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Quantitative and qualitative aspects of dissolved organic carbon leached from senescent plants in an oligotrophic wetland

NAGAMITSU MAIE[1,*], RUDOLF JAFFÉ[1], TOSHIKAZU MIYOSHI[2] and DANIEL L. CHILDERS[3]

[1]*Environmental Geochemistry Laboratory, Southeast Environmental Research Center & Department of Chemistry & Biochemistry, Florida International University, University Park, Miami, FL 33199, USA;* [2]*Research Center of Macromolecular Technology, National Institute of Advanced, Industrial Science and Technology, Tokyo Waterfront, 2-41-6 Aomi, Kohtoh-ku, Tokyo, 135-0064 Japan;* [3]*Southeast Environmental Research Center & Department of Biological Sciences, Florida International University, University Park, Miami, FL 33199, USA; *Author for correspondence (e-mail: nagamits@fiu.edu; phone: +1-305-348-6085 fax: +1-305-348-1667)*

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Abstract. We conducted a series of experiments whereby dissolved organic matter (DOM) was leached from various wetland and estuarine plants, namely sawgrass (*Cladium jamaicense*), spikerush (*Eleocharis cellulosa*), red mangrove (*Rhizophora mangle*), cattail (*Typha domingensis*), periphyton (dry and wet mat), and a seagrass (turtle grass; *Thalassia testudinum*). All are abundant in the Florida Coastal Everglades (FCE) except for cattail, but this species has a potential to proliferate in this environment. Senescent plant samples were immersed into ultrapure water with and without addition of 0.1% NaN_3 (w/ and w/o NaN_3, respectively) for 36 days. We replaced the water every 3 days. The amount of dissolved organic carbon (DOC), sugars, and phenols in the leachates were analyzed. The contribution of plant leachates to the ultrafiltered high molecular weight fraction of DOM (>1 kDa; UDOM) in natural waters in the FCE was also investigated. UDOM in plant leachates was obtained by tangential flow ultrafiltration and its carbon and phenolic compound compositions were analyzed using solid state ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectroscopy and thermochemolysis in the presence of tetramethylammonium hydroxide (TMAH thermochemolysis), respectively. The maximum yield of DOC leached from plants over the 36-day incubations ranged from 13.0 to 55.2 g C kg^{-1} dry weight. This amount was lower in w/o NaN_3 treatments (more DOC was consumed by microbes than produced) except for periphyton. During the first 2 weeks of the 5 week incubation period, 60–85% of the total amount of DOC was leached, and exponential decay models fit the leaching rates except for periphyton w/o NaN_3. Leached DOC (w/ NaN_3) contained different concentrations of sugars and phenols depending on the plant types (1.09–7.22 and 0.38–12.4 g C kg^{-1} dry weight, respectively), and those biomolecules comprised 8–34% and 4–28% of the total DOC, respectively. This result shows that polyphenols that readily leach from senescent plants can be an important source of chromophoric DOM (CDOM) in wetland environments. The *O*-alkyl C was found to be the major C form (55 ± 9%) of UDOM in plant leachates as determined by ^{13}C CPMAS NMR. The relative abundance of alkyl C and carbonyl C was consistently lower in plant-leached UDOM than that in natural water UDOM in the FCE, which suggests that these constituents increase in relative abundance during diagenetic processing. TMAH thermochemolysis analysis revealed that the phenolic composition was different among the UDOM leached from different plants, and was expected to serve as a source indicator of UDOM in natural water.

Polyphenols are, however, very reactive and photosensitive in aquatic environments, and thus may loose their plant-specific molecular characteristics shortly. Our study suggests that variations in vegetative cover across a wetland landscape will affect the quantity and quality of DOM leached into the water, and such differences in DOM characteristics may affect other biogeochemical processes.

Introduction

Wetlands, which occupy 7–9 million km^2 worldwide and correspond to about 4–6% of the land surface of the Earth, are important sources, sinks, and transformers of many kinds of organic and inorganic materials. Wetlands also enhance water quality and enrich biodiversity (Mitsch and Gosselink 2000). In wetland environments, both dissolved organic matter (DOM) and particulate organic matter (POM) play pivotal roles in biogeochemical processes such as elemental cycling, microbial loop energetics, and the transport of materials across landscapes. DOM also influences physicochemical characteristics of aquatic environments (e.g. Findlay and Sinsabaugh 2003). Thus, investigating the characteristics, sources, and fate of DOM is a key topic for understanding wetland ecosystems. In this paper, we present results of a DOM leaching study conducted with senescent plants from an oligotrophic, neotropical wetland landscape.

The Everglades is one of the world's largest subtropical wetlands and is located in the southern part of the Florida peninsula, USA. It is oligotrophic and phosphorus limited. We focused our work here on Everglades National Park (ENP), which includes over 4000 km^2 of freshwater and estuarine wetlands as well as estuarine seagrass beds. The southern Everglades landscape of ENP includes sawgrass/periphyton dominated freshwater marshes, mangrove wetlands, and a seagrass-dominated subtidal estuary. Spikerush is an important vegetation in both the freshwater marshes and in the oligohaline ecotone (where freshwater marsh transitions into mangrove wetlands). Cattail is only dominant in areas where P-rich canal water enters the marsh (Childers et al. 2003), and is currently a minor component of the southern Everglades landscape. However, this plant is the dominant vegetation in the northern Everglades where nutrients have been enriched through agricultural runoff (Qualls and Richardson 2000). The southern Everglades which includes ENP is also threatened by the invasion of cattail due to the increase of phosphorous levels. Water enters this landscape from canal inputs and rain, and flows through freshwater marshes and mangrove wetlands to Florida Bay. Virtually all the organic matter in this water is in dissolved form, and thus most of the phosphorus and a major portion of the nitrogen occur in dissolved organic forms (Rudnick et al. 1999; Noe et al. 2001; Sutula et al. 2003; Childers et al. in press).

The major autochthonous sources of DOM in wetlands are leachates from (senescent) plants, exudates from phytoplankton, microalgae, or macrophytes,

and pore water from sediments and soils (Ziegler and Benner 1999; Bertilsson and Jones 2003). Among these DOM sources, freshly produced DOM from wetland plants via leaching, exudation, and secretion is considered to be very important in the southern Everglades wetlands (Lu et al. 2003; Maie et al. 2005). Several other studies have also inferred the importance of DOM leached from vegetation to Everglades ecosystems by reporting positive correlations between surface water dissolved organic carbon (DOC) concentrations and plant primary productivity (Davis 1991; Qualls and Richardson 2003).

Many studies have reported the rapid loss of weight and constituents during the early stages (a few weeks) of plant decomposition, and have attributed this observation to leaching processes (e.g. Rublee and Roman 1982; Wilson et al. 1986; Blum and Mills 1991; Fourqurean and Schrlau 2003; Davis et al. 2003). However, only a few studies have focused on the production (rate) of DOM from wetland macrophytes, and even fewer have characterized the composition of these materials (Benner et al. 1990a, b; Ziegler and Benner 1999; Hernes et al. 2001; Bertilsson and Jones 2003; Davis et al. 2003). Furthermore, little information is available on the molecular characteristics of the ultrafiltered high-molecular weight component (UDOM) of the leachate (Engelhaupt and Bianchi 2001), despite the biogeochemical importance of UDOM in aquatic ecosystems. Thus, our objective in this study was to better understand the quantity and quality of DOM leached from several important wetland plants in ENP wetlands. We focused on freshwater marsh plants (sawgrass, *Cladium jamaicense*, spikerush, *Eleocharis cellulose*, and cattail, *Typha domingensis*), wet- and dry- periphyton mat assemblages, and estuarine plants (red mangrove, *Rhizophora mangle*, and turtle grass, *Thalassia testudinum*). To better assess the contribution of plant leachates to the UDOM fraction in the surface waters of ENP, we also compared the carbon and phenolic compound compositions of leached UDOM with that in surface water, using ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectroscopy and thermochemolysis in the presence of tetramethylammonium hydroxide (TMAH thermochemolysis).

Materials and methods

Sample collection

Senescent yellow leaves of red mangroves (*Rhizophora mangle*) were hand-picked from trees along the mangrove fringe of northeast Florida Bay (TS/Ph7 = 25°19′ N, −80°64′ W). Senescent above-ground plant samples of sawgrass (*Cladium jamaicense*), spikerush (*Eleocharis cellulosa*), cattail (*Typha domingensis*), and both dry and wet periphyton mats were collected from a freshwater prairie in ENP (TS/Ph2 = 25°40′ N, −80°61′ W). Periphyton is an assemblage of many species of microalgae and heterotrophic microorganisms, and its microbial community structure was found to be

different between dry and wet periphyton mats (Gottlieb et al. 2005). Additionally, both dry and wet periphyton mats were investigated in this experiment because dry periphyton, which is formed in the slough during the dry season, is considered to contribute DOM when it is rewetted at the beginning of the rainy season, while wet periphyton from high hydroperiod areas produce DOM throughout the entire season. Seagrass (*Thalassia testudinum*) was collected from Florida Bay (TS/Ph10 = 25°02′ N, −80°68′ W). Sawgrass, spikerush, and cattail sheaths and blades were cut into fragments of about 8 cm in length, while dried periphyton samples were passed through a 5-mm mesh sieve to minimize the variation in the quality between replicates. Mangrove leaves and wet periphyton were used without any pretreatment. Seagrass blades were used after a gentle rinsing with Milli-Q® water (Millipore, Billerica, MA, USA).

Sample incubation

Ten grams of senescent plant sample (20 g for periphyton) were placed into 250-ml Nalgene® brown bottles (Nalge Nunc International, Rochester, NY, USA). The bottles were filled with 200–250 ml of Milli-Q water and incubated in the dark. Milli-Q water was used as the extractant to minimize post-leaching processing of DOM such as complexation/precipitation and oxidation/polymerization, which can affect the quantitative and qualitative analyses of the leachates. In particular, polyphenols, especially those leached from mangrove leaves, can be unstable under high salinity conditions (unpublished data). In addition, we wanted to avoid contamination of UDOM leached by plants with that present in natural waters. To unify the experimental conditions, seagrass was also immersed into Milli-Q water. We added 1 mg ml^{-1} of NaN_3 as a bacteriostat to half of the bottles (referred as to w/ NaN_3, and w/o NaN_3 treatments, respectively) to test the role of microbial activity on the leaching rates and composition of leachate. The incubations ran for 36 days, and every three days, the water was decanted from each bottle and replaced with fresh Milli-Q® water (w/ or w/o NaN_3). The decanted samples were first filtered through pre-combusted (470 °C for 4 h) GF-F glass fiber filters (nominal pore size, 0.7 μm; Whatman International, Maidstone, England), and then through a 0.22 μm Durapore® membrane filter (Millipore). Water samples decanted from the periphyton treatments were centrifuged to remove suspended solids at 3000 rpm for 10 min before filtration. The rather small amounts of precipitation obtained by centrifugation were returned to the sample bottle. The filtered water samples were stored in the dark at 4 °C for no more than 1 week before analyses.

DOC measurement

The DOC content of the water samples was analyzed using a total organic carbon analyzer (TOC-5000, Shimadzu, Kyoto, Japan) after acidifying the

sample (pH < 2) with HCl and purging with N_2 gas for 5 min. Although some mangrove leachates produced fine particulates upon acidification, most probably due to aggregation of polyphenols, they were uniformly dispersed through purging with N_2 gas, resulting in an average standard deviation (SD) for the measurements of less than 3%.

Colorimetric analysis of sugars

The sugar content of the water samples was analyzed colorimetrically using the Phenol–Sulfuric Acid Method (Dubois et al. 1956; Liu et al. 1973). Briefly, 1 ml of water sample and 1 ml of 5% phenol aqueous solution (w/v) was pipetted into a test tube, and then 5 ml of concentrated sulfuric acid was added. After mixing vigorously with a vortex mixer, the solution was shaken on a reciprocating shaker for 30 min and the absorbance at 490 nm was measured using a UV-Vis scanning spectrophotometer (Shimadzu UV-2101PC UV-visible spectrophotometer, Shimadzu, Kyoto, Japan). Glucose solutions, of concentrations ranging from 4 to 40 mg C l^{-1}, were used as calibration standards. The detection limit of this method (absorption = 0.01) was ~0.4 mg C l^{-1} with a 1-cm pathlength quartz cuvette.

Colorimetric analysis of total phenols

Total phenol content was measured colorimetrically using the Folin–Denis Method (Waterman and Mole 1994). Briefly, a 0.2–3.4 ml aliquot of sample was pipetted into a test tube and the volume was increased to 3.4 ml by adding Milli-Q® water when necessary. Then 0.2 ml of Folin–Denis reagent (Sigma-Aldrich, St. Louis, MO, USA) and 0.4 ml of saturated sodium carbonate solution were added in sequence. After standing for 30 min, the absorbance at 760 nm was measured on a Shimadzu UV-2101PC UV-visible spectrophotometer. Tannic acid solutions, of concentrations ranging from 1 to 5 mg C l^{-1}, were used as calibration standards. The detection limit of this method (absorbance = 0.01) was ~0.25 mg C l^{-1} using a 1-cm pathlength quartz cuvette.

Ultrafiltration of DOM

DOC leaching rates for most of the plant species examined were modeled by a one-stage exponential decay plus constant model or a two-stage exponential decay model (see Result section). Each of which consisted of an initial fast and a subsequently slower DOM release processes, respectively. Accordingly, UDOM leached with a short half-life, and UDOM leached with a long half-life, were collected to investigate the chemical characteristics and possible

changes during early diagenetic processing of plant materials. For that purpose, subsamples of the leachate obtained during 3–12 days and 18–36 days of incubation were collected, separately, and stored on ice in the dark until ultrafiltration. The ultrafiltration was conducted using a Pellicon 2 Mini tangential flow ultrafiltration (TFF) system equipped with a nominal 1 kDa molecular weight cut-off regenerated cellulose membrane (Millipore; see Maie et al. 2005 for details). The concentrated samples (100 ml) were diafiltered with 1 l of Milli-Q water three times to remove small molecular weight organic materials (and NaN_3 when present), freeze-dried, and powdered for ^{13}C CPMAS NMR and TMAH thermochemolysis analyses. Natural water UDOM samples were collected in Aug. 2003 from representative sites of the same dominant vegetation types as used in the leaching experiment; sawgrass and periphyton marsh environment (TS/Ph3 = 25°25′ N, −80°67′ W), mangrove dominated (TS/Ph7), and seagrass dominated (TS/Ph10) (for detailed site information, see Maie et al. 2005 or http://fcelter.fiu.edu). Cattail is not abundant in the FCE yet, although it has a potential to be a major species in the FCE, should the FCE undergo significant eutrophication. Therefore, natural water UDOM surrounded by cattail was not collected.

Solid state ^{13}C NMR spectroscopy

^{13}C CPMAS NMR spectra of UDOM samples were obtained at a ^{13}C resonance frequency of 75.4 MHz on a Bruker ASX300 NMR spectrometer (Bruker, Rheinstetten, Germany) equipped with a commercial 4 mm CPMAS probe using a ramp pulse sequence. ^{13}C chemical shifts were expressed with respect to tetramethylsilane by using the carbonyl carbon of glycine (176.48 ppm) as an external reference. Analytical conditions were as follows: rotation frequency, 13 kHz; contact time, 1 ms; recycle delay, 2 s; scans accumulated, 3000–20,000; spectral width, 25 kHz; filter frequency, 32 kHz; Lorentzian line-broadening, 120 Hz. NMR spectra were divided into six regions according to the representative form of chemical shifts as follows: 0–45 ppm (alkyl C), 45–60 ppm (methoxyl C (OCH_3) + *N*-alkyl C), 60–110 ppm (*O*-alkyl C), 110–140 ppm (aromatic C), 140–160 ppm (phenolic C), and 160–210 ppm (carbonyl C) (Maie et al. 2005).

TMAH thermochemolysis

This method hydrolyzes ether and ester bonds followed by methylation of acidic hydroxyl groups and yields a variety of phenolic compounds (Clifford et al. 1995; McKinney et al. 1995; Filley et al. 1999). TMAH thermochemolysis of UDOM samples was performed according to Hatcher et al. (1995; see also Maie et al. 2005). Briefly, 4–10 mg of powdered UDOM samples were placed in a 5-ml glass ampule (Chemglass, Vineland, NJ, USA), and 200 µl of a

solution of 25% TMAH in methanol (Sigma-Aldrich, St. Louis, MO, USA) and 200 µl of internal standard, *n*-eicosane (50 µg ml^{-1} in methanol), were added. The methanol was evaporated under vacuum for a minimum of 4 h, and the ampoule was flame sealed and placed in a gas chromatographic oven at 250 °C for 30 min. After cooling, the ampoule was cracked open, and the inner glass surface was rinsed with 1 ml of methylene chloride three times. Then the sample was concentrated to dryness under a gentle stream of nitrogen and re-dissolved in 200 µl of methylene chloride. The GC/MS analysis of this extract was performed on a Hewlett Packard 6890 GC–MS series gas chromatograph (GC) coupled to a Hewlett Packard 5973 mass selective detector. One micro litre of the solution was injected into a DB5MS capillary column [(5%-Phenyl)-methylpolysiloxane; 30 m length × 0.25 mm I.D. × 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA]. Helium served as the carrier gas. The GC oven temperature was programmed as follows: initial temperature at 40 °C, ramped at 10 °C min^{-1} to 120 °C, followed by 3 °C min^{-1} to 200 °C, and thereafter by 4 °C min^{-1} to 300 °C (Mannino and Harvey 2000; Maie et al. 2005). Mass spectra were recorded under electron impact ionisation conditions (70 eV) in the m/z = 50–500 range. The assignment of peaks was based on the comparison of mass spectra with a spectral library (Wiley 275, Gaithersburg, MD, USA) and/or mass spectral interpretation. A response factor (1.25) for phenolic compounds to eicosane was calculated by averaging the response of methylation products of vanillin (Sigma-Aldrich), vanillic acid (Sigma-Aldrich), and acetovanilone (Sigma-Aldrich) to that of the internal standard. The measurement was conducted in duplicate. The coefficient of variation (CV) for all the major compounds was 4.6 ± 4.8% (mean deviation, 0.23 ± 0.28 mg g C^{-1} UDOM) whereas that for trace compounds was 16 ± 25% (mean deviation, 0.08 ± 0.10 mg g C^{-1} UDOM). The relative abundance of phenolic products was calculated by dividing the peak area of individual compounds by the total area of the 36 total TMAH products. In an attempt to investigate the phenolic signatures of UDOM, relative abundance of phenolic compounds belonging to groups V, DV, S, and DS were calculated (see Table 4; Maie et al. 2005). Groups V and S contain vanillyl and syringyl type phenolic compounds, respectively, that have been reported as lignin-derived phenols (Hatcher et al. 1995; del Rio et al. 1998). Groups DV and DS contain vanillyl and syringyl units, respectively, that have been reported for lignin in a highly degraded state (del Rio et al. 1998).

Statistical analysis

The total yields of DOC, sugars, and phenolic compounds leached over a 36-days incubation was compared among plant species and treatments (w/ and w/o NaN_3) using two-way ANOVA with interaction followed by the Bonferroni test at a significance level of 0.05 using SPSS version 12 software (SPSS Inc., Chicago, IL, USA). Curve-fitting of the DOC leaching rates was carried

out using SigmaPlot 2001 software (SPSS Inc.). Since the plant leachate was collected and replaced by fresh extractant every 3 days, diurnal DOC leaching rates were calculated by dividing the DOC concentration of the leachates by three. As such, the middle day of each period was used to prepare the graphs and corresponding curve-fitting calculations. A nonhierarchical principal component analysis (PCA) was performed with unnormalized data on the JMP version 5 software to investigate statistically whether natural water UDOM possesses phenol signatures that can be traced back to specific Everglades plants. Although cattail is a dominant species in the northern Everglades where phosphorous levels have increased through runoff from agricultural areas (Qualls and Richardson 2003), this species is not yet abundant in the oligotrophic marshes of ENP. Furthermore, since the phenolic composition of cattail UDOM was drastically different from that of other plants studied here, its inclusion into the PCA analysis would significantly lower the resolution of the method, and was therefore excluded.

Results

Amount of DOC leached from senescent plants

The total amounts of DOC (g C kg^{-1} dry weight (DW)) leached from plant samples during the 36 days of incubation (ΣDOC_{36d}) was variable among sample types and NaN_3 treatment (Table 1). The ΣDOC_{36d} was generally higher for the w/ NaN_3 treatment compared to w/o NaN_3. The DOC leaching yields (w/ NaN_3 treatment) from mangrove (51.5), seagrass (55.2), and cattail (27.6) were significantly higher than those for the dry periphyton mats (8.9), wet periphyton mats (13.0), spikerush (18.7), and sawgrass (15.3) (Table 1). For the dry periphyton, the ΣDOC_{36d} was higher for w/o NaN_3 treatments, suggesting a microbially enhanced leaching process. For most other samples, part of the DOC leached from senescent plants was eliminated from the DOC pool through microbial activity in a relatively short period (3-d) after leaching, suggesting that a portion of plant leached DOC is very labile (based on the comparison of ΣDOC_{36d} for w/ and w/o NaN_3 treatments; Table 1).

Total sugars and total phenols in leachate

The total amount of sugars leached during 36 days of incubation, $\Sigma sugars_{36d}$, and the proportion of the sugar fraction to ΣDOC_{36d} varied among plant types (Table 1). The sugar leaching yield (g C kg^{-1} DW) from w/ NaN_3 treatment was highest for the mangrove (6.87), seagrass (7.22) > cattail (4.80) > dry periphyton (3.02), spikerush (2.65), sawgrass (2.39) > wet periphyton (1.09). While the $\Sigma sugars_{36d}$ was higher in the w/NaN_3 treatment for seagrass, spikerush, and sawgrass, it was lower for periphyton and mangrove, probably

Table 1. Total amount of DOC, total phenols, and total sugars leached from biomass during 36-days of incubation.

Plant species and treatment	DOC (g C kg^{-1} DW[a])	Total sugars (g C kg^{-1} DW[b])	Total phenols (g C kg^{-1} DW[c])	% Total sugars	% Total phenols
Mangrove	50.9 (5.5)[d] A[e]	**9.26**[f] (0.91) A	12.1 (1.3) A	**18** (2) D	24 (2) C
Seagrass	21.5 (3.8) B	4.92 (0.29) C	8.90 (1.25) B	**23** (3) C	**42** (2) A
Cattail	13.7 (1.8) C	4.59 (0.44) C	5.14 (0.50) C	**34** (2) B	**38** (2) B
Periphyton (dry)	**13.4** (0.2) C	**6.12** (0.18) B	0.37 (0.03) D	**46** (2) A	3 (0) F
Periphyton (wet)	13.0 (2.2) C	**3.31** (0.30) D	0.22 (0.01) D	**26** (2) C	2 (0) F
Spikerush	10.2 (0.7) C	2.30 (0.11) D	0.92 (0.03) D	**23** (1) CD	9 (0) E
Sawgrass	7.9 (0.4) C	1.54 (0.03) D	1.53 (0.10) D	**20** (1) CD	19 (0) D
Mangrove w/ NaN_3	51.5 (6.1) a	6.87 (0.64) a	12.4 (1.6) a	13 (0) c	24 (1) b
Seagrass w/ NaN_3	**55.2** (3.9) a	**7.22** (0.73) a	8.24 (0.26) b	13 (1) bc	15 (1) d
Cattail w/ NaN_3	**27.6** (2.1) b	4.80 (0.47) b	**7.76** (0.47) b	17 (0) b	28 (0) a
Periphyton (dry) w/ NaN_3	8.9 (0.3) c	3.02 (0.12) c	0.38 (0.01) d	34 (0) a	**4** (0) f
Periphyton (wet) w/ NaN_3	13.0 (0.4) c	1.09 (0.07) d	0.48 (0.01) cd	8 (1) d	**4** (0) f
Spikerush w/ NaN_3	**18.7** (0.6) c	2.65 (0.01) c	**2.12** (0.03) cd	14 (0) bc	**11** (0) e
Sawgrass w/ NaN_3	**15.3** (0.2) c	**2.39** (0.05) c	**3.05** (0.09) c	16 (0) bc	20 (1) c

[a]DW, dry weight of plant biomass.
[b]Glucose basis.
[c]Tannic acid basis.
[d]Number in parentheses is SD of triplicate.
[e]Levels not connected with same alphabetical letter indicate significant differences ($p < 0.05$); upper-scale letters are for w/o NaN_3 treatment while lower scale for w/ NaN_3 treatment.
[f]Numbers in bold show significantly higher values ($p < 0.05$) for w/ or w/o NaN_3 treatments.

reflecting a difference in the balance of production and consumption rates. The $\Sigma sugars_{36d}$ and its proportion to ΣDOC_{36d} was higher for dry periphyton than wet periphyton w/o NaN_3 (6.12 vs. 3.31 g kg^{-1} DW or 46% vs. 26% of total DOC; Table 1), suggesting that the desiccation of periphyton during the dry season changes the quality of DOM that can potentially be leached after rehydration to a DOM more enriched in carbohydrates. Except for periphyton, $\Sigma sugars_{36d}$ represented 13–17% and 18–34% of ΣDOC_{36d} for w/ NaN_3 and *w/o* NaN_3 treatments, respectively, with the highest value in the cattail leachate.

The total amount of phenols leached during 36 days of incubation, $\Sigma phenols_{36d}$, was more variable than $\Sigma sugars_{36d}$ between plant types, which ranged from 0.22 to 12.4 g C kg^{-1} DW (Table 1). While mangroves yielded the highest amount (for w/ NaN_3 treatment; g C kg^{-1} DW) (12.4), followed by seagrass (8.24), cattail (7.76), and sawgrass (3.05), periphyton leached only small amounts of phenols (0.38–0.48). The proportion of $\Sigma phenols_{36d}$ to ΣDOC_{36d} was also more variable than that of sugars, and comprised 11–28% and 9–42% of ΣDOC_{36d} for w/ NaN_3 and w/o NaN_3 treatments, respectively (excluding periphyton). The relative abundances of ($\Sigma sugars_{36d}$ + $\Sigma phenols_{36d}$) to ΣDOC_{36d} were generally higher for w/o NaN_3 treatments, suggesting the presence of other classes of highly bioavailable organic substances in the leachates.

DOC leaching rates

Mangrove

The leaching rate (A(t)) was best described with a single-stage exponential decay model with a half-life of 10-d (Figure 1a; Table 2). No significant difference was observed in the leaching rate between w/ and w/o NaN_3 treatments.

Periphyton

The leaching rates of periphyton w/ NaN_3 was best described by a single-stage exponential decay with a constant model (Table 2; Figure 2a, b). However, the leaching rates of periphyton w/o NaN_3 did not fit exponential decay models and instead, a prolonged DOC leaching was observed until the end of the 5-week incubation period. The DOC leaching rate from wet periphyton was lower than that from dry periphyton for the w/o NaN_3 treatment at the beginning of the incubation (Figure 2b). Since the wet periphyton had already been submerged in natural water prior to the leaching experiment (no rehydration needed), the easily leachable DOM had already been removed. For both wet and dry periphyton, the DOC leaching rate increased for the w/o NaN_3 treatment during incubation. This may be associated with an increased microbial activity after acclimatization to the new environmental conditions of the incubation, effectively aiding the leaching process.

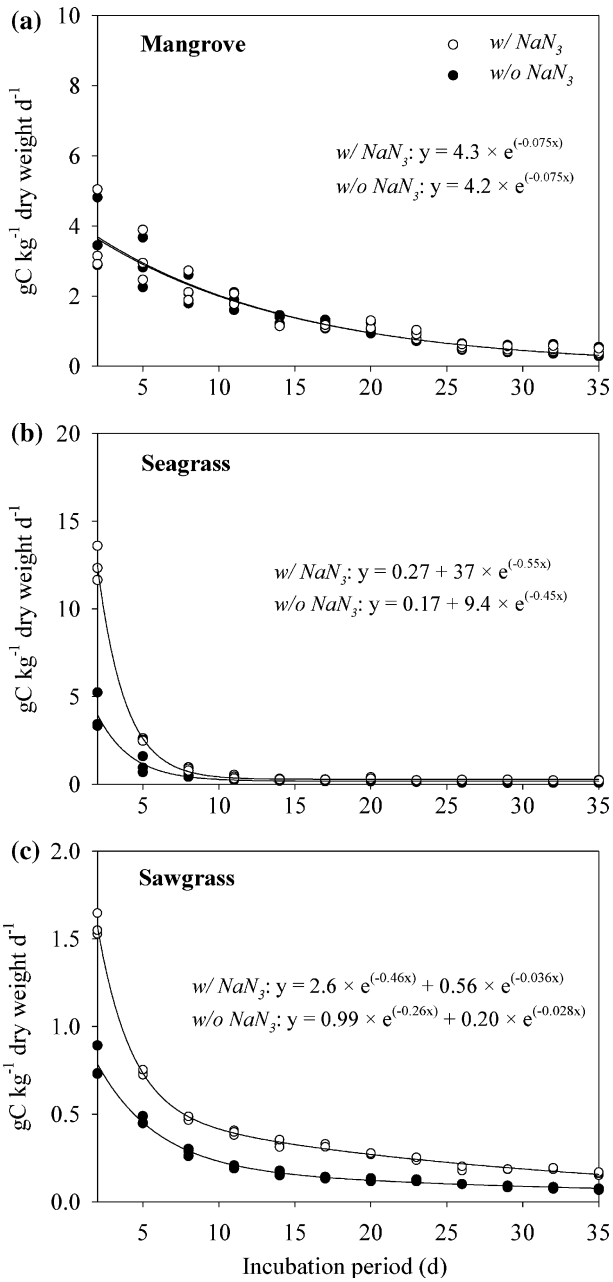

Figure 1. Examples of periodical change of DOC leaching rate (g kg^{-1} dry weight d^{-1}) for (a) mangrove, (b) seagrass, and (c) sawgrass. ○, w/ NaN_3 treatment; ●, w/o NaN_3 treatment.

Table 2. Curve-fitting to DOC leaching rate of senescent plants.

Plant species and treatment	Curve-fit[a]	$T_{1/2}$S(d)[b]	$T_{1/2}$L(d)[b]	r^{2c}	%(1-w)[d]	%(2-w)[d]	% Short[e]
Mangrove	Single	9.9 (2.7)[f]	0	0.98 (0.02)	43 (7)	69 (7)	100 (0)
Mangrove w/ NaN_3	Single	9.8 (2.6)	0	0.98 (0.01)	44 (7)	69 (7)	100 (0)
Seagrass	Single + const.	1.6 (0.7)	Constant	0.99 (0.00)	77 (4)	84 (2)	74 (4)
Seagrass w/ NaN_3	Single + const.	1.3 (0.0)	Constant	1.00 (0.00)	85 (0)	90 (0)	84 (0)
Cattail	Single + const.	4.2 (0.7)	Constant	0.99 (0.00)	50 (3)	71 (2)	62 (2)
Cattail w/ NaN_3	Single + const.	3.0 (0.3)	Constant	0.99 (0.01)	50 (1)	68 (2)	51 (4)
Periphyton (dry)	Unable[g]	N.D.[h]	N.D.	N.D.	24 (1)	43 (2)	N.D.
Periphyton (dry) w/ NaN_3	Single + const.	2.9 (0.5)	Constant	0.91 (0.01)	43 (1)	60 (1)	25 (3)
Periphyton (wet)	Unable	N.D.	N.D.	N.D.	15 (1)	32 (1)	N.D.
Periphyton (wet) w/ NaN_3	Single + const.	15.1 (6.5)	Constant	0.94 (0.04)	37 (1)	63 (1)	55 (8)
Spikerush	Single + const.	3.8 (0.3)	Constant	1.00 (0.00)	65 (3)	85 (1)	86 (1)
Spikerush w/ NaN_3	Double	1.8 (0.2)	18.8 (4.1)	1.00 (0.00)	57 (1)	73 (1)	41 (6)
Sawgrass	Double	2.7 (0.2)	24.8 (1.2)	1.00 (0.00)	52 (2)	71 (2)	43 (4)
Sawgrass w/ NaN_3	Double	1.5 (0.1)	19.1 (0.7)	1.00 (0.00)	50 (1)	68 (1)	29 (2)

[a]Single, single-stage exponential decay model: $A(t) = A_i \times \exp(-\lambda \times T)$; Single + const., single-stage exponential decay with constant model: $A(t) = A_S \times \exp(-\lambda_S \times T) + y_0$; Double, two-stage exponential decay model: $A(t) = A_S \times \exp(-\lambda_S \times T) + A_L \times \exp(-\lambda_L \times T)$; where $A(t)$ is a leaching rate at incubation period T (g C kg^{-1} DW day^{-1}). λ is attenuation coefficient and A and y_0 are constants. T refers to incubation period (d).

[b]$T_{1/2}$ refers to half-life that is calculated by $T_{1/2} = ln\ 2/\lambda$. S and L refer to short and long half-life components, respectively.

[c]Regression coefficient.

[d]%(1-w) and %(2-w) refer to the percentage of DOC leached during the first 1- and 2-week of the 5-week incubation, respectively.

[e]Percentage contribution of the short half-life components to ΣDOC_{36d}.

[f]Number in parentheses is SD of triplicate.

[g]Unable to fit to any exponential decay model.

[h]Not determined.

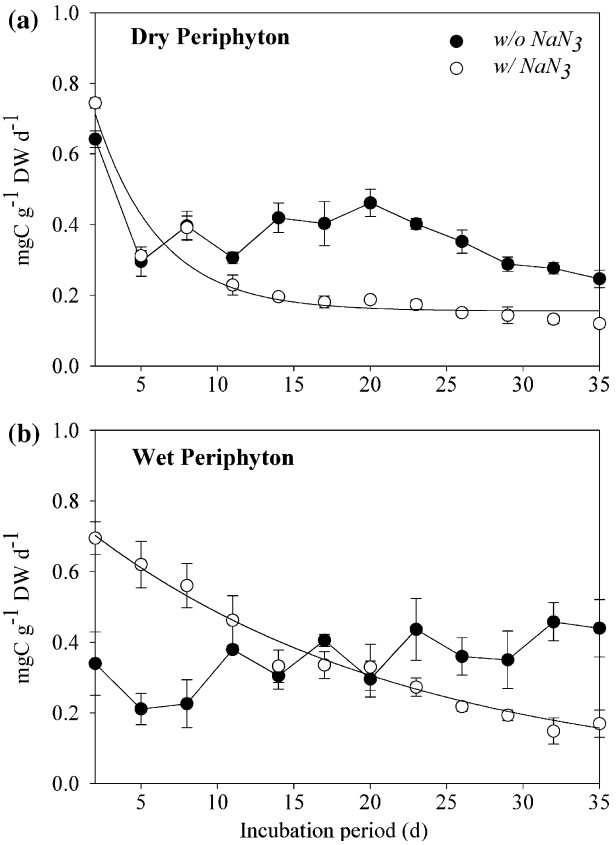

Figure 2. Periodical change of DOC leaching rate for (a) dry and (b) wet periphyton (g kg^{-1} dry weight d^{-1}). ○, w/ NaN_3 treatment; ●, w/o NaN_3 treatment.

Other plant species

Leaching rates were best modeled as either a single-stage exponential decay with a constant models (seagrass, cattail, and spikerush w/o NaN_3, Figuer 1b; Table 2) or by a two-stage exponential decay with short (S) and long (L) half-life components models (spikerush w/NaN_3, sawgrass, Figure 1c; Table 2). The leaching rates of DOC reached a plateau by the end of 5-weeks of incubation (e.g. Figure 1b, c; Table 2). DOC leaching appears to be controlled by a two-step processes; an initial, rapid release followed by a slower release. The half-life of very rapidly leaching components was 1–4 days, while that of slower leaching components was 19 days or longer. The contribution of these rapidly leaching compounds to ΣDOC_{36d} ranged from 29 to 86% (Table 2). Microbial activity may accelerate the apparent leaching rate of DOC because the percentage contribution of rapidly-leaching compounds was higher for w/o NaN_3 treatment for cattail, spikerush, and sawgrass (Table 2). However, since

microorganisms can also consume DOC, the net amount of DOC leached from plant samples was lower in the w/o NaN_3 treatment.

^{13}C CPMAS NMR spectra of UDOM

The ^{13}C CPMAS NMR spectra of the UDOM leached from senescent plants (w/o NaN_3) are shown in Figure 3. A large, sharp peak at around 72 ppm was the major peak for all plant types, and together with a peak at around 102 ppm, is attributed to *O*-alkyl C, which is mainly assigned to carbohydrates. In the case where samples contain condensed tannins, the latter peak also originates from their quaternary C (~105 ppm) (Preston 1999). Other significant peaks observed were a peak at around 18 ppm, representing methyl C; a broad peak at around 42 ppm, representing branched alkyl C and C_α of amino acids in proteins (Kögel-Knabner 1997); a peak at 58 ppm, representing methoxyl C; peaks at 115 ppm and 130 ppm, representing H-substituted and C-substituted aromatic C, respectively; peaks at 146 ppm and 157 ppm, representing phenolic C; and a peak at 170 ppm, representing carbonyl C. The ^{13}C NMR spectra of mangrove UDOM showed two distinct phenolic peaks at 146 and 157 ppm, which are typical for condensed tannins with vicinal OH groups on the B ring (Czochanska et al. 1980). The ^{13}C NMR spectra of cattail showed a single peak in the phenolic region (157 ppm) and a strong peak at 129 ppm (Figure 3). These signatures agree with those of propelargonidin (Chang et al. 2003).

The ^{13}C NMR spectra were not appreciably different between the UDOM leached during the early and the latter stage of incubation (Figure 3; Table 3), suggesting only minor changes if any in the bulk C composition during the 36 day leaching incubation. An exception was seagrass UDOM, of which aromaticity was higher by 5–12% for the early leaching period. This suggests seagrass leaches phenolic compounds relatively rapidly during senescence. The C composition of UDOM was similar between w/ and w/o NaN_3 treatments, except that the *O*-alkyl C (60–110 ppm) and aromatic C (110–160 ppm) concentrations were generally slightly higher (by $5 \pm 2\%$) and lower (by $4 \pm 2\%$) in w/o NaN_3 treatment, respectively. An exception was periphyton UDOM, for which *O*-alkyl C concentrations were higher for the w/ NaN_3 treatment (by 2–8%). While carbohydrates (*O*-alkyl C; 60–110 ppm) made up the highest abundance for all the UDOM samples (41–75%, Table 3), aromatic C + phenolic C (110–160 ppm) concentrations ranged from 2 to 31%.

TMAH thermochemolysis of UDOM

Thirty-six different phenolic compounds were identified by TMAH thermochemolysis and the composition varied depending on plant types (Table 4). The total phenol concentration ranged from 13 to 58 mg g C^{-1} UDOM

(Table 4), and showed a positive correlation with the percentage of ^{13}C CPMAS NMR-based aromatic C in UDOC (C fraction of UDOM) ($n = 6$, $r = 0.84$, $p < 0.05$). Dominant phenolic compounds of mangrove UDOM were 1,3,5-trimethoxybenzene, 2,4,6-trimethoxytoluene, 3,4-dimethoxybenzoic acid methyl ester, and 3,4-dimethoxybenzeneacetic acid methyl ester. These compounds are also the major components of TMAH thermochemolysis products of catechin/epicatechin (Garnier et al. 2003) as well as

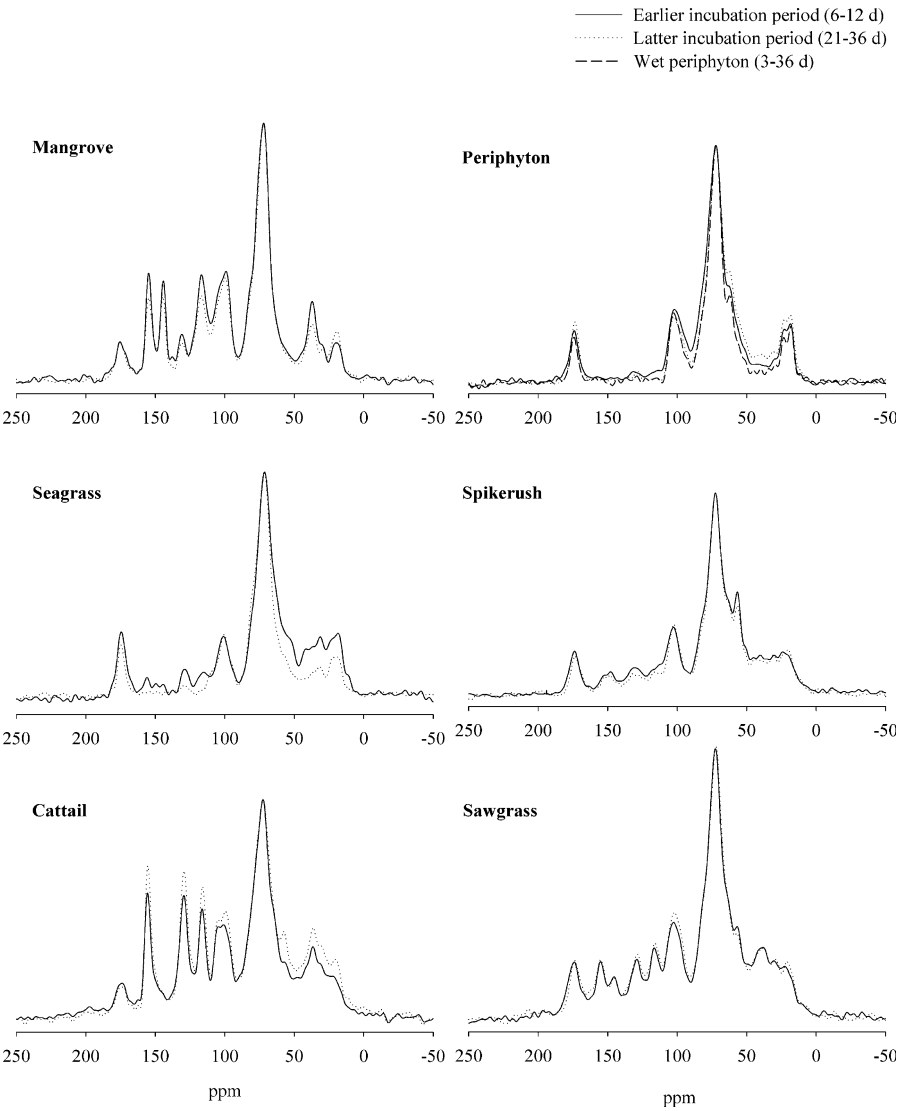

Figure 3. ^{13}C CPMAS NMR spectra of UDOM leached from senescent plant materials.

Table 3. ^{13}C-NMR spectroscopy based carbon composition (%) of UDOM fraction leached from senescent plants.

UDOM source	Alkyl C 0–45 ppm	OCH_3, *N*-alkyl C 45–60 ppm	*O*-alkyl C 60–110 ppm	Aromatic C 110–140 ppm	Phenolic C 140–160 ppm	Carbonyl C 160–210 ppm	Alkyl C/(OCH_3, *N*-alkyl C + *O*-alkyl C)	Aromaticity[a] (%)
Plant-leached UDOM								
Mangrove	13/13[b]	4/4	52/54	15/13	11/11	5/5	0.19/0.19	28/26
Mangrove w/ NaN_3	14/14	4/4	47/48	17/15	14/11	5/8	0.21/0.21	32/28
Seagrass	21/15	9/7	52/67	6/4	3/1	8/6	0.26/0.17	10/5
Seagrass w/ NaN_3	21/18	8/8	44/61	11/4	5/1	11/7	0.29/0.20	18/6
Cattail	14/18	6/7	47/43	18/18	9/9	6/5	0.21/0.26	29/29
Cattail w/ NaN_3	14/16	6/6	44/41	20/21	11/11	5/5	0.22/0.25	32/33
Periphyton (dry)	14/13	7/8	71/67	2/3	1/1	6/7	0.15/0.15	3/5
Periphyton (dry) w/ NaN_3	12/12	7/7	72/75	2/1	1/0	5/5	0.13/0.13	3/2
Periphyton (wet)	18/–[c]	10/–	63/–	2/–	1/–	6/–	0.20/–	3/–
Spikerush	16/17	12/11	52/54	9/8	4/4	7/6	0.20/0.21	14/12
Spikerush w/ NaN_3	15/17	12/13	50/46	11/12	5/6	6/6	0.20/0.23	18/19
Sawgrass	16/16	9/8	50/50	12/13	7/6	7/7	0.21/0.21	20/21
Sawgrass w/ NaN_3	15/17	8/8	48/44	14/15	7/9	8/7	0.21/0.25	23/25
ENP surface water UDOM								
Taylor Slough[d]	20(1)[e]	12(1)	42(3)	9(2)	4(1)	13(2)	0.37(0.01)	15(4)
Florida Bay[d]	13(1)	8(1)	66(4)	1(1)	1(1)	10(1)	0.18(0.03)	2(2)

[a]Aromaticity was calculated by (aromatic C + phenolic C)/(alkyl C + *N*-alkyl C + OCH_3 + *O*-alkyl C + aromatic C + phenolic C)×100.
[b]C composition of the UDOM leached during the early period (3–12 days, left) vs. the latter period (18–36 days, right) of incubation.
[c]Not determined.
[d]Data from Maie et al. (2005).
[e]Number in parenthesis is standard deviation of 4 different sites along Taylor Slough and 3 different sites in Florida Bay.

Table 4. Relative abundance of phenolic compounds released from UDOM samples by TMAH thermochemolysis (%).

No.	Compounds[a]	UDOM leached from plant materials						Mangrove tannins[b]	UDOM in ENP surface water		
		Mangrove	Seagrass	Cattail	Periphyton	Spikerush	Sawgrass		TS/Ph3	TS/Ph7	TS/Ph10
1	Phenol, 2-methoxy-	2.5	4.3	0.3	**5.9**[c]	2.4	1.7	1.8	4.7	2.4	**11.3**
2	1,2-benzenediol, 3-methoxy-	0.0[d]	3.2	0.5	**5.2**	1.2	1.6	0.0	0.0	0.0	0.0
3	1,4-benzenediol, 2-methoxy-	0.2	0.6	0.2	3.7	0.8	0.8	0.0	1.7	2.1	0.0
4	Benzene, 1,2-dimethoxy-	2.4	3.4	0.6	3.6	2.4	1.9	2.6	4.8	3.5	**10.5**
5	Benzene, 1-ethenyl-4-methoxy-	0.4	2.1	0.7	0.0	1.8	1.1	0.0	0.0	0.0	0.0
6	Benzene, 1,4-dimethoxy-	1.4	**6.0**	2.3	**26.3**	1.6	1.4	0.0	**9.2**	**6.0**	**23.2**
7	3,4-dimethoxytoluene	2.3	0.0	0.0	0.0	0.0	0.0	3.7	2.8	3.7	**5.1**
8	2,5-dimethoxytoluene	0.2	0.7	0.2	2.7	0.6	0.4	0.0	0.0	0.0	4.5
9	Benzaldehyde, 4-methoxy-	0.1	**5.7**	**9.9**	0.0	1.4	3.8	0.0	0.0	0.0	0.0
10	Benzene, 1,2,3-trimethoxy-	0.6	1.2	0.6	0.0	**5.3**	2.2	0.4	**5.2**	**5.0**	**7.5**
11	Benzoic acid, 3-methoxy-, ME [e]	1.6	1.9	1.1	4.3	1.0	2.2	0.0	3.7	3.4	**9.5**
12	Phenol, 2,6-dimethoxy-,	0.5	1.4	0.4	1.9	3.9	1.2	0.1	0.0	0.0	0.0
13	Ethanone, 1-(4-methoxyphenyl)-	0.0	2.5	3.6	0.0	1.0	2.3	0.0	0.0	0.0	0.0
14	Benzene, 4-ethenyl-1,2-dimethoxy-	2.6	2.4	0.6	0.0	**5.0**	2.9	1.6	1.8	0.0	0.0
15	1,2,4-trimethoxybenzene	3.8	**13.7**	1.7	**26.5**	**5.7**	3.0	1.7	**11.8**	**14.0**	**24.2**
16	Benzoic acid, 4-methoxy-, ME	0.7	**5.0**	**26.5**	0.0	3.8	**7.6**	0.0	**7.5**	**5.9**	0.0
17	2-propanone, 1-(4-methoxyphenyl)-	0.0	0.0	0.9	**5.6**	0.0	0.0	0.0	0.0	0.0	0.0
18	Benzoic acid, 2-5-dichloro-, ME	0.0	4.1	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	Benzene, 1,3,5-trimethoxy-	**24.8**	3.4	**10.5**	0.0	1.8	**10.4**	**32.6**	3.2	**9.7**	0.0
20	Benzeneacetic acid, 4-methoxy-, ME	1.0	**8.6**	**14.9**	1.9	1.0	4.8	0.0	2.0	0.0	0.0
21	1,2-benzenedicarboxylic acid, di-ME	0.7	0.0	0.8	**5.6**	2.0	4.9	0.0	0.0	0.0	0.0
22	Benzaldehyde, 3,4-dimethoxy-	3.0	0.9	0.0	0.0	2.9	1.5	0.0	0.0	0.0	0.0
23	2,4,6-trimethoxytoluene	**13.9**	0.9	**6.6**	0.0	0.3	**6.9**	**21.6**	2.1	**6.2**	0.0
24	2,5-dimethoxy-4-ethylbenzaldehyde	0.0	0.0	0.4	0.0	2.7	1.0	0.0	0.0	0.0	0.0
25	Ethanone, 1-(3,4-dimethoxyphenyl)-	1.9	4.0	0.4	0.0	2.5	4.9	2.3	2.9	3.2	0.0
26	2′,4′-dihydroxy-3′-methylbutyrophenone	1.6	0.5	0.0	3.1	0.9	1.0	0.0	0.0	0.0	0.0
27	Benzoic acid, 3,4-dimethoxy-, ME	**16.6**	4.4	4.2	2.5	**13.0**	**12.0**	**15.0**	**21.6**	**23.7**	0.8
28	Benzeneacetic acid, 3,4-dimethoxy-, ME	**9.2**	3.2	0.0	0.0	0.0	2.8	**12.3**	2.3	3.5	3.3

Table 4. Continued

No.	Compounds[a]	UDOM leached from plant materials						Mangrove tannins[b]	UDOM in ENP surface water		
		Mangrove	Seagrass	Cattail	Periphyton	Spikerush	Sawgrass		TS/Ph3	TS/Ph7	TS/Ph10
29	Benzenepropanoic acid, alpha-hydroxy-4-methoxy-, ME	0.0	3.0	**7.2**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	Benzene, 1,4-dimethoxy-2,3,5,6-tetramethyl-	3.1	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
31	Ethanone, 1-(3,4,5-trimethoxyphenyl)-	0.6	0.0	0.6	0.0	**7.8**	2.0	0.0	0.0	0.0	0.0
32	2-propenoic acid, 3-(4-methoxyphehyl)-, ME	0.0	3.2	0.7	0.5	3.1	2.6	0.0	3.0	0.0	0.0
33	Benzoic acid, 3,4,5-trimethoxy-, ME	0.9	0.0	1.9	0.7	**12.6**	**5.4**	1.7	**9.6**	**7.6**	0.0
34	Benzenepropanoic acid, 3,4-dimethoxy-, ME	0.6	**6.3**	0.5	0.0	0.8	0.7	0.0	0.0	0.0	0.0
35	2-propenoic acid, 3-(3,4-dimethoxyphenyl-), ME	2.8	3.3	0.7	0.0	0.0	3.3	2.7	0.0	0.0	0.0
36	2-propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, ME	0.0	0.0	0.0	0.0	**10.5**	1.2	0.0	0.0	0.0	0.0
Grouping of phenolic compounds											
	V (14 + 22 + 25 + 27 + 28 + 34 + 35)[f]	37	25	6	2	24	28	34	29	30	4
	S (31 + 33 + 36)	2	0	3	1	39	11	2	10	8	0
	DV (1 + 4 + 7)	7	8	1	9	5	4	8	12	10	27
	DS (2 + 10)	1	4	1	5	7	4	0	5	5	7
	(S + DS):(V + DV) ratio	0.05	0.14	0.59	0.50	1.55	0.45	0.05	0.36	0.31	0.24
	(DV + DS):(V + S) ratio	0.2	0.5	0.2	4.7	0.2	0.2	0.2	0.5	0.4	8.4
	Total benzoic acids (11 + 16 + 18 + 21 + 27 + 33)	20.4	15.4	34.9	13.1	32.4	32.1	16.7	42.4	40.6	10.3
Yield (mg g C^{-1})		39.3 ± 0.0	13.3 ± 0.2	58.0 ± 0.5	16.8 ± 0.4	40.0 ± 2.8	35.6 ± 1.6	N.D.[g]	9.4 ± 0.8	5.8 ± 1.0	0.3 ± 0.8

[a]Phenolic compounds of relative abundance less than 2% for all plant species were excluded.
[b]Extracted from red mangrove leaves according to Maie et al. (2003).
[c]Concentrations of phenolic compounds higher than 5% are shown in bold.
[d]0.0 includes compounds of which concentration was lower than detection limit and those could not be identified due to coelution.
[e]ME, methyl ester.
[f]Numbers in parentheses correspond to the number on the left of compound name.
[g]Not determined.

from condensed tannins extracted from mangrove leaves (Table 4). Major phenolic compounds released from cattail were 4-methoxyphenol derivatives, which are probably derived from propelargonidin, a building block of condensed tannins often found in monocotyledons (Ellis et al. 1983). This interpretation is also corroborated by ^{13}C NMR spectra (see ^{13}C NMR section). Spikerush was characterized by a high abundance of vicinal trimethoxybenzene derivatives such as 3,4,5-trimethoxybenzoic acid methyl ester, and 3-(3,4,5-trimethoxyphenyl)-2-propenoic acid methyl ester. One such derivative, 3,4,5-trimethoxybenzoic acid methyl ester is reported to be derived from galoylated condensed tannins (Garnier et al. 2003) or from lignin-degradation products (del Rio et al. 1998; Vane et al. 2001). Periphyton was characterized by a high abundance of benzene, 1,4-dimethoxy- and 1,2,4-trimethoxybenzene, the sum of which exceeded more than half of total phenolic compounds released by TMAH thermochemolysis. These phenols are also major components of the UDOM of the ENP surface water (Table 4).

The relative abundance of group V compounds in the leachates was $< 10\%$ for periphyton and cattails, and $> 20\%$ for mangrove, sawgrass, seagrass, and spikerush. The abundance of group S compounds in the leachates was low (1–3%) except for spikerush (39%) and sawgrass (11%) (Table 4). The UDOM in plant leachates also contained phenolic compounds from the DV and DS groups, of which relative abundances ranged from 1 to 9% and from 0 to 7%, respectively. The relative abundance of total benzoic acids ranged from 13 to 35%.

Discussion

Quantitative aspect of DOM leached from senescent plants

Our results show that the maximum yields of DOC leached from senescent plants during the early stage of diagenesis range from 13 to 55 g C kg^{-1} DW for the types of vegetation under study (Table 1). Furthermore, a significant amount of the DOC, 43–85% and 68–90% of ΣDOC_{36d}, was leached by the end of the first and second weeks of incubation, respectively (Table 2). Since our time-series DOC leaching data were well fitted to exponential decay models and less or equal amounts of DOC were leached from w/o NaN_3 treatment (Figures 1 and 2, Tables 1 and 2), the physical leaching of soluble organic matter appears to be the main mechanism for weight loss during the early stage of plant decomposition (the first 5 weeks in this experiment). This interpretation agrees with other studies (Rublee and Roman 1982; Blum and Mills 1991). The exception was periphyton, for which DOC leaching rates remained high during 5-weeks of incubation and were sometimes higher for w/o NaN_3 treatment (Figure 2a, b), suggesting that both physical and microbial processes are important in this case.

The half-life of DOC leaching from seagrass was the shortest, with $\sim 80\%$ of ΣDOC_{36d} leached during the first week of incubation (Figure 1c, Table 2).

However, the osmotic pressure of Milli-Q water used for the incubation is different from that of natural water, especially from that of saline water, whereby cells that could not tolerate the sudden change in osmotic pressure might burst, resulting in a quicker release of DOM at the very beginning of the incubation. However, since a rapid weight loss of seagrass biomass has also been observed in the experiments conducted in seawater (e.g. by 20% decrease of initial dry weight after 24 h (Blum and Mills 1991) and after 5 days (Fourqurean and Schrlau 2003) of incubation), DOC leaching from hand-harvested seagrass leaves is thought to occur on a short time scale regardless of salinity.

Vascular plant materials used in this study were leaves and sheath, while periphyton consisted of an assemblage of microalgae and heterotrophic microbes. As such, the microbial activity was presumably much higher in periphyton samples than in senescent leaves. This was evident in the periphyton DOC leaching study. Although the DOC leaching rate was not different during the first 9 days of incubation for dry periphyton w/ and w/o NaN_3 treatments, it became significantly higher for the w/o NaN_3 treatment after this initial period (Figure 2a). This was probably due to the recovery of the microbial activity after rewetting of the dry periphyton.

Microbial mineralization/immobilization of carbon seems to reduce the apparent leaching of very labile DOC fraction from most of the plant species. Indeed, from 1 to 61% of the leached DOC was quickly removed from the DOC pool in this experiment except for periphyton. Note that the microbial degradation of labile DOC took place within 3 days, since the each extractant was replaced with freshly prepared Milli-Q water every time period. Such quick utilization of bioavailable DOC by microbes might be more significant in natural aquatic ecosystems, since Milli-Q water instead of natural water was used as the extractant in this study. The degradation of DOC in aquatic environments may be affected by a variety of factors including the concentrations of nutrients, bioavailable DOM, and microbial growth inhibitors, and also the microbial community structure and its biomass and activity. Benner et al. (1986), for example, reported the quick utilization of mangrove leachate by microbes while no appreciable degradation was observed in our experiments. As such, more controlled experiments are necessary to investigate the bioavailability and degradability of plant-leached DOM, however, this is beyond the scope of this study, which is focused on the leaching process of DOM.

ΣDOC_{36d} from sawgrass and spikerush, macrophytes that characterize oligotrophic freshwater marshes of ENP, was lower than from cattail, which is more characteristic of P enriched areas (Table 1; Davis 1994; Noe et al. 2001). Qualls and Richardson (2003) reported that ground senescent leaves of cattail contained higher concentrations of total water extractable substances than did sawgrass and spikerush. This observation likely reflects the different life history strategies of these species (Grime 1977; Davis 1994); cattail has higher nutrient requirements, has lower nutrient resorption efficiency (Richardson et al. 1999), and decomposes more rapidly compared with sawgrass and spikerush (Davis

1991; Qualls and Richardson 2000). Therefore, the transition of vegetation from sawgrass to cattail caused by eutrophication may increase the amount of DOM released into the environment. In addition, such a vegetation shift could also influence the DOM composition, as discussed below.

Estimates of the contributions of the various plant species to the DOC pool are important for understanding organic matter dynamics in the Florida Coastal Everglades (FCE) because dominant plant species are likely to change with land use and ecosystem management practices. The data presented here will help the estimation of plant-derived DOC inputs once information on the vegetation cover and primary productivity of macrophytes and periphyton become available. Furthermore, our data show that senescent plants supplies large amounts of DOM in a relatively short period after being exposed to water. This process might be particularly important when wetlands are rewetted after the dry season. At the same time, the degradation of periphyton by microorganisms and grazers is also important for the leaching of DOC (Bertilsson and Jones 2003), and this process may be particularly important as a relatively long-lasting DOC source in the FCE.

Qualitative aspect of DOM leached from senescent plants

Our results showed that the composition of DOC was different between vegetation types. Therefore, both the quantity and quality of leached DOM change as water flows through the Everglades landscape. Different compounds in leachates from plant materials undergo different biogeochemical processing in aquatic environments (McKnight 2003; Obernosterer and Benner 2004; Scully et al. 2004). Plant primary compounds, such as sugars and proteins, are considered to be highly bioavailable, and therefore microbial degradation will be an important diagenetic process. On the other hand, phenolic compounds (plant secondary compounds) are generally more resistant to microbial degradation/mineralization and might suppress microbial activity (e.g. Northup et al. 1998, Schimel et al. 1998; Preston 1999). However, since these compounds are very photosensitive and reactive (Scully et al. 2004), photochemical and physical processes will be important in their diagenesis. In addition, phenolic compounds are also important precursors of humic substances and chromophoric DOM (CDOM). Thus, DOM leached from different vegetation types may have different biogeochemical characteristics, and exert different ecological impacts on the environment.

It has been reported that seagrasses contain a significant amount of phenolic compounds (Agostini et al. 1998), including free and esterified phenolic compounds, which are leached rapidly from this substrate (Opsahl and Benner 1993). Even so, it was surprising that seagrass yielded the second highest amount of phenolic compounds, in spite of (1) a low specific UV absorption (unpublished data) of natural water in Florida Bay where seagrass covers most of the bottom sediments (Zieman et al. 1989), and (2) very low aromatic C

concentration in its high molecular weight fraction (> 1 kDa; UDOM) (Maie et al. 2005). However, the phenols in our study were found to be mainly comprised of low molecular weight compounds, which seem to be quickly broken down after exposure to sunlight (Scully et al. 2004). Ziegler and Benner (2000) examined photodegradability of DOM in a subtropical seagrass meadow and reported that the photochemical process had very little affect on the diagenetic processing of DOM. However, the DOM examined by these authors may have already undergone extensive photobleaching even if the water sample had been collected at dawn. In contrast, our UDOM was freshly leached from seagrass and thus a higher photoreactivity is not surprising. Therefore, although a large amount of phenols may be generated in subtropical seagrass-dominated shallow estuaries, such as Florida Bay, those leached from seagrass undergo quick diagenesis under strong sunlight conditions and their resulting abundance in natural water is thus very low.

Qualitative aspects of UDOM leached from senescent plants

Composition based on ^{13}C-NMR

Since the C composition of UDOM was not appreciably different among the early and the latter leached UDOM for w/ and w/o NaN_3 treatments for most plant species, its compositional features will be discussed based on their average values (Table 3). Note that the relative abundance of *O*-alkyl C was slightly higher for most plant species in w/o NaN_3 treatment. Carbohydrates (*O*-alkyl C, 60–110 ppm) were the major constituent of UDOM leached from senescent plants (Figure 3 and Table 3). The relative proportion of *O*-alkyl C in this study was higher than an earlier study by Lu et al. (2003), where comparatively lower proportions of *O*-alkyl C were observed for rotary-evaporated whole DOM samples leached from periphyton, sawgrass, and seagrass. This suggests that carbohydrates can be enriched in UDOM fraction for some plant leached UDOM. A positive correlation was observed between total phenol concentration and the % aromatic C of UDOC ($r = 0.70$, $p < 0.001$, $n = 25$), suggesting that a significant portion of phenolic compounds is associated with the high molecular weight fraction in all samples. An exception was the seagrass UDOC sample leached during the early period of incubation for w/o NaN_3, which contained a very low concentration of aromatic C (Figure 3; Table 3), despite the fact that the whole sample contained the second highest concentration of phenols (Table 1). By excluding this sample from the regression, the correlation coefficient increased to 0.88. On the other hand, *O*-alkyl C was highly enriched in seagrass UDOC in spite of its relatively low sugar concentration in its DOC. This high % *O*-alkyl C of seagrass UDOC coincides with the ^{13}C NMR signature of UDOC in natural water collected from Florida Bay, in which *O*-alkyl C concentration was also very high (Maie et al. 2005). Thus, our result supports the idea that seagrass leachate is an important source of high molecular weight carbohydrates in this estuary.

Plant leached DOM is considered an important source of UDOM in natural water in the Everglades (Lu et al. 2003; Maie et al. 2005). The relative abundance of alkyl C and carbonyl C was consistently low in UDOM leached from plant materials compared with surface waters from ENP wetlands, suggesting that these constituents increase during diagenesis of plant-derived UDOM (Table 3; ^{13}C NMR data for ENP were taken from Maie et al. 2005). This agrees with the finding that oxidative degradation of organic matter is usually accompanied by a decrease of *O*-alkyl C and increase of carbonyl C (Knicker and Lüdemann 1995; Orem and Hatcher 1987; Engelhaupt and Bianchi 2001). The alkyl C (0–45 ppm): ($-OCH_3$ + *N*-alkyl C + *O*-alkyl C 45–110 ppm) ratio is often used to assess the degree of decomposition of organic matter in soils where source materials are similar (Baldock et al. 1997). Our result showed that freshly leached UDOM from various plant species had very similar values (0.28 ± 0.02), except for periphyton (0.16–0.25) (Table 3). As such, a higher alkyl C : (methoxyl C + *N*-alkyl C + *O*-alkyl C) ratio of UDOM in Taylor Slough water (0.37; Maie et al. 2005) was likely the result of higher diagenetic reworking of the DOM. Thus, this ratio may also be a useful indicator of the degree of diagenesis of UDOM in aquatic environments.

Carbohydrates were the major form of C in UDOM in both leachates and in natural water samples (Table 3). Carbohydrates are potentially bioavailable, and thus their concentration is expected to decrease through microbial degradation. In a study by Engelhaupt and Bianchi (2001), UDOM leached from plant materials and exposed to microbial activity for 5 weeks had a higher alkyl C abundance than in our study. In ENP wetlands, however, the microbial activity is low due to phosphorus-limiting conditions (e.g. Davis 1994), resulting in a higher *O*-alkyl C abundance.

The relative abundance of aromatic C in UDOM samples collected from both freshwater and estuarine sites in Taylor Slough, ENP, was relatively constant (Maie et al. 2005), while those in plant leached UDOM varied depending on the species (Table,3). A possible explanation for this observation is that the phenolic compounds leached from senescent plants are very reactive and photosensitive, thus, these aromatic compounds may be rapidly removed or transformed through physical, photochemical, and/or physicochemical processes in natural water (Scully et al. 2004).

Composition based on TMAH thermochemolysis

Phenolic compounds derived from lignin have been considered to be the principal source of aromatic moieties of DOM (or CDOM) in natural environments (del Rio et al. 1998; Mannino and Harvey 2000). These compounds have been characterized using TMAH thermochemolysis (e.g. Hatcher et al. 1995; Martin et al. 1995; del Rio et al. 1998). Phenolic compounds that have vanillyl (V) and syringyl (S) moieties are characteristic markers for lignin (e.g. Hatcher et al. 1995; Martin et al. 1995), and the substitution pattern of these compounds is useful to estimate the origin of lignin (angiosperm, gymnosperms, herbaceous tissues; e.g. Challinor 1995). However, lignin is inherently

insoluble in water, and microbial oxidative degradation is necessary for the lignin-constituents to become soluble. Our results clearly showed that rapidly leachable phenols potentially contribute to the so-called 'lignin phenols' and to CDOM in aquatic environments (Table 4). Such rapidly leachable phenolic compounds are unlikely derived directly from lignin, but instead are derived from polyphenols. The later are probably more reactive/labile than lignin (Scully et al. 2004) in natural environments, since the portion of phenolic groups of lignin is inherently methoxylated and protected. As such, caution must be exercised in assigning a lignin source to these phenolic compounds.

Several geochemical proxies were used to assess the phenolic composition differences between the leachates and natural water samples. These consisted of the (S + DS):(V + DV) ratio, the (DV + DS):(V + S) ratio and the abundance of the total acid-type phenols (total benzoic acids). The (S + DS):(V + DV) ratio has been suggested as a tool to assess the origin of lignins (Challinor 1995). In this study, this parameter ranged from 0.05 to 1.55 for the leachates (Table 4) and showed intermediate values for the natural water samples, and no clear correlation was observed between dominant plants and corresponding natural water samples. It seems that diagenetic processes or alternative UDOM sources do hinder the applicability of this parameter as a source indicator in this study. DV + DS are considered to be produced from V + S through extensive photodegradation (Maie et al. 2005; del Rio et al. 1998). As such, the (DV + DS): (V + S) ratio is considered to be useful to assess the diagenetic state of UDOM. The (DV + DS):(V + S) ratio was low (from 0.2 to 0.5) for all plant leachates except for periphyton (4.7). The freshwater marsh or mangrove influenced sites (TSPH3 and TSPH7) showed correspondingly low values (0.5 and 0.4, respectively). In contrast, the Florida Bay site (TSPH 10), where extensive light exposure is expected, showed the highest values (8.4). Therefore, although plant leachates also contribute DV + DS, the ratio can be useful in investigating the photodegradation state of DOM. Finally, the relative abundance of total benzoic acids indicative of oxidative degradation of DOM, were higher in marshes than for the plant leachates, while they were lowest for Florida Bay (Table 4). This data suggests diagenetic processing of UDOM in the freshwater marsh and mangrove areas increases the abundance of acid-type phenols, and further extensive photodegradation reduces their abundance in Florida Bay (del Rio et al. 1998; Maie et al. 2005). Therefore, and not unexpectedly, the molecular composition of the phenolic substances in the natural water UDOM was very different from that in the UDOM leached from plants (Table 4). Overall, our data suggests that the use of these rather traditional phenol-based geochemical proxies in these studies seems to be limited.

In an attempt to further explore the use of the polyphenols as UDOM source and degradation indicators, PCA was applied to the data shown in Table 4. By applying PCA to the relative abundance of the phenol composition, the UDOM samples from different plants were clearly separated on PC1–PC2 score plots and/or PC1–PC3 score plots (Figure 4). Phenolic compounds with high

positive/negative loading values were abundant in UDOM in plant leachate that had high positive/negative scores on a given PC (e.g. phenolic compounds that have large loading values for PC1 are abundant in periphyton, Table 4 and

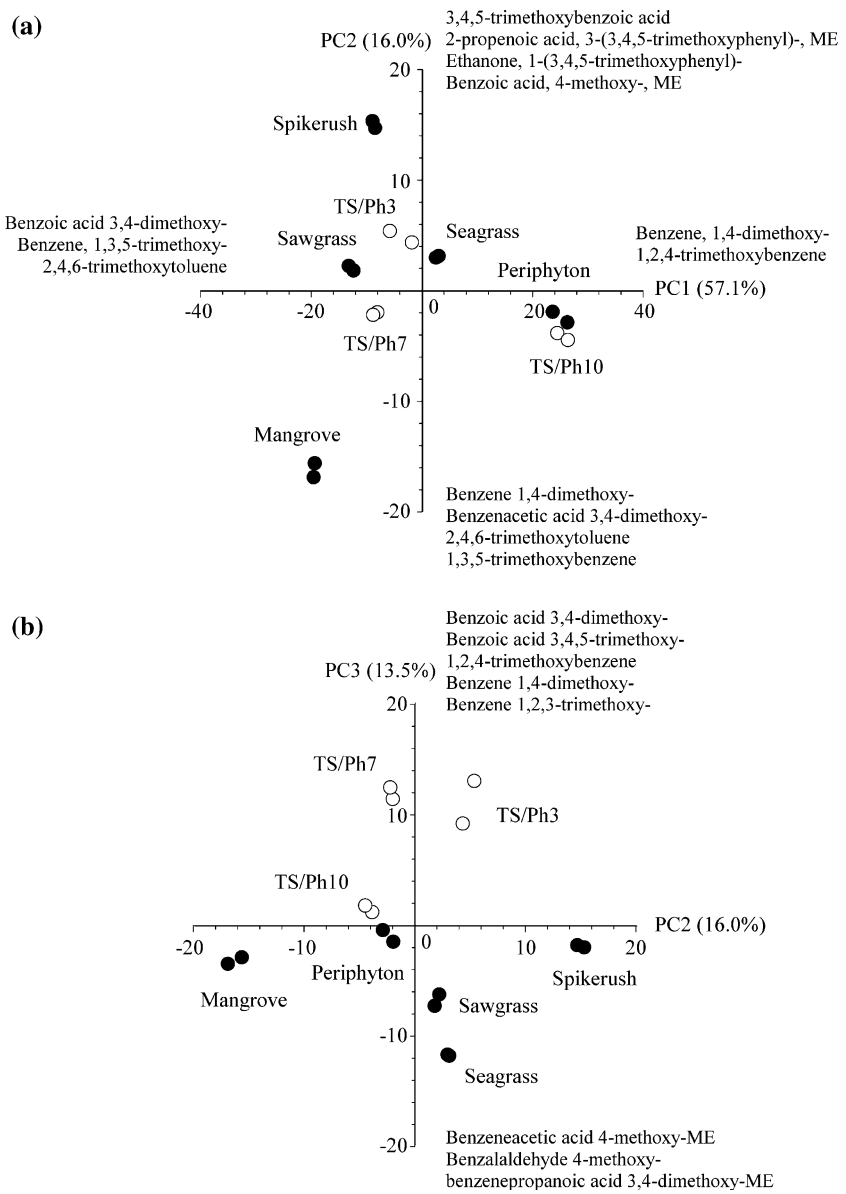

Figure 4. PC1–PC2 (a) and PC2–PC3 (b) score plots of UDOM samples based on the principal component analysis (PCA) of phenolic compounds released by TMAH thermochemolysis. Each UDOM sample was analyzed in duplicate. Cattail was excluded in this analysis since it is not the major vegetation in the studied area.

Figure 4). In this analysis, cattail was not included because this species is not yet a major component of the landscape of the ENP. PCA analysis displayed that the UDOM samples from ENP surface waters did not have a strong signature of phenolic substances from surrounding vegetation (Figure 4), and are clearly separated from plant leached UDOM on PC2–PC3 plots (Figure 4b). This decoupling between leachate and natural water UDOM was ascribed to (1) a high reactivity and photosensitivity of phenolic compounds leached from senescent plants, which may lead to a rapid diagenetic alteration and coagulation (Scully et al. 2004), and (2) contribution of phenolic compounds from other sources such as highly degraded plant materials, soils/sediments, and unexamined vegetation. However, phenolic compounds in Everglades UDOM varied between sampling sites, indicating variations in source and diagenetic processing. Further studies on diagenetic processing of these phenolic substances will be necessary to better understand the relationship of phenolic compounds leached from plant materials and present in natural water.

Implications and conclusions

Submerged and emergent aquatic plants of the FCE have the capacity to contribute significantly to DOM and UDOM in this subtropical wetland system, through leaching and exudation. Our results show that both the quantity and quality of leached DOM change as water flows through the Everglades landscape. Everglades Restoration is expected to increase water flow to this landscape, which will increase both water depth and hydroperiods in freshwater marshes (http://www.evergladesplan.org/). Change in the water regime may change the productivity and vegetation cover in the ecosystem (Childers et al. submitted), and therefore the quantity and quality of DOM being produced. An increase of water level, for example, would create a more open marsh (Childers et al. submitted), which is more favorable for periphyton production. Such shift might reduce the input of phenolic compounds and therefore the chromophoric DOM concentration. Carbohydrates are the major constituents of the UDOM leached from senescent plants as well as the UDOM in natural water in the FCE, which strongly suggests that the senescent plants is an important contributor of UDOM in this ecosystem. Molecular characteristics of phenolic compounds varied among UDOM leached from different plant types, and as such this class of compounds could be used as a source indicator. However, since the phenolic compounds leached from plant materials are highly reactive and photosensitive (Scully et al. 2004), these compounds undergo diagenetic processing shortly after their input to natural environments.

Our results will prove useful in estimating the contribution of DOM derived from the early stage of decomposition of various ecosystem components in spatially articulate models. However, several important questions remain to be explored for more comprehensive understanding of the source of DOM in the

FCE. The quality and quantity of DOM leached from plant materials may vary depending on the physiological state of plants (e.g. nutrient limitation, light and water stress) and senescent stage of plant materials (Agostini et al. 1998; Northup et al. 1998; Richardson et al. 1999). The relative contribution of living biomass exudation of DOM also needs to be explored further (Bertilsson and Jones 2003 and references therein; Ziegler and Benner, 1999), as do DOM fluxes between surface waters and both wetland soils and subtidal sediments.

Knowledge on the commonality and variation types of DOM leached from various vegetation in a wide range of environments, and the fate of each constituent (e.g. sugars, phenols, and proteins) in aquatic environments is a key topic to understand dynamics of DOM in ecosystems. This study shows a large difference in the quality and quantity of DOM leached from various plants living under similar climate and morphological conditions, and represents the first detailed study which compares the kinetics and molecular characteristics of DOM produced by various senescent macrophytes and periphyton from a subtropical wetland.

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