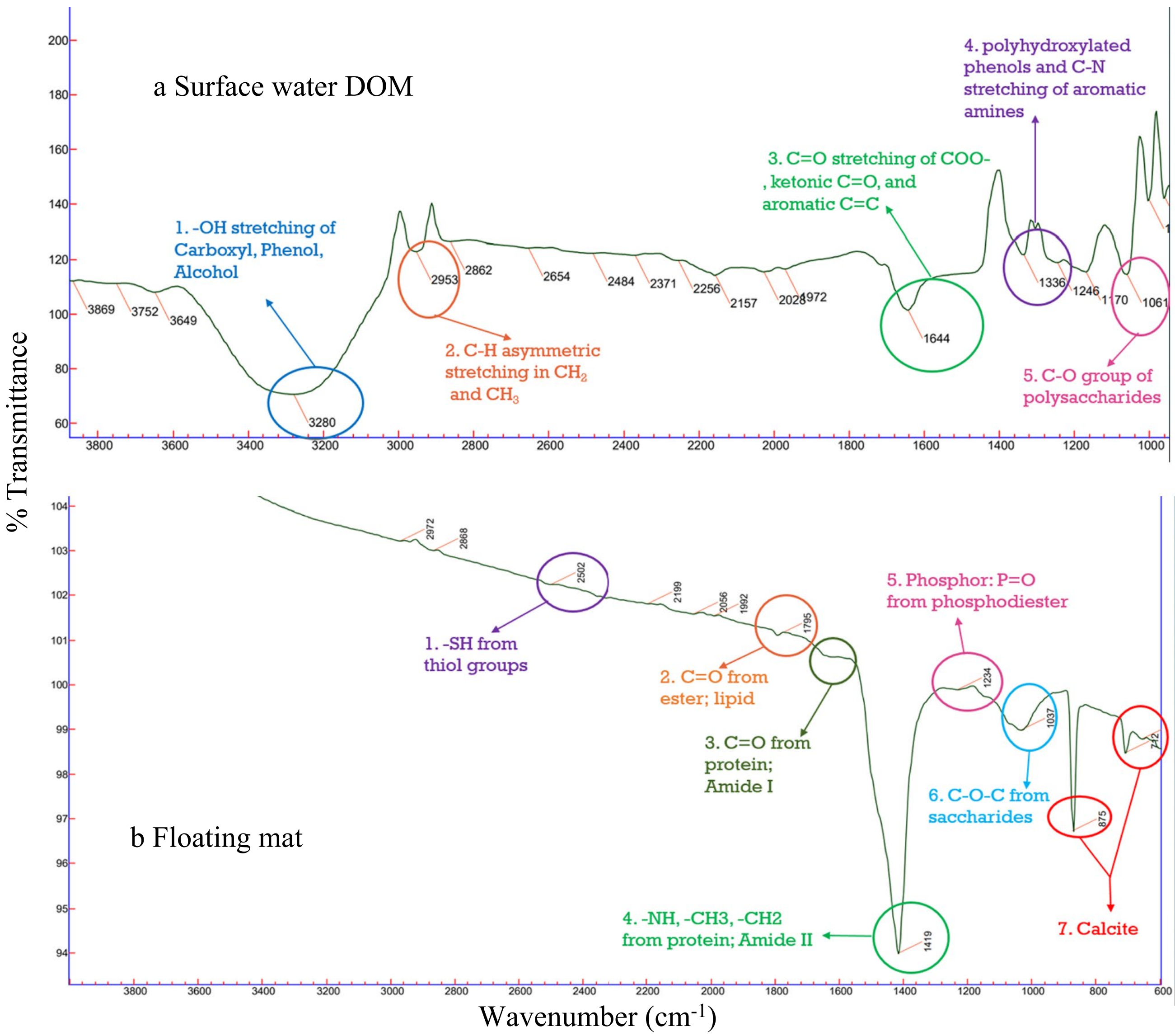

Fig. 3 Functional groups and fingerprint regions of surface water dissolved organic matter (DOM) and periphyton in the Everglades using ATR-FTIR spectroscopy

Conclusion

The higher DOC content in periphyton pore water, in comparison to the surface and overlying waters, is likely attributed to the fact that large quantity of DOM is produced from periphyton. While fluorescent DOM analysis implies that the surface and periphyton overlying water DOM is strongly influenced by inputs from terrestrially derived organic matters from the higher plants, the periphyton pore water DOM originates from microbial sources and is likely leached from periphyton resulting in the prevalence of mostly microbial humic-like DOM. The ATR-FTIR analysis on functional groups and fingerprint regions demonstrates periphyton to be rich in protein, carbohydrate, thiols, and lipids, correlating to compositional measurements indicating periphyton pore water contains more total protein, carbohydrate, and thiol contents than surface water DOM. On the other hand, the alkaliphilic condition (pH 7.7) in Everglades also implies that the surface water DOM functional groups are deprotonated and possibly prone to form complexation with metals and minerals. Periphyton is an algae-bacteria-interlinked niche ubiquitously present in aquatic environments, contributing significantly to primary production and to aqueous organic matter. Shallow lakes and wetlands (like the Everglades) with slowly moving water are in particular inducible to the development of various types of periphyton, where floating mats on the water surface readily receiving sunlight may be photo-synthetically more productive than epiphyton attached to submerged phytoplankton, as observed in this study. Periphyton organic matter influences the carbon cycle by both of its autotrophic and heterotrophic activities, with the former releasing organic compounds into the aquatic environment as a result of primary productivity and the latter linked to microbial assimilation and respiration of OM.

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Author contribution Afia Anjuman: Investigation, data curation, writing-original draft preparation. Yuping Xiang: investigation, methodology. Guangliang Liu: Conceptualization, data curation, writing—reviewing and editing. Yong Cai: Conceptualization, writing (reviewing and editing), supervision. All authors have read and approved the final manuscript.

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Data availability The data included in the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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