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# Early diagenesis of plant-derived dissolved organic matter along a wetland, mangrove, estuary ecotone

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*Abstract*

We studied the role of photochemical and microbial processes in contributing to the transformation of dissolved organic matter (DOM) derived from various plants that dominate the Florida Everglades. Plant-derived DOM leachate samples were exposed to photochemical and microbial degradation and the optical, chemical, and molecular weight characteristics measured over time. Optical parameters such as the synchronous fluorescence intensity between 270 and 290 nm (Fnpeak I), a strong indicator of protein and/or polyphenol content, decreased exponentially in all plant leachate samples, with microbial decay constants ranging from $-1.0$ d$^{-1}$ for seagrass to $-0.11$ d$^{-1}$ for mangrove (half-life [$t^{1/2}$] = 0.7–6.3 d). Similar decreases in polyphenol content and dissolved organic carbon (DOC) concentration also occurred but were generally an order of magnitude lower or did not change significantly over time. The initial molecular weight composition was reflected in the rate of Fnpeak I decay and suggests that plant-derived DOM with a large proportion of high molecular weight structures, such as seagrass derived DOM, contain high concentrations of easily microbially degradable proteinaceous components. For samples exposed to extended simulated solar radiation, polyphenol and Fnpeak I photochemical decay constants were on average $-0.7$ d$^{-1}$ ($t^{1/2}$ 1.0 d). Our data suggest that polyphenol structures of plant-derived DOM are particularly sensitive to photolysis, whereas high molecular weight protein-like structures are degraded primarily through physical–chemical and microbial processes. Furthermore, microbial and physical processes initiated the formation of recalcitrant, highly colored high molecular weight polymeric structures in mangrove-derived DOM. Thus, partial, biogeochemical transformation of plant-derived DOM from coastal areas is rapid and is likely to influence carbon and nutrient cycling, especially in areas dominated by seagrass and mangrove forests.

Dissolved organic matter (DOM) plays a pivotal role in the ecological structure of aquatic ecosystems (Hessen and Tranvik 1998; Findlay and Sinsabaugh 2003). The presence of DOM can influence microbial growth, light penetration, the complexation of metals, and the availability of nutrients (Tranvik 1992; Scully and Lean 1994; Morris et al. 1995; Tranvik 1998; Lu and Jaffé 2001; Qualls and Richardson 2003). The source of this material is especially important in determining the structural characteristics of DOM. Plant-derived organic matter from wetlands, for example, can greatly influence the optical and chemical characteristics of estuarine DOM, especially in those estuaries with significant external loading of DOM (Benner and Opsahl 2001; Dittmar et al. 2001). Coastal marine systems may subsequently receive significant quantities of allochthonous DOM from wetlands via river discharge (Kattner et al. 1999; Miller 1999). Leaf litter organic matter export from mangrove forests may also greatly influence DOM cycling in estuaries (Del Castillo et al. 2000; Dittmar et al. 2001). The organic matter from the leaching of mangrove leaves in coastal mangrove forests

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contains high concentrations of phenolic compounds such as tannin polymers that can undergo rapid transformation (Benner et al. 1990; Hernes et al. 2001).

The chemical and optical properties of plant-derived organic matter are transformed through microbial, physical, and photochemical processes (Benner et al. 1990; Ziegler and Benner 2000; Hernes et al. 2001). Microbial degradation of plant-derived organic matter is an essential process in the diagenesis of DOM in aquatic ecosystems. The polyphenolic structures found in vascular plants can be efficiently transformed by the oxidizing action of microbial enzymes phenol oxidase and peroxidase (Pfaender 1988). Furthermore, exudates from seagrass can be used by bacteria for metabolism and growth and alter nutrient cycling in estuaries dominated by seagrass (Ziegler and Benner 1999*a*,*b*).

Microbial processes are modulated by the photochemical degradation of DOM and by the release of bioavailable low molecular weight structures (Kieber and Mopper 1987; Kieber et al. 1990). Furthermore, Miller and Moran (1997) demonstrated that the microbial degradation of DOM can be increased when DOM has previously undergone photolysis. Photochemical diagenesis of DOM may also have negative effects on bacterial growth (Benner and Biddanda 1998; Tranvik and Kokalj 1998; Anasio et al. 1999; Tranvik and Bertilsson 2001). Phenolic compounds released from DOM may be responsible for this decrease in DOM bioavailability (Freeman et al. 2001). Polyphenolic compounds, for example, are known to react and bind to labile protein structures and enzymes, effectively reducing the availability of previously usable DOM (UDOM) (Muller-Wegener 1988; Hagerman et al. 1998; Northup et al. 1998). Furthermore, condensation reactions can form large recalcitrant polymers through oxidation of low molecular weight polyphenol structures and cross-linking reactions between polyunsaturated lipids (Hedges 1988, and references within). Thus, the presence of these polyphenols at high concentrations affects the bioavailability of DOM through physical processes and, by so doing, inhibits microbial growth. Although there is no direct evidence, polymerization of DOM may be enhanced photochemically and be partly responsible for earlier reports of DOM bioavailability loss through ultraviolet radiation (UV-R) exposure.

The composition and transformation of DOM may be characterized using optical, chemical, and molecular techniques. Absorption and fluorescence spectroscopic techniques have been used effectively to characterize DOM from inland and marine waters (Ferrari and Mingazzini 1995; Lu et al. 2003; Reynolds 2003; Jaffé et al. 2004). Many recent studies of the transformation of DOM in aquatic ecosystems have been conducted using a molecular approach (Benner et al. 1990; Hernes et al. 2001; Benner and Opsahl 2001; Lu et al. 2003). Chemical budget approaches have been used effectively to study the cycling of DOM in aquatic ecosystems (Qualls and Richardson 2003). The export of organic bound nutrients in the Florida Everglades was found to be controlled by plant production, microbial degradation, and photochemical processes.

The Florida Coastal Everglades ecosystem contains several unique ecotones, such as the freshwater marsh region containing large quantities of periphyton and sawgrass, the fringe mangrove forest, and the Florida Bay estuary with areas dominated by the presence of seagrass (Porter and Porter 2002). Sources of DOM in each of the ecotones have distinct chemical and optical characteristics (Clark et al. 2002; Qualls and Richardson 2003; Jaffé et al. 2004). Because of these different optical and chemical characteristics, their response to photochemical and microbial degradation may also be unique and, as such, provide an ideal model system to study the diagenesis of plant-derived organic matter across a hydrologically linked landscape.

In this study we conducted a series of laboratory-based experiments with defined controls in an attempt to elucidate the mechanisms involved in the combined photochemical and microbial diagenesis of plant-derived organic matter of the Florida Coastal Everglades. Chemical, optical, and physicochemical characterization techniques were used to quantify the changes in DOM structural composition of plant-derived organic matter that was degraded microbially and photochemically. Specific objectives of this study were to (1) assess the chemical and optical changes of plant-derived DOM from the Florida Coastal Everglades caused by photochemical and microbial processes; (2) determine the effect of photochemical processes on the microbial degradation rate of this plant-derived DOM; (3) based on the decomposition kinetics, estimate the reactivity of different molecular weight DOM fractions of the plant DOM toward microbial and photochemical processes; and (4) develop a better understanding of the underlying photochemical and microbial mechanisms involved in the transformation of plant-derived DOM of the Florida Coastal Everglades.

## Materials and methods

*Microbial digenesis experiment*—Senescent plant materials of *Rhizophora mangle* (red mangrove), *Cladium jamaicense* (sawgrass), *Eleocharis cellulosa* (spikerush), freshly picked *Thalassia testudinidum* (seagrass), dry matted periphyton, and peat soil were collected May 2002 from Everglades freshwater marsh and estuarine sites and leached at ca. 20 g $L^{-1}$ (wet basis) for 24 h in 2 liters of Milli-Q water (Millipore) at 20°C. The plant leachates were then filtered through a Whatman glass fiber GF/F filter followed by a 0.22-$\mu$m Durapore membrane filter (Millipore) and diluted to ambient DOC concentrations of approximately 20 mg $L^{-1}$ DOC. Nutrients were then added in the form of 1 $\mu$mol $L^{-1}$ $PO_4^{3-}$ and 10 $\mu$mol $L^{-1}$ $NO_3^-$ (final concentration) to eliminate N and P limitation, followed by a 2.0-ml bacterial inoculum of GF/F filtered pond water. The addition of sodium azide ($NaN_3$) served as a biological control but initiated the formation of color and polymerization reactions. Hence, initial values in this study were used as negative treatment controls.

Samples were placed in the dark at 20°C and sampled for DOM optical and molecular size characteristics at 0, 2, 5, 9, 14, and 28 d. DOM optical parameters measured included synchronous fluorescence and UV visible absorbance. Molecular size characteristics were obtained by size exclusion chromatography (SEC). Dissolved organic carbon (DOC) and total polyphenol concentration, as well as bacterial abundance, were obtained at each of the sampling times.

To estimate the relative effect of photochemical processes on the microbial DOM degradation, half of each plant leachate was preexposed to simulated sunlight before being diluted to ambient DOC concentrations. Subsamples (800 ml) were irradiated for 24 h with a Suntest XLS+ solar simulator (Atlas Material Testing Technology LLC) set at 765 W $m^2$ (~solar noon sun at midlatitude) in 20°C water cooled in 30 × 10 × 5 cm Plexiglas irradiation vessels (2.7-cm optical pathlength). The containers were covered and sealed with UV-R transmitting polyvinylidene chloride (PVDC). The absorption spectrum of the PVDC in the 300- to 400-nm region was relatively constant (%T 78 ± 3%). Samples receiving no light treatments were placed in the dark for 24 h at 20°C. The volume and exposure surface area of the samples were chosen to optimize UV-R absorption while still minimizing the irradiation period and internal light absorption effects (i.e., high surface to volume ratio) (Blough 1997; Miller 2000; Whitehead and de Mora 2000; Osburn and Morris 2003). The total absorbed UV-R dose ($E_a$ μmol $cm^2$) was approximately equal for the spikerush, sawgrass, mangrove, and seagrass samples at 968, 1,031, 1,121, and 1,187 μmol $cm^2$ but, because of decreased DOM leaching, was lower for the peat and periphyton samples at 204 and 516 μmol $cm^2$, respectively. $E_a$ was calculated following the equation

$$E_a = \sum E_{a\lambda} \times t \quad (1)$$

where $\Sigma E_{a\lambda}$ is the photon absorption rate ($E_{a\lambda}$) integrated over the full UV-R waveband (mol photons absorbed $cm^2$ $s^{-1}$) and $t$ is the irradiation time (s). The $E_{a\lambda}$ was calculated using the following equation (Miller 2000).

$$E_{a\lambda} = E_{0\lambda}(1 - 10^{-(A\lambda l)}) \quad (2)$$

where $E_{0\lambda}$ is the irradiance at the surface of the sample (mol photons $cm^2$ $s^{-1}$) (irradiance provided by Atlas Material Testing Technology LLC), $A$ is the spectrophotometric absorption ($cm^{-1}$), and $l$ is the optical pathlength (cm). $E_{0\lambda}$ values were corrected for absorption by the PVPC cover using absorption spectrum data. The above $E_{a\lambda}$ values are up to three times higher than a typical midlatitude daily cumulative UV-R ($E_0$) dose for natural sunlight of 370 μmol $cm^2$ (122 J $m^2$) (Scully et al. 2000).

*Photochemical transformation experiment*—In order to obtain photochemical decay rate constants and associated half-life values of plant-derived DOM, we conducted separate experiments where plant DOM leached in natural waters were irradiated over an extended 7-d period and the chemical, optical, and molecular weight properties measured over time. Natural waters were used rather than distilled water to better simulate the chemical conditions (pH, salts, trace metals, degraded DOM) found in natural waters. Senescent plant material of sawgrass, mangrove leaves, freshly picked seagrass, and dried-matted periphyton (50 g) were placed in 2 liters of 0.2-μm filtered natural waters representative of the sampling site (i.e., mangrove leaves in natural waters from a mangrove estuary) and incubated in the dark at 20°C for 24 h. The leachates were again filtered through a 0.22-μm Durapore filter and 600-ml aliquots irradiated for 7 d at 20°C with the solar simulator set at 765 W $m^2$ in the three water-cooled Plexiglas vessels (2-cm optical pathlength). A treatment control sample was incubated separately in the dark. Treated samples were analyzed for chemical and optical parameters as described below at days 0, 1, 3, and 7. The sample volume (i.e., optical length) was reduced because of the higher UV-R absorbance. The $E_a$ values for periphyton, mangrove, sawgrass, and seagrass samples were 1,371, 5,763, 5,589, and 8,207 μmol $cm^2$, respectively, and approximately 4 to 22 times higher than a typical daily midlatitude natural sunlight UV-R dose.

The $E_{a\lambda}$ values were also used to calculate a broad waveband (300 to 400 nm) apparent quantum yield ($\Phi_\lambda$) for polyphenol photodegradation similar to that calculated for hydrogen peroxide ($H_2O_2$) and dissolved inorganic carbon (DIC) photoproduction (Scully et al. 1996; Bertilsson and Tranvik 2000). The $\Phi_\lambda$ was calculated as the number of moles of polyphenol carbon per mole of photon absorbed between 300 and 400 nm and can be represented by Eq. 3.

$$\Phi_\lambda = \frac{\mathrm{PP}}{E_{a\lambda}} \quad (3)$$

where $\Phi_\lambda$ is the apparent quantum yield for polyphenol photodegradation, $E_{a\lambda}$ the photon absorption rate between 300 and 400 nm (moles photons absorbed per milliliter per second), and PP the polyphenol photodegradation rate (moles polyphenol carbon lost per milliliter per second).

*Analytical methods*—Optical characteristics: Synchronous excitation-emission fluorescence spectra of the water samples were obtained from 250 to 550 nm, in 1-cm quartz fluorescence cells at room temperature (20°C), using a Perkin Elmer LS50B spectrofluorometer equipped with a 150-W Xenon arc lamp using a constant offset value between excitation and emission wavelengths ($\delta\lambda = \lambda\mathrm{em} - \lambda\mathrm{ex}$). All spectra were recorded at an offset value of 30 nm with slit width of 10 nm (*see also* Lu and Jaffé 2001; Lu et al. 2003). Fluorescence values were corrected for internal absorbance quenching following the procedures outlined in McKnight et al. (2001). Synchronous fluorescence was also standardized using quinine sulfate and the Raman peak following a modified method similar to that outlined in Hoge et al. (1993) for emission fluorescence as represented by the equation

$$\mathrm{Fn}(\lambda) = \left[\frac{F(\lambda)}{\mathrm{bl}} \times \left(\frac{\mathrm{Fqs}}{\mathrm{Rqs}}\right)^{-1}\right] \times 10 \quad (4)$$

where Fn(λ) is the standardized synchronous excitation-emission fluorescence at λ, $F(\lambda)$ is the synchronous excitation-emission fluorescence at λ, Fqs is the peak synchronous fluorescence of a 0.01 mg $L^{-1}$ solution of quinine sulfate in 1 N $H_2SO_4$, and Rbl and Rqs are the Raman signal of Milli-Q water and the quinine sulfate solution, respectively. Absorption spectra were measured with a Hewlett-Packard photodiode array spectrophotometer fitted with a 1-cm quartz cell. The filtered water samples were blanked with Milli-Q water and baseline corrected at 750 nm.

Molecular weight distribution: The molecular weight distribution was determined by SEC (Maie et al. 2003). Analytical conditions were as follows: column, YMC-Pack Diol-120G

(pore size 12 nm, inner diameter 8.0 mm × length 500 mm; YMC Inc.); eluent, 0.05 M Tris(hydroxymethyl)aminomethane (THAM) adjusted to pH at 7.0 with phosphoric acid; flow rate, 0.7 ml $min^{-1}$; detection, absorption at 280 nm; injection volume, 150 $\mu$l; temperature, 22°C. The void volume ($V_0$, 14.5 min) and void volume plus inner volume ($V_0 + V_I$, 32.3 min) were determined using Blue Dextran 2000 (Pharmacia) and phenylalanine, respectively. The column was calibrated with several kinds of dextran standards of known molecular weight (Sigma Chemical). Weight average molecular weight ($M_w$) was calculated as follows:

$$M_w = \sum (a_i M_i) / \sum A_i \quad (5)$$

where $A_i$ and $M_i$ are the absorbance in arbitrary units and the molecular weight distribution estimated from the calibration curve, respectively, at elution volume *i*.

In order to obtain an estimate of the effect of the treatment on several molecular weight subfractions of the DOM, relative change in the elution peak area was also calculated for the very high molecular weight (VHMW), high molecular (HMW), medium molecular weight (MMW), and low molecular weight (LMW) fractions where VHMW is >15,000 (<15.54 min), HMW is <15,000 to >7,500 (>15.54 to <21.57 min), MMW is <7,500 to >5,000 (>21.57 to < 26.89 min), and LMW is <5,000 (>26.89 min).

Determining molecular weight distributions of DOM by SEC with UV detection has been commonly reported in the literature (Maie et al. 2003; Her et al. 2003). However, the facts that adequate calibration standards are not readily available, that some DOM components do not properly absorb at 280 nm, and that substances such as polyphenols may absorb onto the column matrix may result in $M_w$ values that are not completely accurate. Thus, the $M_w$ values that appear in this paper do not refer to the absolute $M_w$ and should be regarded as an index to compare the average size of DOM measured under the standard conditions.

DOC, polyphenol, and bacteria counts: Samples for dissolved organic carbon (DOC) analysis were filtered in acid-washed syringes through a GF/F swinette adaptor into a 125-ml Nalgene sample bottle and stored at 4°C until processing. Concentrations of DOC were quantified by acidifying the filtered samples to pH < 2 and purging with $N_2$ gas then directly injecting samples onto a hot platinum catalyst in a Shimadzu TOC-5000. Total polyphenol content was measured using the Folin-Denis method (Anderson and Ingram 1996). Briefly, aliquots of 0.2 ml of Folin-Denis reagent (Sigma) and 0.4 ml of saturated $Na_2CO_3$ solution were added in sequence to 3.4 ml of the sample solution. After incubating for 30 min at room temperature, the absorbance of the solution was measured using a UV–visible scanning spectrophotometer (UV-2101PC, Shimadzu) at 760 nm. Samples were standardized with calibration curves of tannic acid at concentrations ranging from 0.01 to 10 mg $L^{-1}$. Bacteria enumeration was determined using 4′,6-diamino-2-phenylindole (DAPI) epifluorescent counts (Turley 1993). Twenty milliliters of water were collected from each sampling and preserved with phosphate buffered formalin to a final concentration of 2% and stored in the dark at 20°C until processing. Slides were prepared using sterile filtration towers with black polycarbonate filters (0.22 $\mu$m) and counted within 2 weeks of collection. Bacteria were counted (200–500 per slide) with a Zeiss microscope equipped with a Hg lamp and a blue excitation filter.

*Molecular weight and chemical degradation kinetics*—Decay rate constants (*k*) for DOC, polyphenol, Fpeak I, and $A_i$ of molecular weight fractions VHMW, HMW, MMW, and LMW were calculated using nonlinear regression analysis. These were calculated by measuring the concentration, fluorescence, or absorbance (*y*) over time (*t*), fitting the data to an exponential decay equation, and using the slope (*b*) as *k* ($d^{-1}$) ($y = y^0 + a \times \exp[-b \times t]$), where *a* is the *y* intercept and $y^0$ is the baseline residual of *y* that is resistant to microbial and photochemical degradation. Similarly, production constants were also calculated using nonlinear regression analysis. Statistical analysis for differences between irradiated and control samples and microbial and photochemical decay rate constants were tested using a paired *t*-test and a one-way analysis of variance (ANOVA) at a 0.05 significance level with SYSTAT, version 10. The half-life ($t^{1/2}$) for the various parameters of the samples was also calculated using the relationship of $t^{1/2} = \ln 0.5\ k^{-1}$.

## Results

*Transformation of DOM in the presence of bacterioplankton*—Polyphenol, DOC, and bacterial abundance: Microbial processes caused significant changes to the chemical, optical, and molecular structure characteristics of the plant DOM leachates. The polyphenol concentrations of the incubated samples decreased through the course of the experiment. The decay rate constants for polyphenol were highest in the periphyton sample (−0.54 $d^{-1}$) ($p < 0.05$) and did not change significantly over time for the peat sample (Table 1). Preexposure to light influenced the initial polyphenol concentration. There was a significant overall decrease in polyphenol concentration after light exposure (paired *t*-test; $p < 0.05$) (average decrease of 17%). The 24-h preexposure to light, however, did not have a significant overall effect on the decay rate constants over the 28-h incubation (paired *t*-test; $p > 0.05$) (Table 1). Although there was no overall effect of preexposure to UV-R on the PP decay constants, there was a substantial increase in the seagrass sample (−0.15 to −0.26 $d^{-1}$) and decrease in the periphyton sample (−0.54 to −0.19 $d^{-1}$). This response may be due to variable sources of polyphenols between the leachate samples.

With the exception of the dark mangrove sample (0.17 $d^{-1}$, $t^{1/2} = 4.0$ d) and the preexposed seagrass (0.23 $d^{-1}$, $t^{1/2} = 3.0$ d), the decrease of DOC over time was very slow (<0.07 $d^{-1}$, $t^{1/2} > 9.9$ d) or not significant (Table 1). The ratio of total polyphenol (milligrams per liter) over DOC concentration (milligrams per liter) ranged between 1 and 1.9 for the plant material leachates but was much lower for the periphyton and peat samples at ~0.2 (Table 1). This observation is not surprising, since polyphenols are easily degraded in the environment and periphyton does not contain tannins.

Although preexposure to 24 h of simulated solar radiation did not influence the decay rate of DOC and polyphenol,

Table 1. Decay rate constants ($d^{-1}$) for total polyphenol (PP), dissolved organic carbon (DOC), and Fnpeak I of plant leachate samples incubated in the dark for 28 days ± SE. The initial PP over DOC concentration ratio values (PP/DOC) (mg $L^{-1}$/mg $L^{-1}$), the change in bacterial abundance (Δbac) from day 0 to 2 ($d^{-1}$), the initial weight, average molecular weight ($M_W$), and difference in $M_W$ between day 0 and 28 ($\Delta M_W$) are also provided. No significant change over time (NS).

| Plant type | PP $k_{PP}$ ($d^{-1}$) | DOC $k_{DOC}$ ($d^{-1}$) | PP/DOC | Δbac | Fnpeak I $k_{Fn}$ ($d^{-1}$) | $M_W$ | $\Delta M_W$ |
|---|---|---|---|---|---|---|---|
| *R. mangle* | −0.10±0.06 | −0.17±0.04 | 1.02 | 0.48 | −0.13±0.04 | 6116 | 1381 |
| *R. mangle* + UV | −0.13±0.07 | −0.07±0.06 | 1.43 | 1.86 | −0.11±0.05 | 5579 | 1892 |
| *C. jamaicense* | −0.07±0.06 | −0.002±0.05 | 1.24 | 0.52 | −0.12±0.06 | 7764 | 261 |
| *C. jamaicense* + UV | −0.11±0.08 | NS | 1.08 | 2.57 | −0.16±0.08 | 7525 | 297 |
| *E. cellulosa* | −0.08±0.05 | NS | 1.21 | 0.68 | −0.20±0.05 | 7815 | 116 |
| *E. cellulosa* + UV | −0.03±0.03 | NS | 1.12 | 2.72 | −0.17±0.03 | 7321 | 482 |
| Peat | NS | NS | 0.24 | 0.40 | NS | 8108 | −147 |
| Peat + UV | NS | NS | 0.16 | 1.50 | NS | 8022 | −229 |
| Periphyton | −0.54±0.16 | NS | 0.25 | 2.58 | −0.48±0.08 | 8244 | −531 |
| Periphyton + UV | −0.19±0.08 | NS | 0.14 | 1.81 | −0.27±0.10 | 8142 | −594 |
| *T. testudinidum* | −0.15±0.04 | NS | 1.87 | 0.36 | −0.51±0.19 | 9053 | −1300 |
| *T. testudinidum* + UV | −0.26±0.10 | −0.24±0.11 | 0.80 | 2.70 | −1.02±0.44 | 9403 | −1146 |

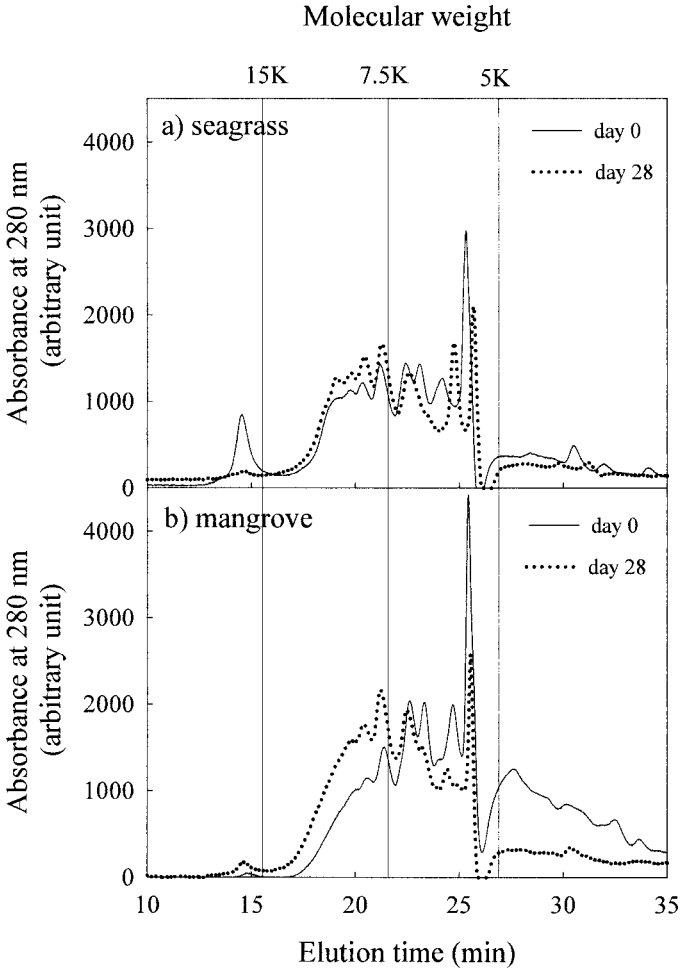


Fig. 1. Elution curve of (a) seagrass and (b) light-exposed mangrove leachate. The solid line represents day 0, whereas the dotted line is after 28 d of incubation.

light exposure did have a significant effect on the bacterial numbers of the incubated samples (Table 1). Increases in bacterial abundance between day 0 and 2 (Δbac) of the incubation experiment were calculated with $\ln(bac_0/bac_2)/t$ where $bac_0$ and $bac_2$ are bacteria numbers on day 0 and day 2, respectively. Initial Δbac for irradiated samples of 2.05 $d^{-1}$ were significantly higher than samples without preexposure to light (0.84 $d^{-1}$) (paired *t*-test; $p < 0.05$) (Table 1). Bacterial abundance decreased after day 2 in all samples, which was possibly due to a depletion of the bioavailable DOM.

Fnpeak I and molecular weight distribution: Optical parameters such as the Fnpeak I, a strong indicator of protein and/or polyphenol content (Miano and Senesi 1992; Reynolds 2003; Wu et al. 2003; Yamashita and Tanoue 2003), decreased for all samples with microbial decay rate constants ranging from −1.0 $d^{-1}$ for light preexposed seagrass DOM to −0.12 $d^{-1}$ ($t^{1/2}$ 16 h to 6 d) for sawgrass and red mangrove (Table 1) ($p < 0.05$). DOM material from sawgrass, red mangrove, and spikerush with relatively high concentrations of polyphenol (>2 mg $L^{-1}$) seemed more resistant to microbial degradation relative to that derived from seagrass and periphyton with presumably a higher relative proportion of labile protein material. Preexposure of the samples to simulated sunlight did not influence the intensity of the Fnpeak I decay rate constants (paired *t*-test; $p > 0.05$).

Molecular weight analysis demonstrated that seagrass and mangrove samples containing relatively large proportions of proteinaceous materials and polyphenols, respectively, were especially susceptible to microbial degradation processes. This was apparent when examining the SEC elution curves for seagrass and mangrove DOM before and after the 28-d incubation (Fig. 1). The changes in the molecular size of the seagrass DOM sample during the 28 d of incubation were quite remarkable, especially the decrease of the elution peak at 14.8 min and, to a lesser extent, the elution peaks with long retention time >26 min that corresponded to very high and smaller molecular size region, respectively. The opposite trend was clear for the mangrove DOM, where LMW and

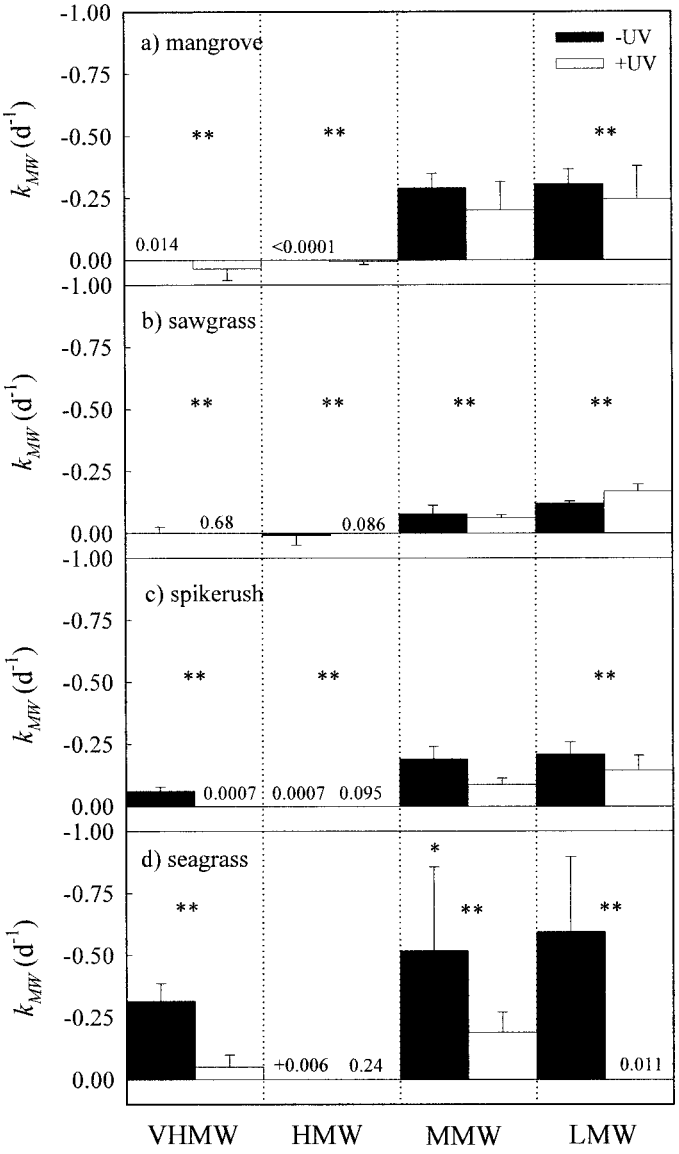


Fig. 2. The decay and formation rate constants (d$^{-1}$) for the molecular weight fraction >15,000 (VHMW), <15,000 to >7,500 (HMW), <7,500 to >5,000 (MMW), and <5,000 (LMW) of filter sterilized plant leachates of (a) mangrove, (b) sawgrass, (c) spikerush, and (d) seagrass incubated in the dark for 28 d with (+ UV) and without (− UV) a 24-h preexposure to light with a solar simulator. Error bars are the standard error. *Significant change over time at $p < 0.06$. **Significant difference in mean $A_i$ (peak area) of VHMW, HMW, MMW, and LMW between light-exposed sample and control, paired *t*-test at 0.05 level of significance. The slope values for samples that did not change significantly over time are also provided.

MMW compounds decreased over the incubation period for most samples but the decay rate constants were especially high for seagrass, red mangrove, and spikerush with values ranging from −0.12 to −0.61 d$^{-1}$ ($t^{1/2}$ = 5.7 to 1.1 d) (Fig. 2). The decay rate constant for the MMW fractions was significant in all samples and was most prominent, in descending order, in seagrass, mangrove, spikerush, and sawgrass ($p < 0.05$). The decay for the VHMW fraction was most prominent in the dark seagrass and spikerush samples with decay rate constants of −0.32 and −0.06 d$^{-1}$ ($t^{1/2}$ = 2.1 and 8.6 d), respectively. The absorbance increased significantly in both light-exposed VHMW and HMW fractions of mangrove leachate and resulted in production rate constants of 0.03 and 0.006 d$^{-1}$ and indicates that preexposure to light may have enhanced polymerization reactions and produced high molecular weight chromophores.

Preexposure to simulated sunlight also had a significant effect upon the degradation of the seagrass DOM. Changes in the four molecular weight classes over the incubation period for the seagrass sample preexposed to light were markedly different from the dark experiments. The decay of light-exposed VHMW structures over time was low (−0.05 d$^{-1}$; $R^2 = 0.83$; $F_{2,3} = 13.61$; $p < 0.05$) relative to the unexposed sample (−0.32 d$^{-1}$; $R^2 = 0.96$; $F_{2,3} = 65.20$; $p < 0.05$) and did not change significantly for the LMW fraction ($R^2 = 0.65$; $F_{2,3} = 2.82$; $p > 0.05$). It is possible that preexposure to light may have effectively removed labile polyphenols and other precursor materials.

The general responses to microbial degradation of the four different molecular weight DOM fractions of sawgrass and spikerush were similar (Fig. 2bc). The degradation of MMW and LMW structures dominated the transformation of DOM in these two sources. The decay rate constants for the VHMW and HMW fractions were generally low or did not change significantly ($p > 0.05$). These results are in agreement with the low decay rate constants for Fnpeak I, polyphenol, and DOC and indicative of DOM that is relatively resistant to microbial degradation.

The peat and periphyton samples responded in a similar manner. The periphyton samples were characterized by no significant changes in any size fractions ($p > 0.05$). The change in abundance over time for the peat sample was also not significant ($p > 0.05$). The relatively low initial polyphenol concentrations in the samples (~2 mg L$^{-1}$) are likely the reason for the lack of change in the LMW to MMW compounds in the peat and periphyton samples. In this respect, the peat sample may have already undergone extensive microbial and physical degradation, resulting in DOM that is resistant to transformation processes. Surprisingly, the periphyton sample, which showed the highest decay rate constants for Fnpeak I and polyphenols, only after the seagrass DOM, fell into this category. The microbial lability of the periphyton samples may have been further underestimated using SEC, since polysaccharides, found at high concentrations in periphyton, do not absorb at 280 nm.

*Effect of light on DOM transformation in the absence of bacterioplankton*—Polyphenol and DOC: Extended exposure to simulated solar radiation had a strong effect on the chemical parameters of DOM samples. Polyphenol decay rate constants for the samples ranged from −0.46 to −1.83 d$^{-1}$ (Table 2) ($t^{1/2}$ = 22–9 h). Dark decay of the polyphenol was not significant with the exception of the periphyton sample with a dark decay rate constant of −0.57 d$^{-1}$ ($t^{1/2}$ = 29 h). Based on the $\Phi_\lambda$ for polyphenol photodegradation, the mangrove sample was the most sensitive to extended light exposure, with a $\Phi_\lambda$ value of $24.0 \times 10^{-4}$, followed in descending order by periphyton ($10.4 \times 10^{-4}$), seagrass (7.2

Table 2. Decay rate constants ($d^{-1}$) for total polyphenol (PP) and Fnpeak 1 of plant leachate samples irradiated over a 7-d period. The initial PP over DOC concentration ratio values (PP/DOC) (mg $L^{-1}$/mg $L^{-1}$) and decay rate constants for dark controls (dark) are also provided. No significant change over time (NS).

| Leachate type | PP $k_{PP}$ ($d^{-1}$) | Fnpeak I $k_{Fn}$ ($d^{-1}$) | PP/ DOC |
|---|---|---|---|
| *R. mangle* | −1.83±0.23 | −0.61±0.04 | 0.48 |
| *R. mangle* (dark) | NS | NS | 0.04 |
| *C. jamaicense* | −0.46±.0525 | −0.71±0.07 | 0.24 |
| *C. jamaicense* (dark) | NS | NS | 0.05 |
| Periphyton | −0.76±0.08 | −0.71±0.07 | 0.07 |
| Periphyton (dark) | −0.57±0.08 | NS | 0.01 |
| *T. testudinidum* | −0.76±0.08 | −0.93±0.06 | 0.41 |
| *T. testudinidum* (dark) | NS | NS | 0.05 |

$\times 10^{-4}$), and sawgrass ($4.8 \times 10^{-4}$). DOC concentration, however, did not change significantly over time in any of the DOM samples, both irradiated and dark controls ($p > 0.05$). Although some DOC loss is expected to occur through photochemical dissolved inorganic carbon (DIC) production, the production rate for similar waters are relatively low (Bertilsson and Tranvik 2000; Scully et al. 2003*a*) and not easily quantified using the disappearance of DOC.

Fnpeak I and molecular weight distribution: The optical parameter, Fnpeak I, for irradiated red mangrove, sawgrass, periphyton, and seagrass samples changed significantly over time ($p < 0.05$) (Table 2). The calculated decay rate constants ranged from −0.61 to −0.93 $d^{-1}$ (Table 2) ($t^{1/2}$ = 27–18 h) and were also significantly higher than samples exposed to microbial degradation (one-way ANOVA; $p < 0.05$). Presumably, these changes are primarily due to the degradation of the plant-derived DOM that was added to the natural DOM containing water sample. It is assumed that this DOM was already present in a highly degraded form in comparison to the freshly leached plant DOM (*see Fig. 3*). This can be further illustrated by comparing the VHMW/HMW and LMW/HMW ratio values of the mangrove, seagrass, and peat samples from the microbial experiment. The ratio values were substantially lower for the presumably degraded peat sample at 0.02 and 0.06 compared to 0.13 and 0.35 for seagrass and 0.11 and 1.56 for the mangrove sample. Dark decay rate constants did not change significantly over time ($p > 0.05$).

Decay rate constants for the molecular weight fractions were highest in the red mangrove sample, with values in the LMW and VHMW exceeding −1.0 $d^{-1}$ ($t^{1/2} < 17$ h) (Fig. 4a) ($p < 0.05$). Dark decay rate constants exceeded those of light treatment for the VHMW fraction and suggested that the loss of the two molecular weight fractions in the mangrove sample occurred through a physicochemical, rather than a photochemical, process (Fig. 4a). Microbial degradation is unlikely, since the samples were filtered and most of the change occurred during the first 24 h. There was a formation of HMW compounds in the dark as was apparent by a production rate constant of 0.69 $d^{-1}$ ($R^2 = 0.99$; $F_{2,7} = 846$; $p < 0.05$) and the dark control elution curve (Fig. 4a).

There were no significant changes in the dark controls for the sawgrass and periphyton samples (Fig. 4bc) ($p > 0.05$), which indicates that the loss of the various molecular weight fractions was primarily due to photochemical processes. The photooxidation of polyphenol compounds and natural water DOM was most likely the process responsible for the relatively high decay rate constant of −0.54 and −0.86 $d^{-1}$ ($t^{1/2}$ = 31 and 19 h) for the LMW fraction. The decay rate constants for the HMW, MMW, and LMW fractions of the irradiated periphyton sample were also similar to the sawgrass sample, but the decay rate constant for the VHMW sample was higher at −0.79 $d^{-1}$ ($t^{1/2}$ = 21 h).

There was significant dark decay of LMW structures in the seagrass sample, with decay rate values reaching −0.25 $d^{-1}$ ($t^{1/2}$ = 2.8 d) (Fig. 4d). This decrease in LMW compounds for the dark control sample may have been caused by a polymerization reaction resulting in an increase in the MMW fraction (Fig. 5b). This increase is not significant enough to affect the rate constant shown in Fig. 4d because of the high background in the MMW signal.

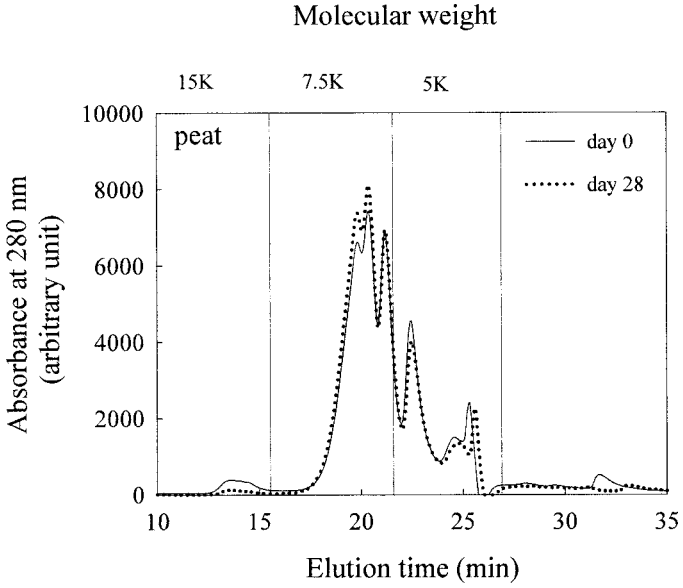


Fig. 3. Elution curve of light-exposed peat leachate. The solid line represents day 0 of the incubation, whereas the dotted line is after 28 d of incubation.

## Discussion

*Biogeochemical processes*—Microbial DOM diagenesis: It is apparent that both polyphenols and protein-like structures play an essential role in the transformation of plant-derived DOM. Considering the above results and that both proteinaceous and polyphenol material have a fluorescence peak at ~280 nm, it is also not surprising that there was a significant relationship between the decay rate constants of the Fnpeak I and the initial average molecular weight ($M_W$) of the plant DOM samples (Table 1) (Fnpeak I = 0.119 + 0.0004 × exp[0.0014 × $M_W$]; $R^2 = 0.91$; $F_{2,7} = 31.15$; $p < 0.05$). Samples such as seagrass with relatively high $M_W$ (>15,000) (most likely due to proteins) had Fnpeak I decay values an order of magnitude higher than red mangrove sam-

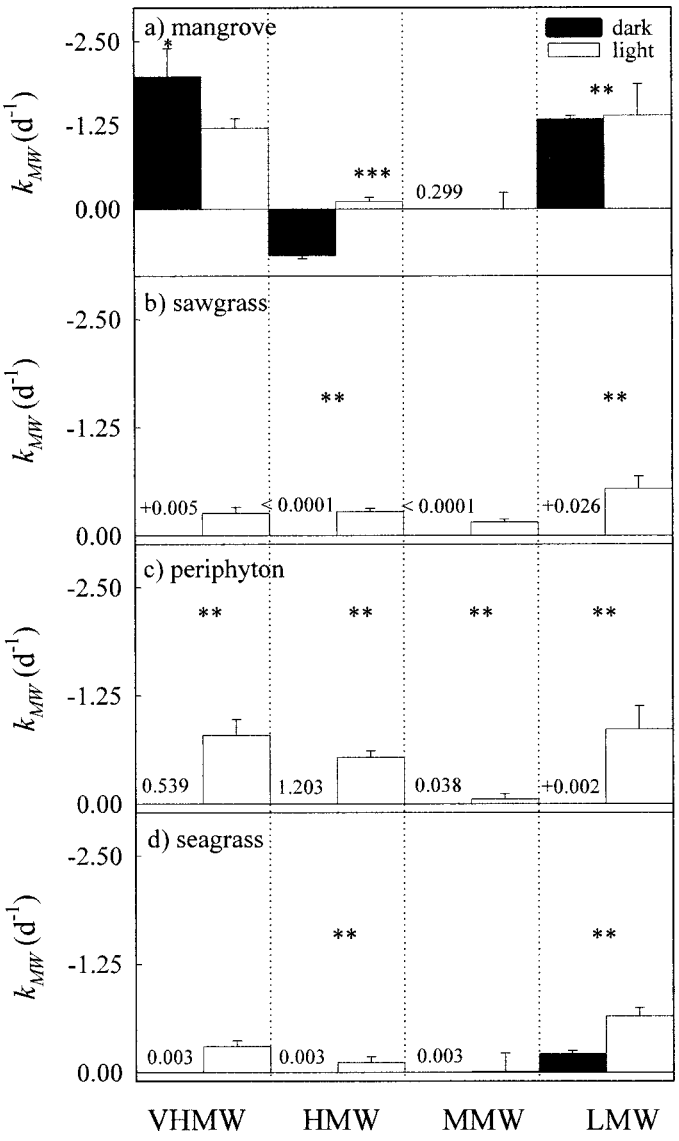


Fig. 4. The decay and formation rate constants ($d^{-1}$) for the molecular weight fraction >15,000 (VHMW), <15,000 to >7,500 (HMW), <7,500 to >5,000 (MMW), and <5,000 (LMW) of filter sterilized plant leachates of (a) mangrove, (b) sawgrass, (c) spikerush, and (d) seagrass irradiated with a solar simulator and dark controls. Error bars are the standard error. *Significant change over time at $p < 0.06$. **Significant difference in mean $A_i$ (peak area) of VHMW, HMW, MMW, and LMW between light-exposed sample and control, paired *t*-test at 0.05 level of significance. The slope values for samples that did not change significantly over time are also provided. ***Decay rate constant obtained between sampling day 1 and 7.

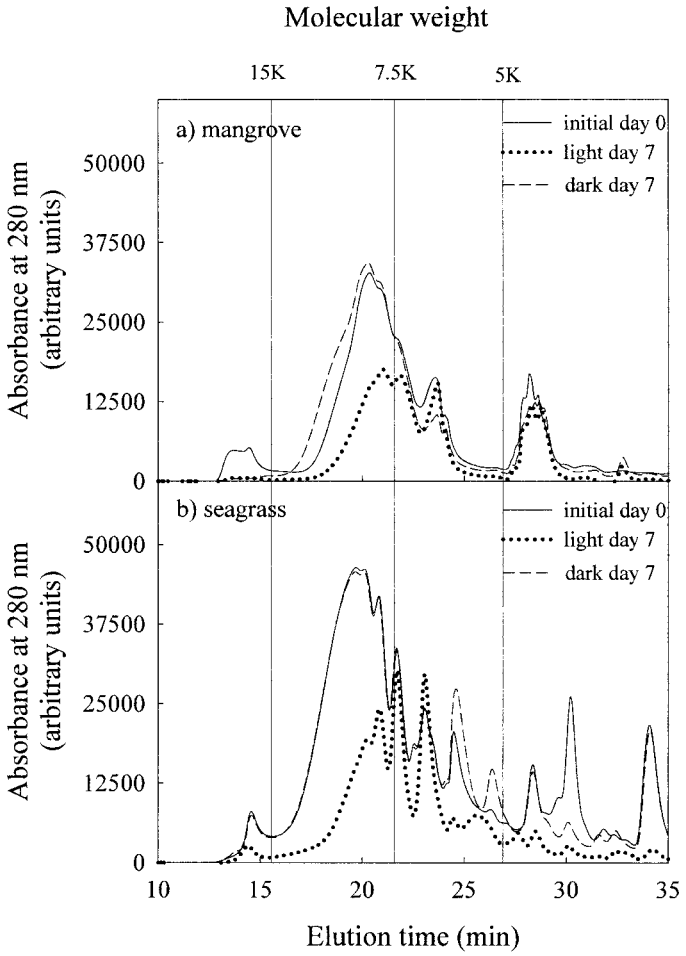


Fig. 5. Elution curve of (a) seagrass and (b) mangrove leachate. The solid line represents day 0 of the incubation, whereas the dotted line is a 7-d irradiation treatment and the broken line the 7-d dark control.

ples with $M_W$ values <5,000 (most likely rich in polyphenols). This suggests that higher microbial degradation rates for proteins compared to polyphenols and/or reduced degradation rates for DOM in polyphenol-rich samples are due to microbial inhibiting effects. Although the degradation rates of the LMW and MMW fractions were rapid, the relatively high concentration of polyphenols reduced the bioavailability by decreasing the rate of degradation of the protein-like VHMW fraction.

Further evidence for the importance of polyphenols and proteinaceous DOM is provided by the significant relationship between the initial $M_W$ and the change (positive and negative) in the $M_W$ over the 28-d incubation period (Table 1) ($[\Delta M_W = 6679 + -0.86 \times M_W]$; $R^2 = 0.96$; $F_{1,10} = 295$; $p < 0.05$). The $M_W$ increased most significantly for the mangrove leachate, followed by sawgrass and spikerush samples. However it decreased for peat, periphyton, and seagrass. The increase in the $M_W$ in some samples is likely the result of the dominance of the degradation of LMW polyphenols, whereas a decrease, particularly in the seagrass sample, would seem to be indicative of the loss of large molecular weight protein-type compounds. It also appears that polyphenols from vascular plants such as sawgrass, spikerush, and red mangrove were generally more resistant to microbial degradation compared to those produced by seagrass and periphyton. These results may be due to a higher abundance of recalcitrant phenols in the emergent vegetation in comparison with the submerged plants.

Photochemical DOM diagenesis: The photochemical degradation of DOM was most prominent in the LMW and the

VHMW fractions (Fig. 4). The photooxidation of polyphenols by reactive oxygen species is likely the primary pathway by which the LMW fraction was eliminated. The photochemical half-lives for the LMW fraction and polyphenol concentration of the plant leachates were both short (9 to 22 h). The relatively low polyphenol concentration in our peat samples and in natural waters in general ($<1.0$ mg $L^{-1}$) supports our findings that there is a rapid turnover of LMW polyphenols from plant material in waters from the Florida Coastal Everglades. This initial photochemical degradation may act as a catalyst in increasing the general bioavailability of DOM and microbial degradation of remaining polyphenols. This was evident only when comparing the microbial Fnpeak I decay rate constant of the seagrass DOM sample to the one preexposed to 24 h simulated sunlight. The microbial decay rate constant for seagrass DOM increased from 0.5 to 1.0 $d^{-1}$ with preexposure to 24 h simulated sunlight (Table 1). A substantial change in the Fnpeak I decay rate constant was, however, only present in the seagrass sample and may be due to a relatively high concentration of VHMW DOM. The importance of the photochemical degradation of polyphenols was also evident when examining the decay of the LMW and MMW fractions. The decay rate constants were generally high in all of the samples at nearly $-1.0$ $d^{-1}$. Although polyphenol structures are a broadly defined term, the response of the mangrove sample to light exposure (i.e., $\Phi_\lambda = 24.0 \times 10^{-4}$) seemed to indicate that condensed tannins are particularly photodegradable. Condensed tannins are especially reactive and are an important structural component of mangrove leaves (Hernes et al. 2001; Hernes and Hedges 2004). Further studies are, however, required to fully a evaluate the photostability of tannins produced by wetland and/or estuarine plants.

Protein-like VHMW compounds also appeared to be easily degraded through photochemical processes. However, it is likely that the mechanisms responsible for the photodegradation of proteinaceous plant-derived DOM are quite different from that responsible for polyphenol transformation. The absorption of UV-R by proteins is generally restricted to wavelengths $<290$ nm, well below the wavelengths that are present in sunlight ($>300$ nm) (Scully et al. 2003*b*). Thus, direct photolysis of the protein-like DOM, generally a major degradation pathway for DOM, is likely negligible. Instead, the primary photochemical degradation pathway of the VHMW fraction was likely through the absorption of sunlight by chromophoric DOM and then subsequently by initiation of physical reactions such as DOM precipitation, polymerization, and production of reactive oxygen species (ROSs) (*see below*).

Physical DOM transformation: During the course of the microbial incubation experiment there was a significant decrease in both VHMW and LMW compounds in the seagrass derived DOM with no preexposure to light (Fig. 2d). The loss of the VHMW and LMW fractions may have been due to microbial and/or physicochemical processes. The two fractions were likely metabolized microbially or precipitated out through the interaction of polyphenols and protein structures (Hagerman et al. 1998; Maie et al. 2003). Precipitation of DOM may have also occurred during the photochemical

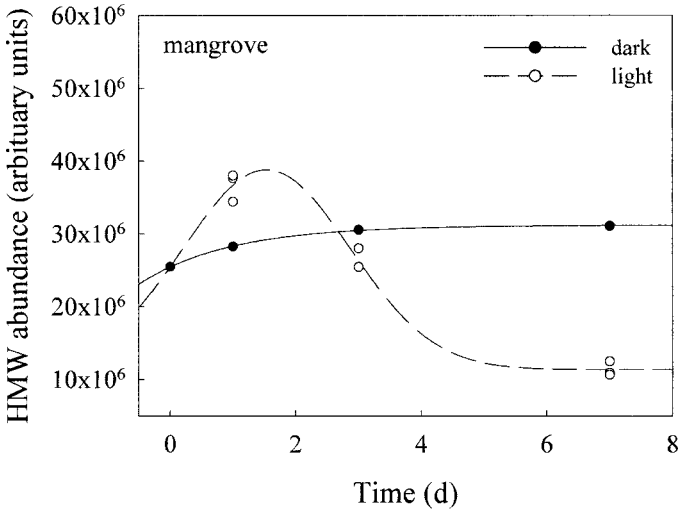


Fig. 6. The HMW abundance ($<15{,}000$ to $>7{,}500$), determined using the integrated absorbance at 280 nm of the elution curve of mangrove leachate exposed to simulated sunlight and in the dark over 7 d.

experiment, where there was a substantial decay of VHMW and LMW fractions and formation of a cloudy appearance in the mangrove sample. In agreement with these findings, DOM precipitation/flocculation was suggested to occur in Whitewater Bay, Everglades National Park, where high abundance of mangrove-derived DOM is exposed to photochemical processes because of increased light exposure in this open and shallow water bay (Jaffé et al. 2004). While these results seem to contradict our microbial degradation experiments, it is important to keep in mind that the photochemical experiments were performed in natural waters (vs. distilled water) and therefore contain a variety of trace metals and already degraded natural DOM, which could induce this precipitation process. In fact, SEC traces (Fig. 5) are clearly different from those in distilled water (Fig. 1) because of the presence of preexisting DOM mixed with freshly leached plant-derived DOM. These chromatograms are a combination of plant-derived DOM and degraded Everglades DOM. These results highlight the importance of considering the role of other chemical constituents present in natural waters when studying the diagenesis of plant-derived DOM.

The most striking feature of the physical transformation of mangrove DOM was the increase in the VHMW and HMW fractions when preexposed to light (Fig. 2a). Polymerization of DOM into larger molecular size may have been responsible for the formation of compounds in the two size fractions. The polymerization reaction was also apparent when examining SEC elution curves and synchronous fluorescence scans for the mangrove leachate with light treatment at day 0 and 28 (Figs. 1b and 5a). Unlike the other DOM leachates, there was an increase in abundance below the elution time of 20 min and the appearance of a distinct synchronous fluorescence peak at 500 nm ($Fn_{500}$ 10 to 47) after incubating both the dark and light-exposed mangrove leachate for 28 d. Exposure to light also accelerated the formation of HMW DOM (Fig. 6). Although the HMW fraction

in the mangrove DOM samples increased over time under both the dark and under simulated sunlight conditions, the kinetic responses of the two samples were substantially different. The light-exposed sample was characterized by a rapid increase over the first 24 h and significant decrease thereafter, whereas in the dark sample, the abundance of the HMW fraction of the mangrove DOM increased at a relatively slower rate and gradually stabilized over the next 6 d.

These results indicate that different processes act synergistically in altering the structure of mangrove-derived DOM by accelerating the decomposition of small polyphenols and also by the stimulation of the production of large molecular weight structures having unique optical and chemical properties. These results also demonstrated the importance of considering the long-term kinetics of the photochemical, physical, and microbial degradation of DOM. Information essential for the proper interpretation of results may be inadvertently overlooked if time course experiments are overly simplified (i.e., time series are too short).

The polymerization of DOM may be responsible for earlier reports of decreased bioavailability of DOM exposed to sunlight (Benner and Biddanda 1998; Tranvik and Kokalj 1998). For example, decreased microbial lability of plant-derived DOM by sunlight exposure was attributed to the actions of high concentrations of photochemically produced hydrogen peroxide ($H_2O_2$) (Farjalla et al. 2001). Hydrogen peroxide acting directly on the plant leachates is, however, unlikely given the low reactivity of $H_2O_2$ in the absence of a catalyst (Kieber et al. 2003). More likely is that the presence of ROSs such as $H_2O_2$ along with a catalyst accelerated the initial oxidation reactions of polyphenols, which resulted in polymerization reactions. Oxidative enzymes leached from the plant material and attached to microorganisms such as phenol oxidase or peroxidase may be one such catalyst that is able to initiate the above reactions. Clearly further work is required to fully understand the underlying processes involved in the transformation of mangrove-derived DOM.

In summary, the results of our study indicate that high molecular weight protein and lower molecular weight polyphenol compounds, two major constituents of the plant-derived DOM of the Florida Coastal Everglades, are consumed via different pathways through unique mechanisms. Polyphenol structures of plant material can undergo photochemical, physical (polymerization), and, to a much lesser extent, microbial degradation, whereas the major sink for larger molecular weight proteins appears to be primarily microbial processes, but significant photochemical degradation was also observed. The removal of proteins through physical processes was found to be important in the mangrove leachates. We also demonstrated that plant-derived DOM from the various ecotones undergoes unique physicochemical, photochemical, and microbial transformation processes, which are dependent on the initial molecular characteristics of the plant-derived DOM and that these three transformation processes do not occur independently of each other. The detailed chemical characterization of DOM components in such ecosystems is needed to further advance our knowledge on the dynamics of DOM in wetlands and estuarine ecosystems.

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