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Photochemical transformations and bacterial utilization of high-molecular-weight dissolved organic carbon in a southern Louisiana tidal stream (Bayou Trepagnier)

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Abstract. UV irradiation of dissolved organic carbon (DOC) in the laboratory can produce small, labile organic compounds utilizable by microbes, but few studies have attempted to document this process *in situ*. ^{13}C nuclear magnetic resonance (NMR) was used to examine the bulk chemical composition of natural and laboratory-irradiated high-molecular-weight DOC (HMW-DOC) from shaded (150 μmol m^{-2} s^{-1} average light in surface water) and open (1500 μmol m^{-2} s^{-1}) field sites over one and a half years. ^{13}C NMR revealed only small differences in carbon functional groups between laboratory irradiated and non-irradiated HMW-DOC. However, bacterial protein productivity per cell (BPP) was enhanced in naturally irradiated samples of HMW-DOC in a field mesocosm experiment ($p < 0.05$), suggesting that bacterial growth was enhanced by photochemical production of labile DOC substrates. Absorbance characteristics such as spectral slope, absorbance at 350 nm, and the absorbance ratio 250 nm/365 nm revealed that HMW-DOC was photoreactive, yet no differences in these values were found between samples irradiated with and without UV-B. In experiments conducted with simulated solar radiation in the laboratory and with natural light in the field mesocosm experiment, UV-A (320–400 nm) and photosynthetically active radiation (PAR; 400–700 nm) were more effective than UV-B (280–320 nm) in HMW-DOC photolysis.

Introduction

Photolysis has been shown to play an important role in regulation of dissolved organic carbon (DOC) cycling in aquatic ecosystems (Kieber et al. 1989; Mopper et al. 1991; Moran and Zepp 1997). Photochemical reactions can result in release of nutrients (ammonium, amino acids, and phosphate) as well as various low-molecular-weight molecules, such as fatty acids and keto acids (Kieber et al. 1990; Wetzel et al. 1995; Bushaw et al. 1996). However, formation rates of the identified photo-

products account for less than 20% of the bleaching rate of DOM, indicating that a large pool of bleached organic matter remains uncharacterized (Miller and Zepp 1995).

The release of these biologically available substrates from otherwise recalcitrant DOC (such as humic substances) has been shown to increase bacterial productivity (Lindell et al. 1995; Wetzel et al. 1995; Bushaw et al. 1996). Moran and Zepp (1997) estimated that identified labile low-molecular-weight DOC photoproducts alone can supply about 4% of the annual bacterial carbon demand and 5% of the bacterial nitrogen demand globally. These values may be much higher if contributions from labile unidentified bleached organic matter are included. For example, photochemically modified high-molecular-weight dissolved organic carbon (HMW-DOC) can be readily assimilated by microbes (Miller and Moran 1997).

The photoreactive components of DOM are often collectively referred to as colored or chromophoric dissolved organic matter (CDOM), and are a major component of HMW-DOC (Nelson and Guarda 1995; Battin 1998). HMW-DOC is often strongly colored in systems markedly influenced by terrestrial inputs (Frimmel and Christman 1988). Coastal wetland systems such as tidal streams can have very high HMW-DOC concentrations (Engelhaupt and Bianchi 2001), and photochemical changes occurring in these systems could affect the composition and lability of DOC before they reach the coastal ocean.

Recent evidence has indicated that increasing levels of UV-B radiation, as a result of stratospheric ozone reduction, (Kerr 1993; Lubin and Jensen 1995; Muller et al. 1997) could lead to accelerated photolytic degradation of DOC macromolecules in natural waters (Zepp et al. 1995; Williamson et al. 1996). The wavelengths of light causing these reactions has been an additional area of interest. Although the importance of high-energy UV-B radiation (280–315 nm) has been demonstrated by action spectra in many studies, recent evidence suggests that UV-A (315–400 nm) and photosynthetically active radiation (PAR, 400–700 nm), which comprise a much larger fraction of the energy of incoming solar radiation at the earth's surface, are also important in DOC photochemistry (Vahatalo et al. 2000; Yocis et al. 2000; Wetzel 2001).

The effects of UV radiation on DOC composition could be especially important in coastal and inland areas, where both primary productivity and terrestrial inputs of CDOM are high. Decomposition of this large pool of DOC could lead both directly (by photolysis of organic matter) and indirectly (via microbial respiration of DOC) to increased release of CO_2 (Miller and Zepp 1995). Relatively few studies of DOC photolysis and subsequent microbial utilization of photoproducts have examined this process *in situ* (Ziegler and Benner 2000).

This problem is partly because of the difficulty of finding field sites with similar hydrologic regimes and organic matter sources but varying light regimes to allow direct comparisons of UV effects. One such site is Bayou Trepagnier, a forested wetland stream which is part of the larger Lake Pontchartrain estuary in southern Louisiana. The long hydraulic residence time, shallow water column, variable light regime, and high DOC concentrations of Bayou Trepagnier make it an excellent location to study the effects of UV photolysis on DOC cycling in a natural system.

Thus, the two primary goals of this study were as follows: (1) to examine differences in carbon functionality and absorbance properties of HMW-DOC at two sites with different natural light regimes in Bayou Trepagnier; (2) to examine *in situ* UV effects on the availability of the HMW-DOM fraction to the natural bacterial community (via bacterial productivity) of Bayou Trepagnier.

Materials and methods

Bayou Trepagnier is a forested tidal stream with an average water depth of 2 m located west of New Orleans, Louisiana and connected to Lake Pontchartrain via Bayou LaBranche (Figure 1). The bayou is slightly brackish (generally < 2.0 ppt) with very high DOC concentrations (averaging 2.8 mM) and drains cypress swamps, freshwater and brackish marshes, and deciduous bottomland hardwood forest. The bayou is tidally influenced by Lake Ponchartrain, which has a small tidal range of 0–14 cm (USGS). Apart from the slight tidal influence from the lake and occasional wind-driven forcing, Bayou Trepagnier has little directional flow; therefore we designate no traditional "upstream" and "downstream" in the bayou. During periods of minimal tidal movement, flow rates are essentially zero in the upper bayou (Flowers et al. 1998); the hydraulic residence time may be as long as months. During the passage of cold fronts the water level of Lake Pontchartrain can vary by as much as 30 cm, and the residence time in the bayou can be much shorter. The upper bayou is shaded by a heavy tree canopy while the lower bayou is generally not shaded closer to the lake. The bayou is moderately turbid, with total suspended particle (TSP) concentrations averaging 23 mg L^{-1} (Table 1).

Two field sites with different light regimes but similar DOC and HMW-DOC concentrations and physicochemical characteristics were chosen. A shaded site with heavy canopy cover was selected at marker 56, and an open site with no canopy was selected at marker 142 (Figure 1). Eight samplings were conducted at mid-day between August 1997 and January 1999. At each site, physicochemical parameters (water temperature, salinity, pH, alkalinity, oxygen, light intensity (PAR) and attenuation, and total suspended solids) were measured. Light intensity (400–700 nm) was measured 1 cm below the water surface with a Li-Cor LI-189 photometer with a spherical underwater sensor. In addition, 20–30 L of water was filtered through a Nuclepore 0.2 μm filter cartridge and brought back to the laboratory. Each 20–30 L sample was ultrafiltered with an Amicon Proflux Tangential System Model M12 consisting of two Amicon spiral ultrafiltration polysulfone cartridges (nominal pore size 1000 Daltons) aligned in series. Samples were concentrated to approximately 1.5 L of HMW-DOM retentate (< 0.2 μm and > 1 kDa), mixed to homogenize the sample, and divided into subsamples for irradiation and analysis. DOC concentrations of each fraction (< 0.2 μm, < 1 kDa, and 0.2 μm > × > 1 kDa) were measured during ultrafiltration, and a carbon mass balance was calculated for each sample. Percent recovery values for ultrafiltered carbon averaged 97%, with most values between 86% and 102%, indicating little net loss or contamination of DOC

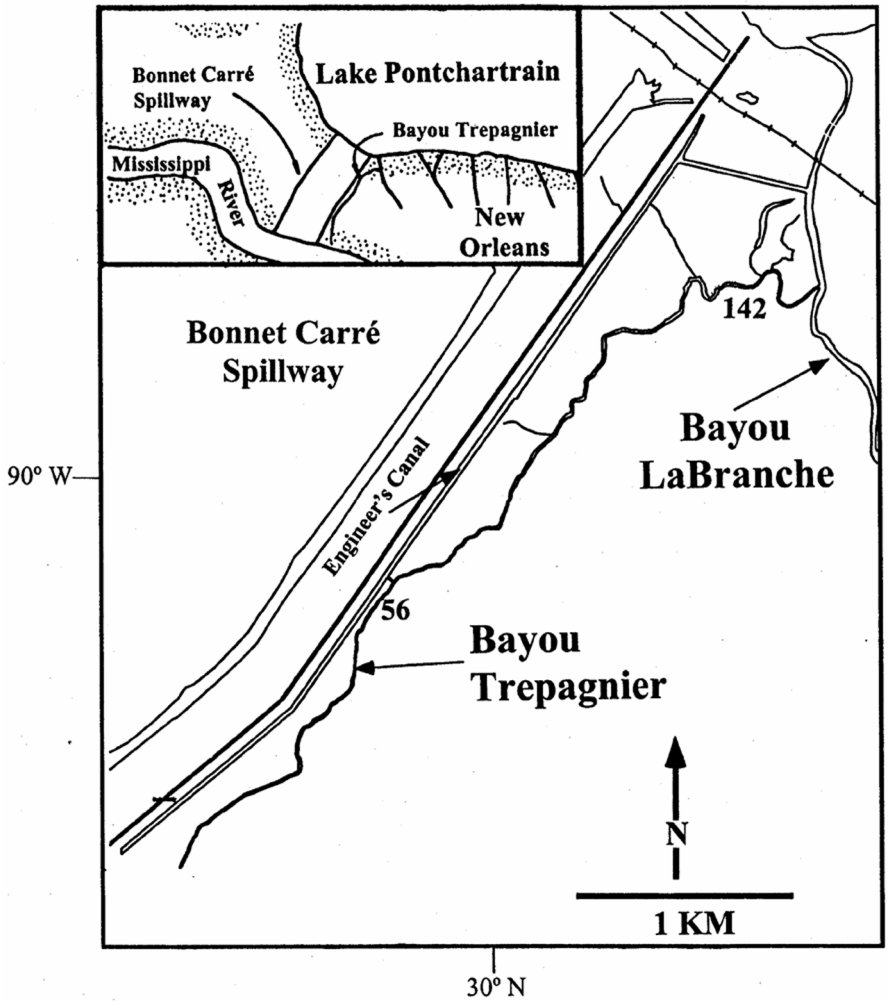

Figure 1. Map of Bayou Trepagnier, LA and the surrounding Lake Pontchartrain estuary.

during ultrafiltration. Subsamples of HMW-DOM solution were irradiated for 24 hours in a Suntest CPS+ solar simulator at full spectrum irradiance of 765 W m^{-2}. Each sample was placed in a specially designed double-walled quartz beaker with a lid and chilled water recirculation (20 °C) through the beakers. Samples were irradiated for 24 hours, the equivalent of approximately 3.8 days of natural sunlight exposure in Miami, FL. Beginning in March 1998, an additional sample was covered with both the quartz lid and a sheet of Mylar-D film, which attenuated over 90% of UV-B. Spectrophotometric analyses (200–800 nm) of all DOM and HMW-DOM samples were conducted before and after solar simulator irradiations. All HMW-DOM was then frozen, lyophilized, and stored for ^{13}C NMR analysis.

Table 1. Physical and chemical properties in Bayou Trepagnier, LA. Surface irradiance of PAR (1 cm below water surface in bayou) and attenuation coefficients (K_t) measured near solar noon. ND = not determined.

Date	Sample	PAR (μmol m^{-2} s^{-1})	K_t (m^{-1})	Water Temp. (°C)	pH	Alkalinity (meq L^{-1})	O_2 (ppm)	TSP (mg L^{-1})	Salinity (ppt)	DOC (mM)	HMW-DOC (mM)
Aug 97	Shaded site	150	ND	32.0	7.2	192	ND	22.4	0.9	ND	ND
	Open site	1650	ND	30.2	7.1	118	ND	ND	1.4	ND	ND
Nov 97	Shaded site	68	5.1	12.5	6.8	153	ND	8.0	3.0	51.6	27.3
	Open site	605	4.4	13.0	7.0	135	ND	22.4	4.0	49.4	30.5
Mar 98	Shaded site	230	4.0	19.0	7.2	125	2.7	ND	0.8	38.2	13.7
	Open site	1740	6.5	22.0	7.2	84	5.2	ND	1.0	26.1	13.1
Jun 98	Shaded site	102	3.7	30.0	7.0	200	5.8	19.6	2.8	12.1	3.4
	Open site	1750	3.3	31.0	7.0	130	6.2	32.0	9.5	24.9	2.9
Aug 98	Shaded site	50	1.4	29.5	6.6	63	4.9	ND	5.0	55.1	36.0
	Open site	410	4.2	32.0	7.1	56	6.9	ND	5.0	47.5	27.8
Oct 98	Shaded site	160	17.5	32.0	7.0	260	1.0	11.6	2.5	27.3	6.1
	Open site	850	24.2	32.5	6.8	239	1.2	15.2	3.1	21.8	11.2
Jan 99	Shaded site	235	ND	15.9	7.2	184	5.7	20.4	1.4	51.6	27.3
	Open site	775	ND	16.9	7.2	125	5.7	36.8	1.6	49.4	30.5

ND = not determined.

^{13}C NMR spectroscopy was performed according to the methods described in Engelhaupt and Bianchi (2001). General parameters of the standard pulse program included a spinning rate of 7000 Hz, contact time of 1 ms, pulse delay of 700 ms, and from 25,000 to 100,000 scans. Natural bacterial communities collected from sediments in Bayou Trepagnier were used in all BPP experiments. Sediments were inoculated into sterile inorganic REM medium (Wetzel et al. 1995) and grown for 72 hr at 20 °C. Samples of the culture solution were then pipetted into sterile cryogenic (Nunc) vials with 20 mL sterile glycerol. Tubes were mixed, frozen, and stored in liquid nitrogen. For experiments, one frozen vial of the consortium was thawed, grown for 48 h in sterile REM medium, and pelleted (10,000 rpm, 10 min). The supernatant was decanted and the pellet was washed with 20 mM MOPS (pH = 7). This procedure was repeated with MOPS and sterile ultra pure water (Millipore Q-pyrogen-free). The pellet was brought up in 10 mL sterile Q-water. One mL of bacteria was added to each 200- mL flask of HMW-DOM.

Bacterial protein productivity (BPP) was measured in HMW-DOM collected from each field site to quantify differences in HMW-DOM lability between the sites. In March 1998, HMW-DOM material was collected from each site, sterile-filtered with a 500 mL Nalgene sterile filtration apparatus containing a 0.2 μm cellulose nitrate filter (previously tested for contamination), and shipped on ice to the University of Alabama for inoculation with Bayou Trepagnier bacterial cultures. BPP was measured using rates of ^{3}H-leucine incorporation (Wetzel and Likens 2000).

A field mesocosm experiment was conducted in Bayou Trepagnier in August 1998 to determine whether natural differences in light levels between shaded and open field sites were sufficient to affect HMW-DOM composition and BPP over the course of one day. An additional treatment examined the relative effects of removing UV-B irradiation. Water was collected from the shaded site and half was transported covered to the open site. Water was placed in 55 L rectangular acid-leached polypropylene containers at each site, with the containers partially submerged in the bayou such that the water level in the container was even with the surrounding bayou. Water within each container reached a depth of $\sim$ 25 cm. Four uncovered replicate containers of water were placed at each field site, and two additional containers covered with Mylar-D sheeting (to remove UV-B) were placed at the open site. The Mylar-D sheeting was raised $\sim$ 4 cm above the container to allow evaporation. Containers were incubated for approximately seven hours, in order to receive one full day of solar exposure. Light, pH, alkalinity, temperature, oxygen, salinity, and total suspended particulates (TSP) were measured in each container at the beginning and end of the experiment. After 7 hours, water from each container was filtered with 0.2 μm Nuclepore filter cartridges. Filtered water samples were returned to the lab and ultrafiltered. Aliquots of colloidal solution from each replicate were sterile-filtered with a Nalgene sterile filtration apparatus with a 0.2 μm cellulose nitrate filter. These samples were inoculated with the Bayou Trepagnier bacteria that had been previously cultured and frozen. Bacterial protein productivity and abundance were measured each day for four days in inoculated HMW-DOM. Analyses were done within one week of sample collection.

Absorbance was measured on a Cary 500 UV-VIS-NIR spectrophotometer with a 1 cm path length using Cary WinUV software. Absorbance spectra were referenced to a deionized water blank. Samples with absorption at 350 nm > 2 absorbance units were diluted and the results scaled accordingly.

All DOC measurements were made by the High Temperature Catalytic Oxidation method (HTCO) with a Shimadzu TOC 5000 Analyzer (Guo et al. 1994). Total carbon and nitrogen percentages were determined using a Carlo Erba CHNS Elemental Analyzer. Soil samples were acidified with 12 N HCl fumes.

BPP was measured using ^{3}H-leucine uptake and conversion to protein as described by Wetzel and Likens (2000). Thirty minute incubations of HMW-DOM solutions were conducted every 24 hours for 4 days with a 10 nM final concentration of ^{3}H-leucine; protein production calculations were made according to Kirchman et al. (1993). Bacterial abundance in each colloidal solution replicate was also measured by direct counts using DAPI staining and epifluorescence microscopy on black 25- mm polycarbonate filters (Wetzel and Likens 2000). Twenty grids were counted for each sample. Bacterial protein productivity was normalized to bacterial abundance (cells L^{-1}) to give bacterial protein productivity per cell (μg cell^{-1} hr^{-1}). This value was converted to units of μg C cell^{-1} hr^{-1} by multiplying by a conversion factor of 0.86 (Wetzel and Likens 2000).

Results

Laboratory irradiation of HMW-DOM

Irradiation of HMW-DOM in a solar simulator resulted in a decreased organic C/N mass ratio ($p = 0.06$) (Table 2). This result was found in HMW-DOM from all field sampling dates for which total carbon and nitrogen were analyzed, with the exception of the October sampling. Although October HMW-DOM had carbon and nitrogen contents within the range found for other sampling dates, it probably had a somewhat different composition as a result of the impacts of tropical storm Frances and may have been less photoreactive. October HMW-DOM appeared to be derived from soil or sediment sources based on its high iron content (data not shown), and appeared gray in color rather than the usual brown.

^{13}C NMR data did not reveal consistent changes in carbon functional groups of HMW-DOC after irradiation in a solar simulator (Figure 2). Although the differences in average relative abundance of functional groups at each site before and after irradiation (Figure 3) were not statistically significant ($P > 0.05$, Student's t-test), closer examination of NMR spectra from each sampling date revealed that HMW-DOC did appear to undergo some changes after irradiation in a solar simulator. In some cases, irradiation resulted in large changes in the relative abundance of a functional group. For example, the percentage of carboxyl carbon increased 340% after irradiation in Nov. 1997, from 5% to 22%. The carboxyl peak (165 – 190 ppm) generally exhibited some splitting after irradiation, with the appearance

Table 2. Optical absorbance, spectral slope (S), and carbon/nitrogen ratios of dissolved organic matter, Bayou Trepagnier, LA.

Date	Sample	Irradiation	a_{350} (cm^{-1})	a_{254}/a_{365}	S	C/N
Aug 97	Shaded site	None	ND	ND	ND	17.8
		Full Spectrum	ND	ND	ND	17.7
	Open site	None	ND	ND	ND	18.5
		Full Spectrum	ND	ND	ND	17.7
Nov 97	Shaded site	None	2.12	ND	0.0124	21.1
		Full Spectrum	2.07	ND	0.0160	20.3
	Open site	None	1.73	ND	0.0162	21.3
		Full Spectrum	1.70	ND	0.0198	19.0
Mar 98	Shaded site	None	1.45	3.83	0.0154	ND
		UV-A	1.33	4.27	0.0161	19.9
		Full Spectrum	1.32	4.25	0.0161	19.8
	Open site	None	1.55	3.65	0.0149	19.5
		UV-A	1.38	4.11	0.0154	19.7
		Full Spectrum	1.38	4.13	0.0154	17.4
Jun 98	Shaded site	None	0.68	ND	ND	ND
		UV-A	0.54	ND	ND	ND
		Full Spectrum	0.54	ND	ND	ND
	Open site	None	0.68	4.42	ND	ND
		UV-A	0.60	4.87	ND	ND
		Full Spectrum	0.60	4.89	ND	ND
Oct 98	Shaded site	None	4.38	3.89	0.0136	16.1
		UV-A	4.06	3.96	0.0139	ND
		Full Spectrum	4.08	3.95	0.0138	16.5
	Open site	None	5.20	3.78	0.0137	19.9
		UV-A	4.92	3.90	0.0141	ND
		Full Spectrum	4.78	3.89	0.0141	21.0
Jan 99	Shaded site	None	ND	ND	ND	ND
		UV-A	ND	ND	ND	ND
		Full Spectrum	ND	ND	ND	ND
	Open site	None	1.51	4.74	0.0171	ND
		UV-A	1.60	4.91	0.0160	ND
		Full Spectrum	1.65	4.91	0.0158	ND

ND = not determined.

of a distinct peak around 166 ppm in addition to the peak located between 175–180 ppm. In addition, the height of a peak located in the aliphatic region between 21–24 ppm increased after irradiation. This increase in peak height occurred more consistently in samples from the shaded site than in those from the open site. The percentage of carbohydrate carbon was lower and the percentage of carboxyl carbon was higher after irradiation in all but one case, Nov. 1997 at the shaded site (Table 3; $p = 0.11$). Aliphatic and aromatic carbon did not show a consistent pattern after irradiation.

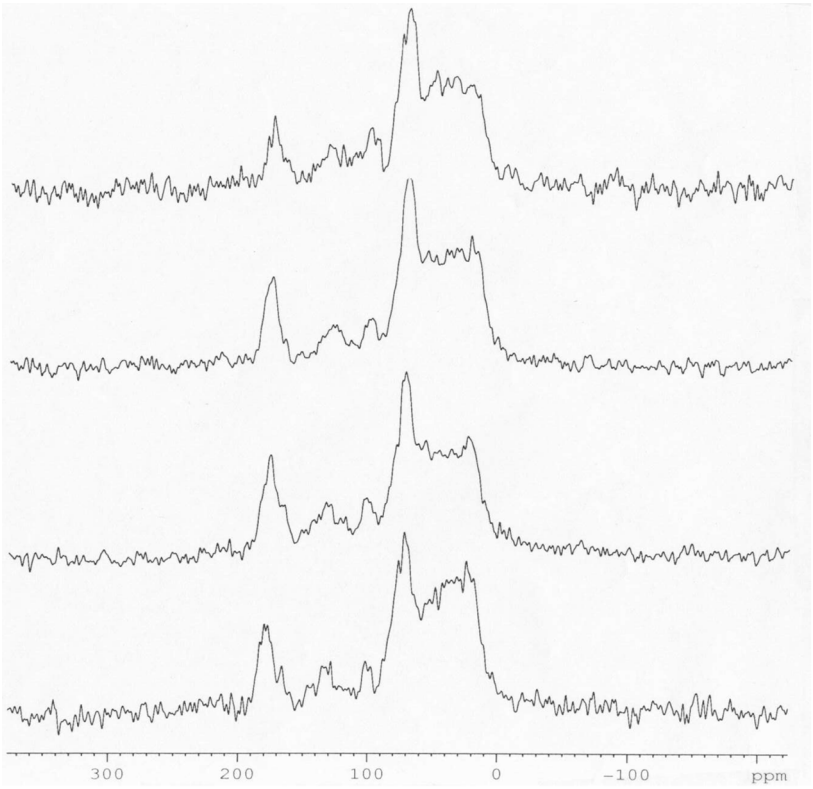

Figure 2. ^{13}C NMR spectra of HMW-DOC from an open site in Bayou Trepagnier A) non-irradiated March 1998; B) irradiated in a solar simulator (PAR+UVA+UVB) March 1998; C) non-irradiated August 1997; and D) irradiated in a solar simulator (PAR+UVA+UVB) August 1997.

UV/PAR absorbance

Most of the color in DOM samples was contained in the HMW-DOM fraction. For example, absorptivity (a = 2.303A/b, where a is absorptivity, A is absorbance, and b is the pathlength in meters) at 365 nm for the open site in Nov. 1997 was 36.8 in the total DOM fraction, 5.9 in the LMW-DOM fraction (low-molecular-weight DOM; < 1 kDa), and 7.9 in the HMW-DOM fraction (LMW-DOM and HMW-DOM values corrected for concentration factor during ultrafiltration). Absorption of UV and visible light by CDOM in the HMW-DOM fraction can be described by an exponential curve with a slight shoulder between 250–300 nm. The shape of this curve was consistent across seasons. Irradiation in the solar simulator for 24 hours resulted in 2–21% fading at 350 nm of HMW-DOM samples (values from Table 2), with greater fading in samples from the shaded site.

An increased fraction of the UV-absorbing HMW-DOM was of low molecular weight after irradiation in the solar simulator, as indicated by an increased ratio of absorbance at 254 nm and 365 nm (Table 2). This ratio, a_{254}/a_{365}, has been shown

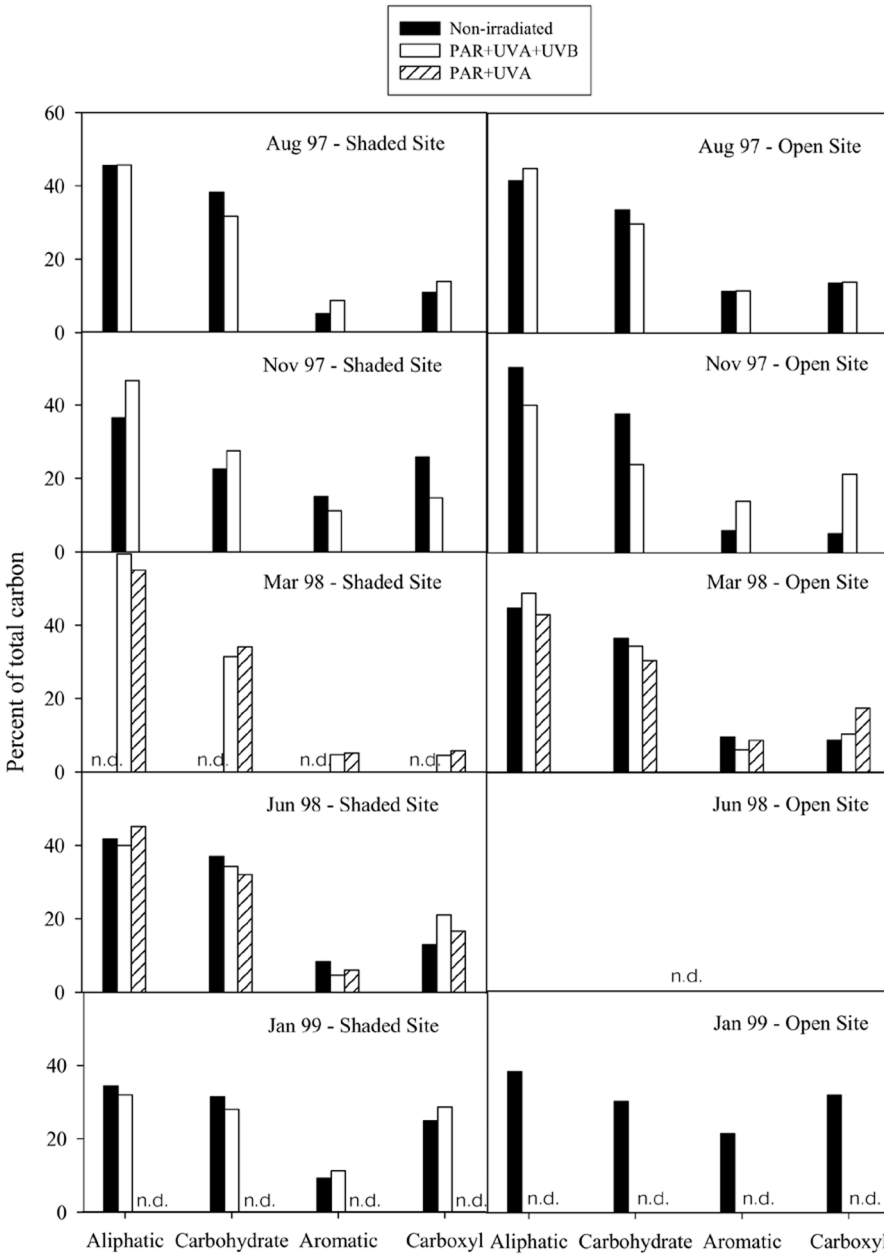

Figure 3. Percent abundance of functional groups in non-irradiated and solar simulator irradiated (PAR + UVA + UVB, PAR + UVA) HMW-DOC from Bayou Trepagnier.

to be higher in samples of lower average molecular weight, including samples that have been irradiated (Strome and Miller 1978; Dahlen et al. 1996). In HMW-DOM

Table 3. Relative abundance of functional groups in ^{13}C NMR spectra of Bayou Trepagnier HMW-DOC collected during the sampling period of August 1997 through January 1999, as percentages of total carbon in each sample. Solar simulator irradiation treatments include non-irradiated, PAR + UVA + UVB, and PAR + UVA irradiated.

Date	Sample	Treatment	% Aliphatic	% Carbohydrate	% Aromatic	% Carboxyl
Aug 97	Shaded site	Non-irradiated	46	38	5.0	11
		PAR + UVA + UVB	46	32	9.0	14
	Open site	Non-irradiated	41	34	11	14
		PAR + UVA + UVB	45	30	11	14
Nov 97	Shaded site	Non-irradiated	37	23	15	26
		PAR + UVA + UVB	47	27	11	15
	Open site	Non-irradiated	51	38	6.0	5.0
		PAR + UVA + UVB	40	24	14	22
Mar 98	Shaded site	Non-irradiated	ND	ND	ND	ND
		PAR + UVA + UVB	59	31	5.0	4.5
		PAR + UVA	55	34	5.0	6.0
	Open site	Non-irradiated	45	37	10	9.0
		PAR + UVA + UVB	49	35	6.0	10
		PAR + UVA	43	31	9.0	18
Jun 98	Shaded site	Non-irradiated	42	37	8.0	13
		PAR + UVA + UVB	40	34	4.6	21
		PAR + UVA	45	32	6.0	17
	Open site	Non-irradiated	ND	ND	ND	ND
		PAR + UVA + UVB	ND	ND	ND	ND
		PAR + UVA	ND	ND	ND	ND
Jan 99	Shaded site	Non-irradiated	34	31	9.0	25
		PAR + UVA + UVB	32	28	11	29
		PAR + UVA	ND	ND	ND	ND
	Open site	Non-irradiated	31	25	18	26
		PAR + UVA + UVB	ND	ND	ND	ND
		PAR + UVA	ND	ND	ND	ND

collected from Bayou Trepagnier over 4 seasons, the average ratio was 4.03, increasing to an average of 4.34 in irradiated samples ($p < 0.01$ in a paired *t*-test). There was no difference in the absorbance ratio of samples irradiated with and without UV-B ($p = 1.0$).

Spectral slope of HMW-DOM samples was calculated by linear regression analyses of natural log transformed absorption coefficients versus wavelength. Regression analyses were performed for the wavelength range of 300–450 nm, the range for which linear values were found and absorbance was above the practical detection limit of the spectrophotometer ($r^2 > 0.99$). Spectral slope values in samples not irradiated in the solar simulator were found to be 0.012 – 0.015 nm^{-1} for shaded site HMW-DOM and 0.014 – 0.017 nm^{-1} for open site HMW-DOM (Table 2).

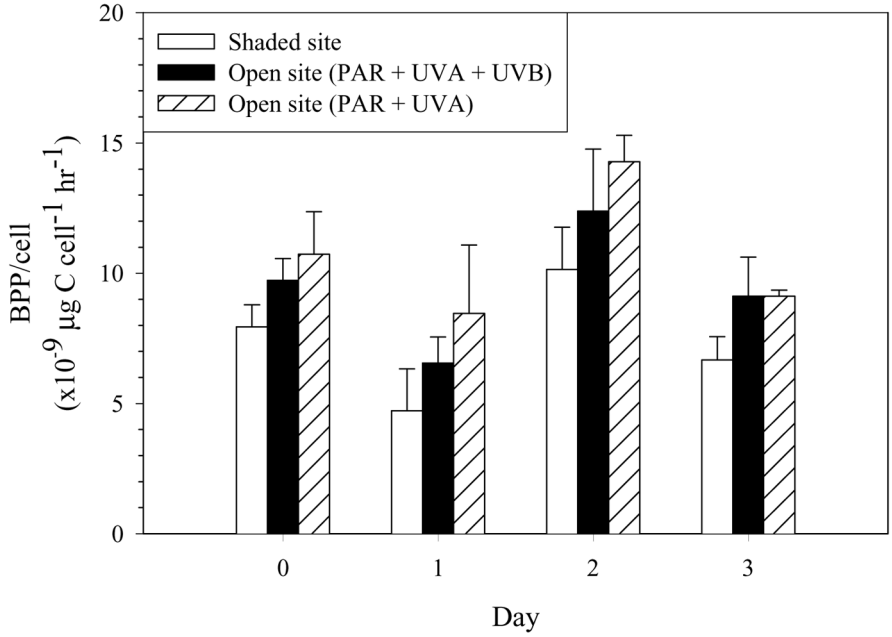

Figure 4. Bacterial protein productivity (BPP) per cell in Bayou Trepagnier HMW-DOC from shaded, open (PAR + UVA + UVB), and mylar-covered treatments (PAR + UVA) in a field mesocosm experiment.

Bacterial utilization

Field Mesocosm Experiment—HMW-DOM from waters irradiated with direct sunlight for one day in Bayou Trepagnier supported significantly higher bacterial productivity than HMW-DOM from waters shaded from direct sunlight. Both treatments from the open site (with and without UV-B) supported significantly higher BPP per cell than the shaded site treatment ($P < 0.05$, ANOVA) (Figure 4, Table 4). There was no significant difference in BPP between treatments at the open site (with and without Mylar-D to block UV-B), although there was a trend of slightly higher BPP/cell from the Mylar treatment.

Field BPP measurements

BPP did not vary significantly between open and shaded field sites in March 1998 ($P > 0.05$, ANOVA). BPP averaged 7.4 μg C L^{-1} h^{-1} at the shaded site and 6.6 μg C L^{-1} h^{-1} at the open site over the course of the experiment (Table 4).

Table 4. Bacterial cell counts and productivity per cell as calculated from ^{3}H-leucine uptake. August 1998 experiment results shown over four days of bacterial growth in samples.

Date	Site	Day	Bacterial abundance (10^6 cells ml^{-1})	Bacterial productivity (μg C L^{-1} hr^{-1})
Mar 98	Shaded	–	ND	7.4
	Open	–	ND	6.6
Aug 98	Shaded	1	0.8 ± 0.1	8.3
	Open	1	1.1 ± 0.2	7.7
	Mylar	1	0.9 ± 0.1	8.9
	Shaded	2	1.3 ± 0.1	7.2
	Open	2	1.6 ± 0.2	8.3
	Mylar	2	1.0 ± 0.4	7.8
	Shaded	3	0.8 ± 0.1	8.7
	Open	3	0.9 ± 0.1	9.5
	Mylar	3	0.6 ± 0.01	8.5
	Shaded	4	1.0 ± 0.2	7.6
	Open	4	1.2 ± 0.2	8.8
	Mylar	4	0.9 ± 0.05	7.9

ND = not determined.

Discussion

UV/PAR absorbance

Laboratory irradiations with simulated natural light resulted in photolysis of CDOM, with UV-A and photosynthetically active radiation (PAR) resulting in the most effective CDOM photolysis. The average molecular weight of Bayou Trepagnier HMW-DOM (as indicated by an increase in the absorbance ratio a_{254}/a_{365}) decreased after irradiation in a solar simulator. In addition, the overall absorbance of irradiated samples decreased and spectral slope (S) values were higher in irradiated relative to non-irradiated samples. Recent work has shown an increase in the a_{254}/a_{365} ratio in response to increased dosing of UV-B radiation (Obernosterer and Herndl 2000). However, no difference was found in a_{254}/a_{365}, fading at 350 nm, or S values between samples irradiated with and without UV-B, indicating that UV-B was less important than UV-A and PAR in photolysis with natural or simulated sunlight. These results supported conclusions reached by investigators measuring increased S values of CDOM with distance offshore, which have been interpreted as resulting from photolytic cleavage of DOC (Carder et al. 1989; Vodacek et al. 1997; D'Sa et al. 1999). Coastal rivers, especially those containing wetland waters with high DOC and CDOM concentrations ("black water" rivers), have been shown to be important contributors of coastal DOM (Valentine and Zepp 1993; Miller and Zepp 1995; Battin 1998).

Changes in bulk composition (^{13}C NMR)

^{13}C NMR revealed only very subtle changes in HMW-DOC bulk composition after exposure to simulated solar radiation. A similar pattern occurred in laboratory-irradiated samples in this study and in a diurnal field experiment conducted in Bayou Trepagnier (Bianchi et al. 2002). More specifically, an aliphatic peak ($\sim$ 20 ppm shift) was observed to increase slightly during daylight hours. These diurnal changes within the bayou could have resulted from either photolytic changes in DOM composition with increasing daylight exposure, or from increased contribution of aliphatic substances from phytoplankton during daylight hours. DOC concentrations changed little (ranging from 16 to 19 mg L^{-1}) during the time period when compositional changes were occurring in the diurnal experiment, which is inconsistent with the idea of increased phytoplankton contribution unless a removal process of the same magnitude was occurring concomitantly. Moreover, samples irradiated in the solar simulator had been filtered with a 0.2 μm filter (and thus contained no phytoplankton) and yet showed similar effects to seen in the diurnal field experiment, indicating that phytoplankton contribution to compositional changes was likely minimal. The composition appeared to be returning to its ″nighttime″ state at the end of the diurnal experiment, suggesting that either of two processes could be occurring, assuming the involved reactions are not reversible. Either the newly-produced DOC components are being selectively removed (perhaps via heterotrophic activity) and thus their contribution is minimized once daytime production ends, or when the new components are no longer being produced at night their contribution to the DOC pool is diluted by inputs of unaltered DOC. The former scenario would be consistent with photoproduction of labile organic compounds readily assimilated by microbes in the water column (Geller 1986; Moran and Zepp 1997).

The aliphatic peak (21–24 ppm) that increased after irradiation in the solar simulator could arise from either terminal methyl groups or methylene groups in alkyl chains (possibly lipids or proteins) (Malcolm 1990; Knicker and Ludemann 1995). The splitting of the carboxylic peak after irradiation could possibly result from the production of low-molecular weight organic compounds during irradiation of high-molecular weight HMW-DOC. All photoproducts of this type identified to date are carbonyl compounds, many of them fatty acids and keto acids (Moran and Zepp 1997).

As for changes in percent abundance of entire functional groups after irradiation in the solar simulator, carbohydrate carbon generally decreased and carboxyl carbon increased after irradiation. The decrease in carbohydrate abundance is consistent with data obtained by Wetzel et al. (1995). If carbohydrates are indeed more susceptible to photolysis, then organic matter derived from carbohydrate-rich plants and algae could be an important source of photolytic products. Since the light-absorbing chromophores in humic and fulvic acids are largely associated with aromatic moieties (McKnight et al. 1994), the fraction of HMW-DOC derived from aromatic humic substances should be susceptible to photolytic degradation. However, this was not recorded as a general trend in Bayou Trepagnier HMW-DOC.

The changes observed within the aliphatic, carbohydrate, and carbonyl functional groups did not translate into statistically significant differences in percentages of the functional groups between non-irradiated and irradiated samples, indicating that photolysis acts on specific types of chromophores rather than on general carbon bond types. It has been estimated that only 20% of the photolysis products have been identified with recent studies suggesting that further information on the chromophore-specific components is needed (Miller and Zepp 1995; Obernosterer and Herndl 2000).

Bacterial protein productivity

Bacterial productivity supported by HMW-DOM was enhanced in naturally irradiated samples from the field mesocosm experiment in August 1998, indicating possible photochemical production and subsequent uptake of more labile DOM by the microbial community, possibly including nitrogen- and phosphorus-containing compounds in addition to carbon substrates. Because the same culture of Bayou Trepagnier bacteria was used to inoculate each sterile sample of HMW-DOM in the lab, the only difference between treatments was the composition of the substrate available to the bacteria; thus, differences between treatments should reflect changes in HMW-DOM composition among the irradiation treatments (Wetzel et al. 1995). Additionally, water used in all three experimental treatments was from the same source at the shaded site; therefore, any background differences in HMW-DOM composition between the open and shaded site were not a factor in the experiment.

Bacterial protein productivity (BPP) was significantly higher in HMW-DOM collected from open relative to shaded treatments in Bayou Trepagnier. Differences between open treatments with and without UV-B were not significant, indicating that UV-A and PAR are more significant than UV-B in DOM photochemistry *in situ*. The observed trend of higher productivity in samples irradiated without UV-B, although not statistically significant, could have resulted from a greater direct loss of bioavailable DOC as CO_2 in the +UV-B samples (see Table 4).

Relative bioavailability of HMW-DOC was calculated as in Ziegler and Benner (2000), (Table 5), by dividing the average bacterial productivity (BP; converted to μM C h^{-1}) by the concentration of HMW-DOC in each sample (DOC; mM). That paper used BP/DOC to compare the relative bioavailability of DOM in waters from different sources and environments and found values ranging from 1.01 for Laguna Madre to < 0.06 for humic pond water. Values in the August 1998 experiment in this study averaged 0.17, similar to values found for *Juncus effusus* leachate and blackwater rivers (Ziegler and Benner (2000) and references therein). This finding demonstrates the low to moderate bioavailability of HMW-DOC in Bayou Trepagnier relative to other systems. The effects of photolysis on bacterial activity may depend upon both the photoreactivity and the bioavailability of DOM, as suggested by others (Ziegler and Benner 2000). In Bayou Trepagnier, HMW-DOM was of relatively low bioavailability but was highly colored and photoreactive, and photolysis at the open site produced more labile HMW-DOM for bacterial uptake. In

Table 5. PAR, [HMW-DOC], and relative bioavailability of HMW-DOC calculated as average bacterial productivity (BP; μM C h^{-1}) on Day 1 of BPP measurements divided by HMW-DOC concentration (mM) in Bayou Trepagnier, LA.

Date	Site	PAR (μmol m^{-2} s^{-1})	BP (μM C h^{-1})	[HMW-DOC] (mM)	BP/[HMW-DOC] (μM C h^{-1})/(mM C)
March 1998	Shaded	230[1]	0.62	1.2	0.51
	Open	1740[1]	0.55	1.2	0.46
August 1998	Shaded	53[2]	0.69	4.8	0.14
	Open	252[2]	0.64	3.6	0.18
	Mylar	268[2]	0.74	3.8	0.19

[1] 1 cm below water surface in open bayou
[2] 1 cm below water surface in experimental chambers

systems where the opposite is true, and HMW-DOM is of relatively lower photoreactivity and higher lability, the effects of natural light on bacterial productivity could potentially be too small to discern.

Because water from the shaded site was used in all treatments, differences in the composition of HMW-DOM between open and shaded treatments could primarily result from two potential mechanisms: (1) the production of more labile organic matter by increased phytoplankton productivity at the open site; or (2) the production of more labile organic matter by photolytic processes. Exploring the first mechanism, such a scenario would result in greater primary productivity at the open versus the shaded field site at all times. This relationship was suggested as the dominant mechanism in a highly productive lagoon which is considerably more dynamic than Bayou Trepagnier (Ziegler and Benner 2000). For example, when chlorophyll and carotenoid phytoplankton pigments were measured in the bayou (Bianchi et al. 2002) pigment concentrations were higher in the vicinity of the shaded site than near the open site, indicating that light is probably not limiting phytoplankton production in Bayou Trepagnier. The higher biomass at the shaded site could result from higher nutrient concentrations (Trepagnier NH_4 = 1 ppm; Lake Pontchartrain = 0.05 – 0.14 ppm), with the open site closer to the lake (Argyrou et al. 1997; Flowers et al. 1998). In addition, although pH increased more in open treatments during the experiment (1.7 pH units versus 0.4 units at the shaded site), oxygen concentrations increased to a greater degree in shaded treatment relative to open treatments, indicating some ambiguity as to which site supported higher phytoplankton growth. Finally, to aid in determining which site supported higher productivity during the mesocosm experiment, net CO_2 fixation was calculated using measured changes in alkalinity during the experiment (Stumm and Morgan 1995). Net CO_2 fixation from photosynthesis was actually higher at the shaded site than at the open site (4.4×10^{-4} M versus 1.5×10^{-5} M). Thus, release of labile substrates from phytoplankton likely did not result in the observed higher bacterial productivity in HMW-DOM irradiated at the open site. It is also interesting to note that CO_2 fixation could be accelerated by photoproduction of NH_4^+ from HMW-

DOM (Wang et al. 2000), and could potentially contribute to phytoplankton production, though further work is necessary to assess the relative importance of this contribution. Although this calculation assumes a closed water body without atmospheric CO_2 exchange or $CaCO_3$ deposition, these factors should have been relatively equal between treatments. Taking into account the above factors, the second mechanism better explains the compositional differences that led to increased BPP in HMW-DOM from the open treatment.

There was no significant difference in the bacterial productivity supported by HMW-DOM between the two field sites in March 1998, presumably as a result of mixing and system heterogeneity (differential DOC inputs, turbidity, bacterial populations, and patchiness of light input). Although the effects of solar irradiation can produce measurable changes in bacterial productivity under controlled conditions, as was observed in our field manipulation experiment as well as in previous laboratory studies, translating these phenomena to the natural environment can still be problematic.

Data from the field mesocosm experiment were used to calculate the average "extra" bacterial carbon production from uptake of photolyzed HMW-DOM that was otherwise unavailable. Because BPP was 30% higher in HMW-DOM from the open site relative to the shaded site, 30% of the average BPP at the shaded site (2.21×10^{-9} μg C cell^{-1} hr^{-1}) multiplied by an assumed bacterial concentration of $1{\times}10^6$ bacteria/mL gives 2.21 μg C L^{-1} h^{-1} of extra bacterial carbon productivity derived from photolysis of HMW-DOM in surface waters (< 25 cm). The assumed bacterial cell concentration is the average value found for mesotrophic lakes (Wetzel 2001). Assuming a conservative growth efficiency of 10% (Coveney and Wetzel 1995; Bertilsson and Tranvik 1998), this estimate is in good agreement with the combined photoproduction rate of carboxylic acids in surface waters from a humic lake with natural sunlight (Bertilsson and Tranvik 1998), as 10% of their production value of 19 μg C L^{-1} h^{-1} would equal 1.9 μg C L^{-1} h^{-1} of productivity expected from uptake of photolytically produced carboxylic acids. Photolytic processes in high-DOC systems, such as humic lakes and wetland streams, can therefore provide a substantial source of energy not otherwise available to heterotrophic bacteria.

Conclusions

^{13}C NMR revealed only very minor alterations in bulk HMW-DOC composition; however, bacterial productivity supported by HMW-DOM was enhanced in naturally irradiated samples in a field mesocosm experiment. The difference in sunlight exposure between a tree canopy-shaded site and an unshaded site was sufficient to result in differences in bacterial response to HMW-DOM substrate after just 7 hours of *in situ* exposure of HMW-DOM at these sites. Results of field experiments and various laboratory analyses suggested that UV-A and PAR radiation were more important to HMW-DOM photolysis *in situ* than UV-B radiation. No significant dif-

ferences existed in absorbance characteristics such as spectral slope, absorbance at 350 nm, or the absorbance ratio a_{254}/a_{365} nm between samples irradiated with and without UV-B. Our data support the idea that photolysis of HMW-DOC may be particularly important to biota in highly colored waters with relatively recalcitrant DOC.

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