



Chemical and optical changes in freshwater dissolved organic matter exposed to solar radiation

CHRISTOPHER L. OSBURN^{1*}, DONALD P. MORRIS¹,
KEVIN A. THORN² & ROBERT E. MOELLER¹

¹*Department of Earth and Environmental Sciences, Lehigh University, Bethlehem, PA 18015, U.S.A.* ²*U.S. Geological Survey, National Water Quality Laboratory, Denver Federal Bldg., Denver, CO 80002, U.S.A. (*Current address: National Research Council-Naval Research Laboratory, Code 6115, Washington, DC 20375, U.S.A.)*

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Abstract. We studied the chemical and optical changes in the dissolved organic matter (DOM) from two freshwater lakes and a *Sphagnum* bog after exposure to solar radiation. Stable carbon isotopes and solid-state ¹³C-NMR spectra of DOM were used together with optical and chemical data to interpret results from experimental exposures of DOM to sunlight and from seasonal observations of two lakes in northeastern Pennsylvania. Solar photochemical oxidation of humic-rich bog DOM to smaller LMW compounds and to DIC was inferred from losses of UV absorbance, optical indices of molecular weight and changes in DOM chemistry. Experimentally, we observed a 1.2‰ enrichment in δ¹³C and a 47% loss in aromatic C functionality in bog DOM samples exposed to solar UVR. Similar results were observed in the surface waters of both lakes. In late summer hypolimnetic water in humic Lake Lacawac, we observed 3 to 4.5‰ enrichments in δ¹³C and a 30% increase in aromatic C relative to early spring values during spring mixing. These changes coincided with increases in molecular weight and UV absorbance. Anaerobic conditions of the hypolimnion in Lake Lacawac suggest that microbial metabolism may be turning over allochthonous C introduced during spring mixing, as well as autochthonous C. This metabolic activity produces HMW DOM during the summer, which is photochemically labile and isotopically distinct from allochthonous DOM or autochthonous DOM. These results suggest both photooxidation of allochthonous DOM in the epilimnion and autotrophic production of DOM by bacteria in the hypolimnion cause seasonal trends in the UV absorbance of lakes.

Introduction

Dissolved organic matter (DOM) represents a substantial portion of the total organic carbon found in aquatic ecosystems ranging from freshwater lakes to the open ocean. Recent investigations into the cycling of DOM in aquatic ecosystems that have identified the importance of photochemical reactions induced by the absorption of solar radiation, primarily in the ultraviolet region

(UVR, 290 to 400 nm: Miller 1994; Zepp 1988; Zepp et al. 1995; DeHaan 1993). Indirect evidence for the importance of photooxidation on carbon cycling in the ocean has been found from analysis of natural radiocarbon of DOM relative to bacteria. Preliminary surveys indicate that photooxidation may contribute up to 20% of old, refractory DOM to bacteria in the Pacific Ocean (Cherrier et al. 1999) and may contribute substantial C to bacteria in the Atlantic Ocean (Kieber et al. 1989), such that the half life of terrestrial DOM in the mixed layer of the ocean maybe as short as 15 years (Kieber et al. 1990). For fresh waters, multiple studies have demonstrated the importance of photochemically produced substrates for microbial metabolism (Moran et al. 2000; Bertilsson et al. 1999; Bano et al. 1998; Bertilsson & Tranvik 1998; Lindell et al. 1995; Wetzel et al. 1995). Photochemical reactions have received much attention in the past decade because of the potential for increased levels of solar ultraviolet radiation (UVR) at the Earth's surface due to depletion of stratospheric ozone (Madronich 1993; Tevini 1993). In freshwater ecosystems, these reactions are especially important due to the large and variable range of dissolved organic carbon (DOC) concentrations ($80 \mu\text{M C}$ for oligotrophic and clear fresh waters to $>1600 \mu\text{M C}$ for humic-rich freshwater lakes and bogs; Thurman 1985; Wetzel 1983).

Photochemical reactions in aquatic ecosystems can alter the chemical structure and optical properties of DOM due to the absorbance of UVR by certain moieties of the bulk DOM, termed *chromophores*. However, it is unclear how the optical and chemical changes are related. Because DOM is heterogeneous in nature, only a small portion may be chromophoric and thus responsible for photochemical reaction. Likewise, these reactions may not be related to the loss of UV absorbance (photobleaching or fading) that has been observed upon exposure of DOM to natural sunlight and in the surface waters of aquatic ecosystems. Morris and Hargreaves (1997) have reported seasonal trends in the UV absorbance of DOM from temperate lakes located in northeastern Pennsylvania and have indirectly demonstrated chemical change from optical indices of molecular weight and molar absorptivity from photooxidation. Bourbonniere et al. (1997), Miller & Zepp (1995), and Valentine & Zepp (1993) have shown that photochemical production of DIC and CO is correlated to loss of UV absorbance. However, few studies correlate chemical alteration of DOM with changes in its absorbance. Moreover, little information exists on the co-variability of chemistry and optical properties of DOM on seasonal scales and spatially in aquatic ecosystems. One reason for this lack of data stems from the difficulty in distinguishing pool sizes of photochemically produced DIC and low molecular weight (LMW, $<1 \text{ kD}$) compounds from other sources, such as bacterial respiration. DIC is generated from multiple sources in aquatic ecosystems and is rapidly consumed (e.g.,

DIC production from respiration and uptake by photosynthesis) or evolved out of the ecosystem (DIC loss in supersaturated systems, Molot & Dillon 1996, 1997; Kling et al. 1991). In addition, the pool size of DIC is large, and measuring photochemical production of DIC *in situ* is difficult. Likewise, measurements of photochemical production of LMW DOM (e.g., monomers and carboxylic acids) in the water column are difficult because of their rapid production by autochthonous processes and consumption by aquatic bacteria.

In this paper, we utilized stable carbon isotope analysis and ^{13}C -NMR spectroscopy as molecular markers for direct evidence of photochemical alteration of DOM. We expected that changes in $\delta^{13}\text{C}$ of DOM in surface waters of lakes will reflect exposure to UVR and that NMR spectra could provide information on the photoreactivity of particular functional groups. These methods provide qualitative descriptions of DOM chemistry at the molecular level that may be used in conjunction with measurements of dissolved UV absorbance and DOC concentration to interpret the effects of photochemical reactions on the bulk DOM pool. We exposed DOM isolated from a *Sphagnum* bog to natural sunlight to determine photochemically induced changes in stable carbon isotope ratio and ^{13}C -NMR spectra of the bulk DOM. These results were compared to changes in the UV absorbance of the bulk DOM. Bog water was chosen as an allochthonous C source because it is rich in humic and fulvic acids, the two moieties of DOM most often identified as chromophores (Strome & Miller 1978; Thurman 1985). Moreover, it is a major source of DOM for lakes in temperate regions where we conducted our study. In addition, we collected seasonal and spatial samples from a humic and non-humic lake for similar optical and chemical analyses and compared those observations with our experimental results.

Methods

Study sites

We compared seasonal observations of optical and chemical properties from two lakes on the Pocono Plateau in northeastern Pennsylvania that had different UVR environments. Samples for seasonal monitoring of changes in optical and chemical properties were collected from 1 m and 10 m depths in Lake Lacawac, a humic lake, from April to November in 1998 and 1999. From April to November of 1998, samples were collected from 3 m and 17 m in Lake Giles, an oligotrophic, optically clear lake located approximately 15 km east of Lacawac. Limnological characteristics of these lakes are presented in Table 1 and the seasonal variation in the UV environments of these lakes has been previously characterized (Morris & Hargreaves 1997). To charac-

Table 1. Optical and chemical characteristics of the study lakes based on surveys from 1993–1995 (Morris & Hargreaves 1997). Chemical data are mean summer epilimnion (mixed layer) values

Variable	Lacawac	Giles
Latitude	41°22'57" N	41°22'34" N
Longitude	75°17'35" W	75°05'33" W
$K_{d,320}$ (m^{-1})*	7.78	0.32
Chl <i>a</i> ($mg\ L^{-1}$)	2.3	1.5
DOC (μM)	398	125
pH	6.03	5.35
Alkalinity ($\mu eq\ L^{-1}$)	30	-1.4

* Attenuation coefficient for 320 nm (Morris et al. 1995).

terize the water chemistry of Lake Lacawac at the end of the summer, we collected a vertical profile (discrete samples collected at 1 m intervals from the surface to 11 m) on 2 September of 1999.

Photooxidation experiments

Water used in photooxidation experiments was collected in May of 1998 at 2 m depth from a lysimeter installed in a *Sphagnum* bog which surrounds nearly one-third of Lake Lacawac. The bog water had abundant DOM (DOC = 4600 μM) and very high UV absorbance (a_d measured at 320 nm approx. 1600 m^{-1}). The anaerobic bog water (strong smell of sulfide) was air-equilibrated for 4 hours (dissolved oxygen = 250 μM) and serial filtered through a single Whatman GF/D and double GF/F glass fiber filters to remove particulates and microbes larger than 1 μm . Before use, the bog water was filtered through 0.2 μm Sterivex spiral-wound filters (Millipore, Cambridge, MA) to achieve mechanical sterilization. Additional inhibition of microbial growth was achieved through the addition of 0.1% (vol./vol.) of NaN_3 .

Samples were placed in clean, pre-leached 4 L polyethylene Ziploc bags, which transmit > 85% of the UVR from 280–400 nm (Figure 1). These large volumes were necessary in order to recover enough carbon (approximately 90 mg dry wt.) for solid-state ^{13}C -NMR analysis. For the light treatment, 2 L of water was placed in each bag and sealed; the dark treatment was wrapped in aluminum foil. We used the Ziploc bags so that when lying flat in our exposure pans, the light pathlength travelling vertically through the bog water was minimized (pathlength, 0.05 m). Because the bog water absorbed strongly in the UV region, we note that most light was absorbed by the sample during the experiment. Each exposure treatment was run in

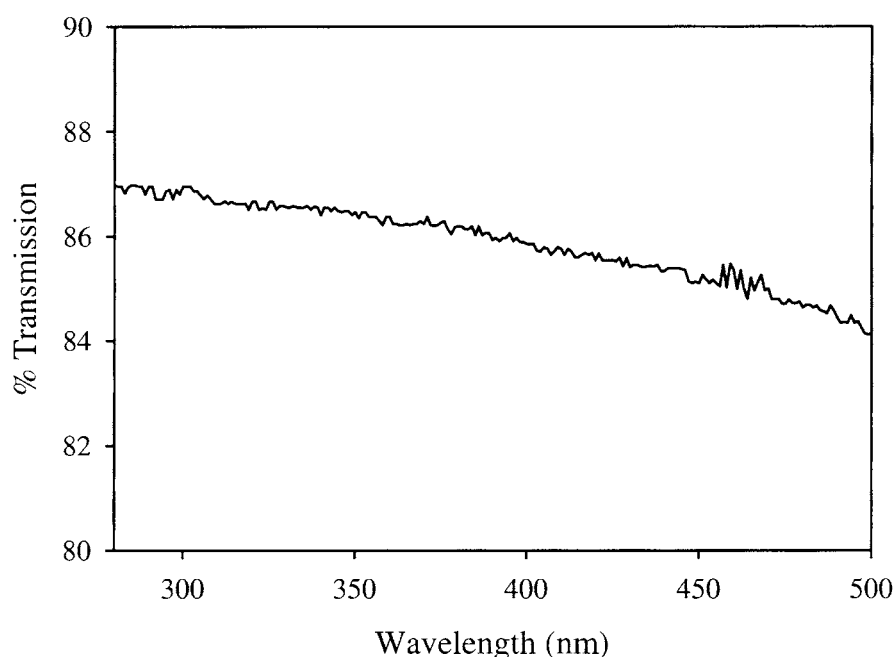


Figure 1. Transmission spectra of the plastic Ziploc bag used for the bog water exposure.

duplicate. Samples were set on the roof of Williams Hall at Lehigh University, Bethlehem, PA and exposed for 7 days. The weather was sunny and hazy during the exposure period.

After each exposure, subsamples were collected for optical analysis and DOC measurements. The remaining DOM was lyophilized and the dried material was stored in airtight, ashed (450 °C, 8 hours minimum) glass vials under desiccant. This dried material was used in the stable carbon isotope and solid-state ^{13}C -NMR analyses.

Seasonal sampling in 1998 and 1999

Bi-monthly samples were collected from the epilimnion and hypolimnion of both lakes from April through November in 1998 and from Lake Lacawac only in 1999. Samples were serially filtered in the manner described above. Aliquots were taken for absorbance and DOC measurements, and the remainder was frozen and lyophilized for measurement of $\delta^{13}\text{C}$.

Vertical profile of Lake Lacawac in 1999

In August and September of 1999, additional samples were collected to better characterize chemically the dissolved and particulate pools of organic carbon as they had diverged during summer stratification. Moreover, we wanted to determine the differences in the ^{13}C -NMR and $\delta^{13}\text{C}$ signals between the epilimnetic and hypolimnetic DOM toward the end of summer stratification after a full summer exposure of surface waters to solar UVR. Twenty liters of water were collected from 1 m and 10 m in August, filtered through ashed Whatman GF/F filters, and then concentrated with reverse osmosis and lyophilized for ^{13}C -NMR and stable isotope analysis of DOM. Mass balance calculations showed C recovery of 93–97%, which is similar to other reported values using reverse osmosis (Clair et al. 1991). We retained the Whatman GF/F filters for stable isotope analysis of particulate organic matter (POM).

In early September of 1999, we collected samples at 1 m intervals for analysis of $a_{d,320}$, pH, DIC, CH_4 , and sulfide. Temperature and dissolved oxygen were also measured at 1 m intervals with a YSI temperature/oxygen meter.

Analytical techniques

Dissolved absorbance (a_d) of samples was determined using a Shimadzu UV 160 U spectrophotometer in 1- or 10 cm quartz cuvetts blanked against air. A low carbon (UV-C, treated) DI water blank was used to subtract the absorbance of pure water plus the cuvet. DOC concentration was measured by high-temperature combustion with a Shimadzu TOC-5000 using methods outlined by Sharp et al. (1993).

The chemical structure of lyophilized samples was determined from solid state CP/MAS ^{13}C NMR spectra which were recorded on a Chemagnetics CMX-200 NMR spectrometer at a carbon resonant frequency of 50.3 MHz using a 7.5mm ceramic probe (zirconium pencil rotor). Acquisition parameters included a 30,000-Hz spectral window, 17.051-ms acquisition time, 2.0-ms contact time, 1.0-s pulse delay, and spinning rate of 5 kHz. Integrated peak areas representing intensities of functional groups were determined according to McKnight et al. (1997) and Thorn (1994) and are reported as the chemical shift (δ) in parts per million (ppm).

In CP/MAS ^{13}C NMR, for peak areas to accurately represent the number of nuclei resonating, the time constant for cross polarization must be significantly less than the time constant for proton spin lattice relaxation in the rotating frame, $T_{\text{CH}} \ll T_{1\rho}\text{H}$. Thus, several considerations are noteworthy in interpreting the solid state spectra. Without an analysis of the spin dynamics or a comparison with direct polarization experiments, the spectra presented in

this paper can only be interpreted semi-quantitatively. Peak areas are reported as percentages of total carbon for each spectrum. With the 2 msec contact time value used in these experiments, aromatic, carboxyl, and carbonyl carbons in the DOM samples may be underestimated quantitatively. Other factors affecting quantitation in the CP/MAS experiment, including the presence of paramagnetic metals, have been discussed in the review of Kinchesh et al. (1995).

Carbon stable isotope ratios of CO₂ gas liberated from lyophilized and particulate samples using a modified Dumas combustion were determined on a Finnigan MAT 252 IRMS (Boutton 1991). Values of carbon stable isotope ratios are reported in the standard per mil ‰ notation:

$$\delta^{13}\text{C}(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$. The precision of the mass spectrometer was 0.1‰ and we found no recoverable signal from the filter blank. Precision and accuracy of our measurements were determined relative to an internal laboratory standard (commercial freeze dried tea as a proxy for tannic acid; $\delta^{13}\text{C}$ of standard = $-26.86 \pm 0.05\text{‰}$).

Concentrations of DIC (and CH₄) from the vertical profile in September of 1999 were measured using a Shimadzu gas chromatograph (Stainton 1973). CH₄ was determined in the gas headspace created for DIC analysis. Sulfide was determined using a colorimetric method (Golterman 1971).

Results

Photochemical oxidation of bog water

Photochemical oxidation of bog water DOM resulted in losses of a_d and DOC, as well as changes in chemical structure and $\delta^{13}\text{C}$ (Figure 2). Dissolved absorbance at 320 nm ($a_{d,320}$) in light treatments decreased by approximately 30%, whereas the dark treatment showed no significant change from the initial ($p = 0.05$). We noticed a DOC decrease of 16% for the light treatment, which corresponded to a quantitative loss of 718 $\mu\text{M C}$ and suggests its photooxidation to DIC. No significant loss of DOC was observed for the dark treatments ($p = 0.05$).

¹³C-NMR spectra of bog water from the bog exposure complement the absorbance and DOC results, and reveal changes in the chemical composition of the bog DOM as a result of exposure to solar UV radiation (Figure 3). In comparing the spectra, it is assumed that the aliphatic carbons, corresponding to the peak from 0 to 60 ppm (AL1), are not photochemically degraded, and

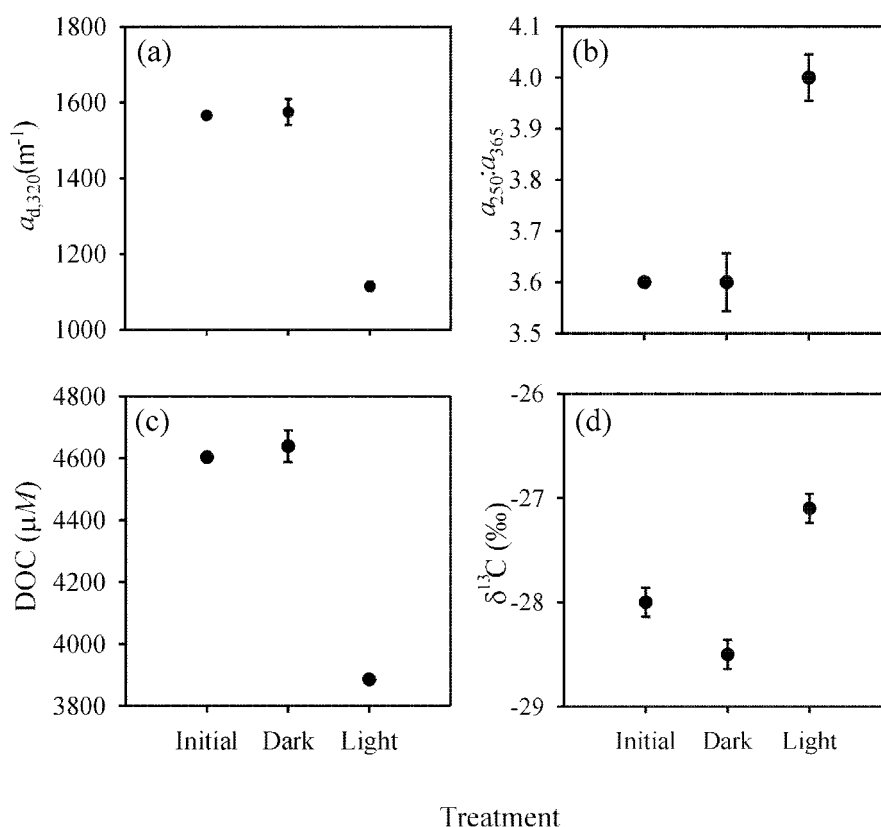


Figure 2. Results of bog water DOM exposure on (a) UV dissolved absorbance at 320 nm, (b) the optical index of molecular weight ($a_{250}:a_{365}$), (c) DOC concentration, and (d) $\delta^{13}C$ ratios. The loss of $a_{d,320}$ was greater than the loss of DOC, corresponding to an enrichment in ^{13}C of the light treatment by 0.9‰; error bars are 95% CI ($n = 3$).

that the absolute intensity of this peak does not change from one spectrum to the other. Therefore, we set the aliphatic peak intensities of each individual spectrum equal to the aliphatic peak of the initial and report peak areas as a percentage of total carbon signal (*see Methods*). The spectra of the bog DOM indicate losses of carbohydrate and secondary alcohol carbons (AL2, 60–90 ppm), anomeric carbons of carbohydrates (AL3, 90–110 ppm), protonated and substituted aromatic carbons (AR, 110–160 ppm), carboxyl, amide, and ester carbons (C1, 160–180 ppm), and ketone and quinone carbons (C2, 180–220 ppm). Peak area descriptions of the spectra are listed in Table 2.

These structural changes in the spectrum can therefore be interpreted as losses of carbon functionalities downfield from ~60 ppm (aliphatic peak) as a result of photochemical degradation. The assumption that the aliphatic

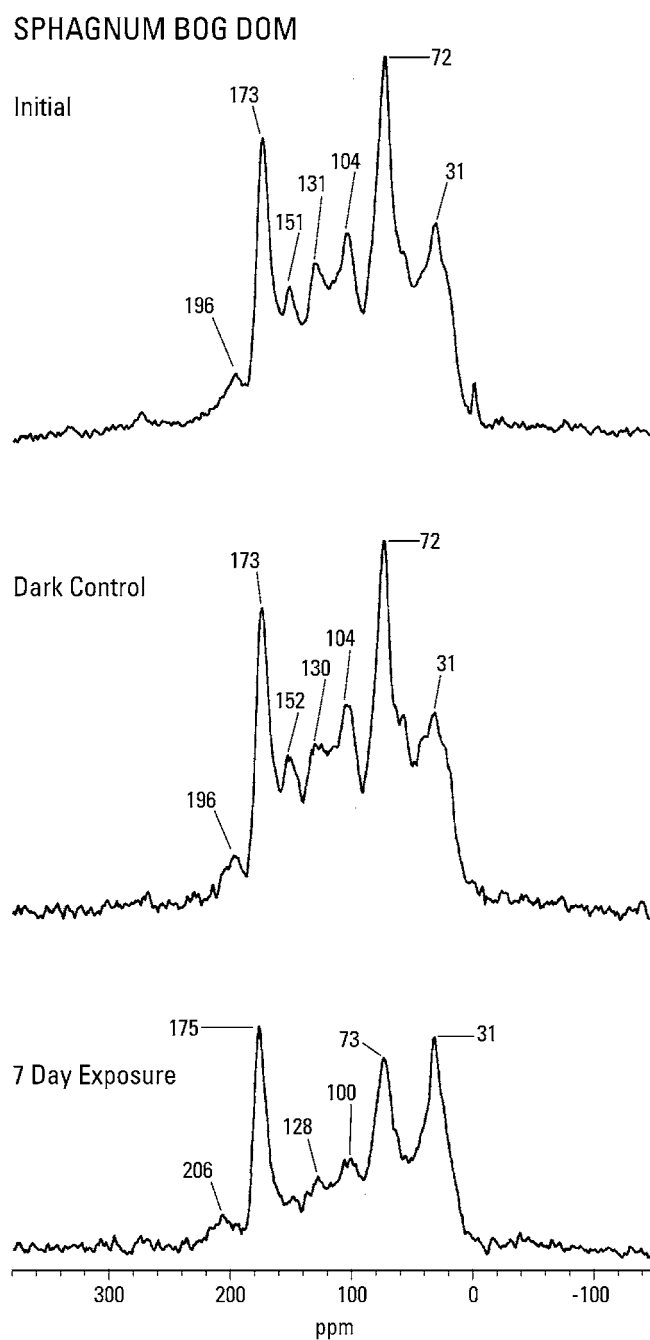


Figure 3. Solid state CP/MAS ^{13}C -NMR spectra of initial, dark control, and exposed DOM from *Sphagnum* bog water. The 7 day exposure (bottom) resembles the Nordic fulvic acid spectra reported by Kulovaara et al. (1996). See Table 2 for a description of peak assignments.

Table 2. Peak areas as a percent of total carbon for solid state CP/MAS ^{13}C -NMR spectra of Lacawac *Sphagnum* bog DOM samples (chemical shift as ppm, in parentheses). Peak assignments are after Thorn (1994)

DOM Sample	C2 (220-180)	C1 (180-160)	AR (160-110)	AL3 (110-90)	AL2 (90-60)	AL1 (60-0)
Initial	5.6	12.9	22.1	10.4	23.4	26.9
Dark	5.4	13.7	23.6	11.1	22.7	24.7
Light	6.1	15.1	15.1	8.7	21.7	34.7
Light*	4.7	11.7	11.7	6.7	16.8	26.9

Key:

C2: second carbonyl peak (ketones & quinones).

C1: first carbonyl peak (carboxyls, amide, and esters).

AR: aromatic peak and olefinic carbons.

AL3: anomeric carbons in carbohydrates and acetal carbons.

AL2: second aliphatic peak (carbohydrates, secondary alcohols, and secondary ethers).

AL1: first aliphatic peak, represents carbons bonded to other carbons, sulfur, and nitrogen.

*Peak area of aliphatic carbons (ALI, 0–60 ppm) set equal to peak area of aliphatic carbons for initial sample, assuming no photochemical degradation of aliphatic C.

peak does not change is based upon liquid phase ^{13}C -NMR analyses of humic, fulvic, and hydrophilic acids isolated from the Okefenokee Swamp and Hellerudmyra Pond (the IHSS Nordic fulvic acid standard) that were subjected to laboratory UV-irradiation experiments (Figure 4; Thorn and Younger, unpublished data). The IHSS Nordic fulvic acid was subjected to laboratory UV irradiation experiments using a 45 watt medium pressure quartz Hg vapor lamp. Continuous decoupled liquid phase ^{13}C -NMR spectra were recorded at 75% and 65% of initial $a_{d,465}$. The spectra revealed several chemical changes also observed in the whole *Sphagnum* bog DOM. While the aliphatic carbons (0–60 ppm) in the Nordic fulvic acid are not degraded, losses in the carbohydrate and secondary alcohol, aromatic, carboxyl, and ketone and quinone carbons occur upon exposure to UV radiation, similar to what we observed for the *Sphagnum* bog DOM.

Changes in the bog water's chemical structure suggested by the NMR spectra are also complemented by an optical index of molecular weight (the ratio of $a_{d,250}:a_{d,365}$; Figure 2). This index has been used to suggest changes in the relative size of DOM that might occur from photochemical degradation, where an increase in the ratio is indicative of a decrease in molecular weight (Morris & Hargreaves 1997; DeHaan & De Boer 1987; DeHaan 1993; Strome & Miller 1978). The index increased by 10%, suggesting a decrease in the molecular weight of the bog water DOM during the exposure experiment.

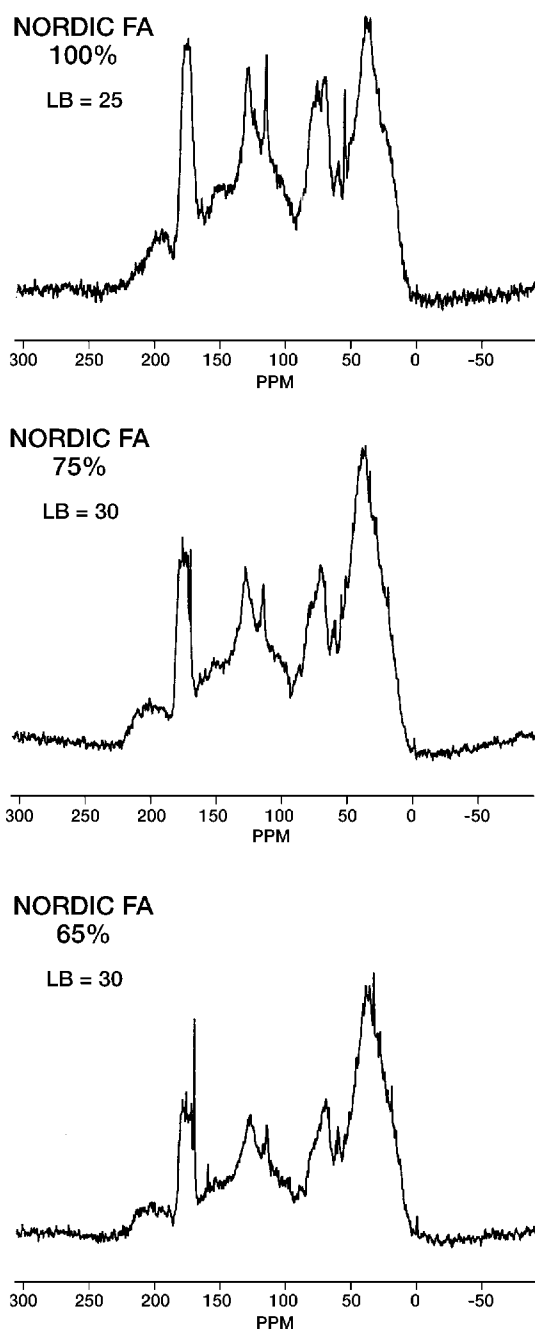


Figure 4. Liquid state ^{13}C -NMR spectra of Nordic fulvic acid exposed to artificial UV light. Samples were recorded at 100, 75, and 65% of initial dissolved absorbance at 465 nm. Notice the decrease in aromatic (140 ppm) and carboxyl (185 ppm) signal intensity with exposure. LB = line broadening. Acquisition parameters described in Thorn et al. 1991: 45 degree pulse angle, 0.2 second acquisition time, 1.0 second pulse delay, continuous decoupling.

Carbon isotope fractionation

Figure 2 also shows that photooxidation of bog DOM led to a 1.2‰ enrichment in $\delta^{13}\text{C}$ relative to dark controls that corresponds with decreases in $a_{d,320}$ nm and [DOC]. The initial $\delta^{13}\text{C}$ value of bog DOM (−28‰) and dark controls were similar to other reported $\delta^{13}\text{C}$ values of DOM from Jones et al. (1998) for Loch Ness, Schiff et al. (1997) for the Harp Lake watershed, and Hedges et al. (1994) and Benner et al. (1987) for riverine DOM. Moreover, the $\delta^{13}\text{C}$ we measured for the bog water DOM is similar to IHSS standards for aquatic humic and fulvic acids (range = −28.2 to −27.6‰). We note no significant difference between the initial and dark control in the $a_{d,320}$, DOC, or $\delta^{13}\text{C}$ ($p > 0.05$). The increase in $\delta^{13}\text{C}$ of the light treatment was significant ($p < 0.001$) compared to the initial and dark treatment appears to result solely from photochemical oxidation of DOM.

Seasonal changes in DOM

In the epilimnion of both Lake Lacawac and Lake Giles, optical properties, DOC, and stable carbon isotope ratios of DOM shifted in accordance with the pattern observed during photooxidation of bog water. Epilimnetic water samples (1 m depth) showed a decrease in $a_{d,320}$ consistent with increased UV transparency as a result of photooxidation of DOM during the summer of 1998 and 1999 (Figure 5), a result previously reported for these two lakes (Morris & Hargreaves 1997). As thermal stratification progressed (late April to October), the UV absorbance of waters in the epilimnion decreased by 62% and 48% in Lake Lacawac (1998 and 1999, respectively) and by 47% in Lake Giles (1998), presumably as a result of photobleaching.

We observed changes in the optical and chemical properties of the hypolimnion of both lakes similar to those observed by Morris and Hargreaves (1997; Figure 5). In the hypolimnion of Lake Lacawac, $a_{d,320}$ increased by 72% in 1998 and by 43% in 1999, whereas in Lake Giles $a_{d,320}$ of the hypolimnion increased by 40% in 1998. In both lakes, $a_{d,320}$ increased to a maximum value in late summer and then decreased. The increase in UV absorbance in the hypolimnion of both lakes suggests additional or new sources of chromophoric material.

We observed little seasonal variation in the DOC concentration of either Lacawac or Giles (Figure 6). The concentration of DOC at 1 m in Lake Lacawac decreased by 125 μM from May to late October of 1998. In contrast, the DOC concentration at 10 m increased gradually by 83 μM over the same period. We observed no real change in the DOC concentration of Lake Lacawac in 1999. In Lake Giles, the DOC concentration decreased at 3 m

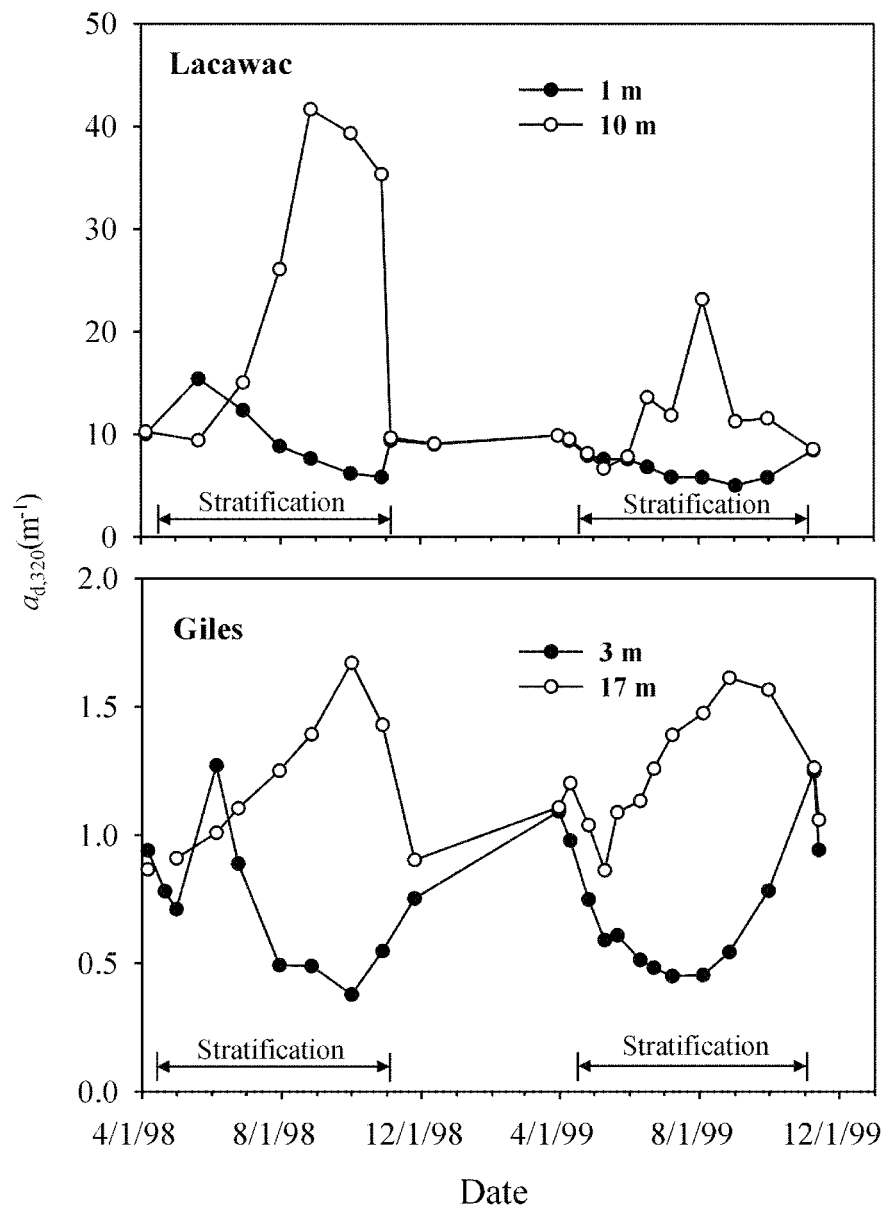


Figure 5. Monthly changes in UV absorbance at 320 nm in the surface and bottom waters of Lake Lacawac (top panel) and Lake Giles (bottom panel) in 1998 and 1999. Approximate periods of summer stratification are indicated with line arrows.

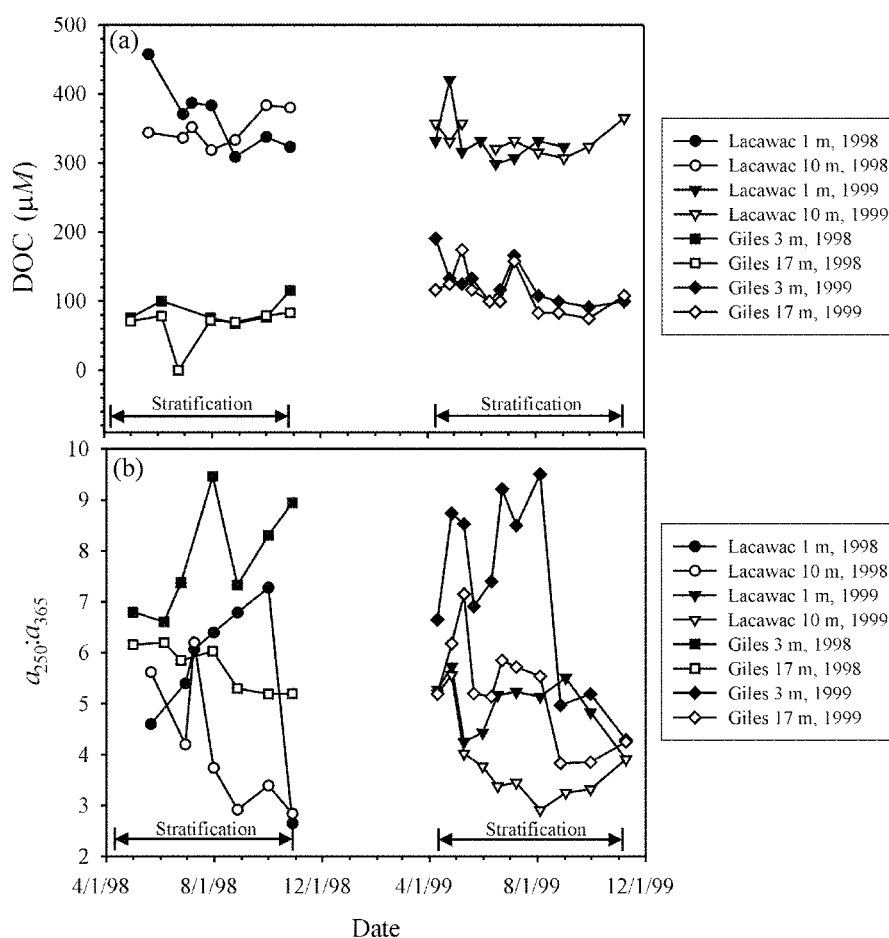


Figure 6. Seasonal patterns of (a) DOC concentration and (b) optical index of molecular weight ($a_{250}:a_{365}$) in Lacawac and Giles. Morris and Hargreaves (1997) reported similar values for these lakes.

by $42 \mu M$ from May to late October of 1998 and by $100 \mu M$ in 1999. We observed no change in the DOC at 17 m in Lake Giles during 1998 or 1999.

However, the $a_{250}:a_{365}$ ratio for the lakes changed in a manner similar to that observed experimentally with the bog water (Figure 6). At 1 m in Lake Lacawac, $a_{250}:a_{365}$ increased by 58% over the course of the summer and into early October then decreased by the end of fall turnover. In 1999 $a_{250}:a_{365}$ at 1 m in Lacawac increased by 30% through mid-September. The index increased at 3 m in Lake Giles in 1998 by 74% to late October where it remained much higher than the value at 17 m, which decreased by 16%.

Carbon stable isotope values for the surface water DOM in Lake Lacawac in both 1998 and 1999 increased during summer stratification (Figure 7). From May to late October 1998, $\delta^{13}\text{C}$ at 1 m increased only slightly by 0.3‰. In 1999, however, $\delta^{13}\text{C}$ at 1 m increased by 2‰ over the same period. In contrast, the $\delta^{13}\text{C}$ of DOM at 10 m increased by 4.5‰ in 1998 and by 3‰ in 1999. In Lake Giles, the surface water $\delta^{13}\text{C}$ of DOM increased by 2‰ from May to late July of 1998, and then decreased by the same amount to its early spring value. At 17 m in Lake Giles, $\delta^{13}\text{C}$ varied only slightly ($\sim 0.2\text{‰}$) but did exhibit two depletions of $\sim 1.5\text{‰}$ in late June and late October of 1998 relative to the early spring. We note that the increases in surface water $\delta^{13}\text{C}$ of both Lake Lacawac and Lake Giles correspond to periods of maximum exposure to solar UVR and that they occur during the period when the surface waters are most transparent.

Late summer DOM dynamics in Lake Lacawac

In order to better understand the carbon dynamics in Lake Lacawac that might explain the stable isotope results, we measured solid state ^{13}C -NMR spectra of DOM in addition to the carbon stable isotope values of DOM and POM in August of 1999 (Table 3). Solid state spectra of these samples are shown in Figure 8. The epilimnetic sample had a lower aromatic content (relative to aliphatic C) than the hypolimnetic sample. ^{13}C -NMR data from the epilimnion on 10 April 1999 (when the lake was mixed) is included for comparison, thus the loss in aromatic C (relative to aliphatic C) in surface water DOM during the summer is apparent in the August sample. In addition, the hypolimnetic spectrum shows a gain in aromatic C relative to both the epilimnetic sample and the 1 m sample from 10 April. $\delta^{13}\text{C}$ values for DOM are enriched ($\sim 2\text{‰}$ and 3‰ , respectively) relative to terrestrial carbon measured from the bog water and 1.5–2.5‰ relative to the 10 April value. $\delta^{13}\text{C}$ ratios for the POM in the lake were isotopically depleted in ^{13}C relative to terrestrial carbon.

Vertical chemical and optical profiles developed in Lacawac during the summer of 1999 (Figure 9). The epilimnion extended for 5 m and remained well oxygenated. Below 5 m, the lake became anaerobic and below 7 m we observed accumulation of DIC, CH_4 , and sulfide. These results demonstrate that the absence of oxygen and UVR in the hypolimnion of Lake Lacawac has a marked effect on the lake's carbon cycle. Figure 9 also shows the increased UV-B absorbance of the anaerobic waters compared to the lower UV absorbance of the epilimnion. The $a_{250}:a_{365}$ ratio suggests that the molecular weight of material in the anaerobic hypolimnion is greater than that in the epilimnion.

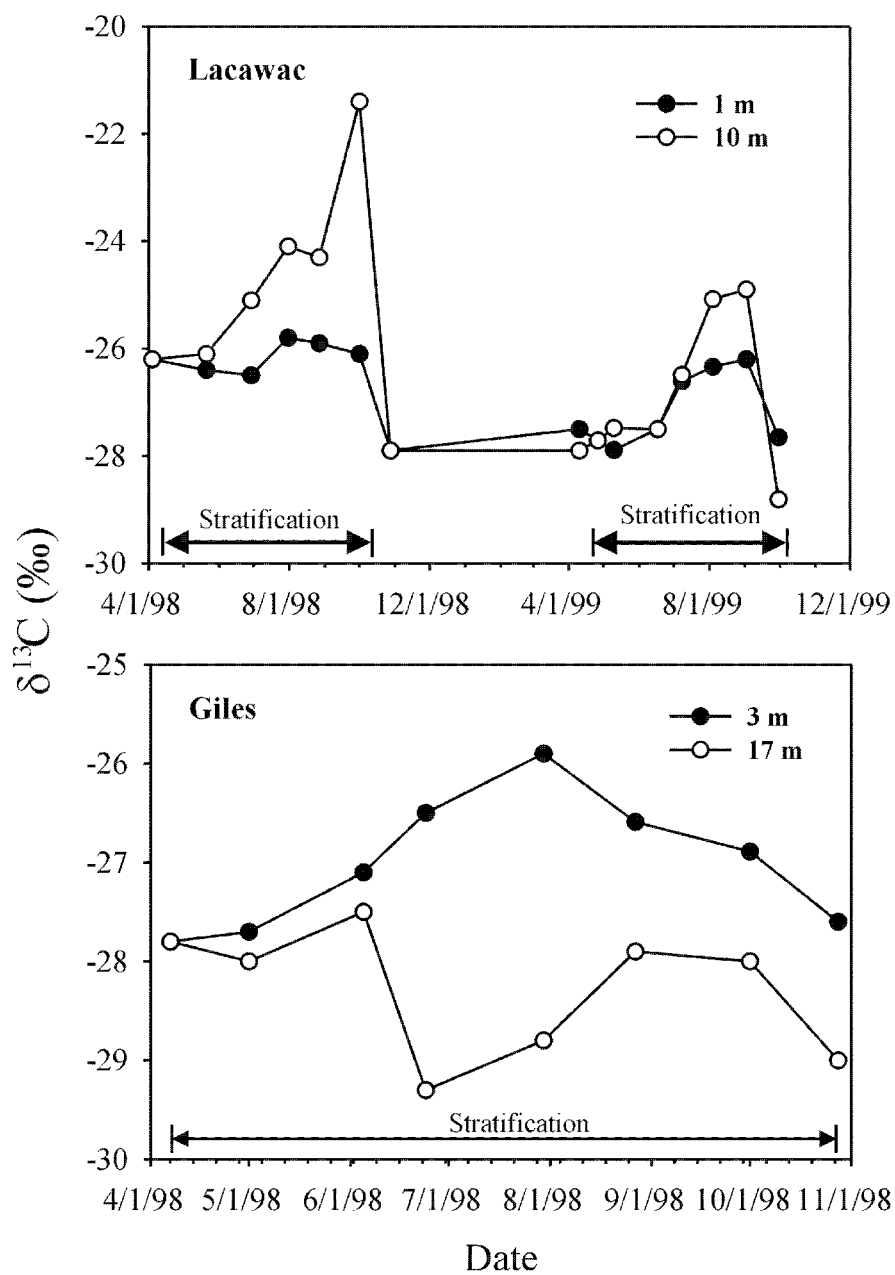


Figure 7. Monthly patterns in $\delta^{13}\text{C}$ of DOM collected from same depths and at same times as samples in Figures 5 and 6. Increases in $\delta^{13}\text{C}$ for the epilimnion waters of both lakes occurred very closely to decreases in UV absorbance.

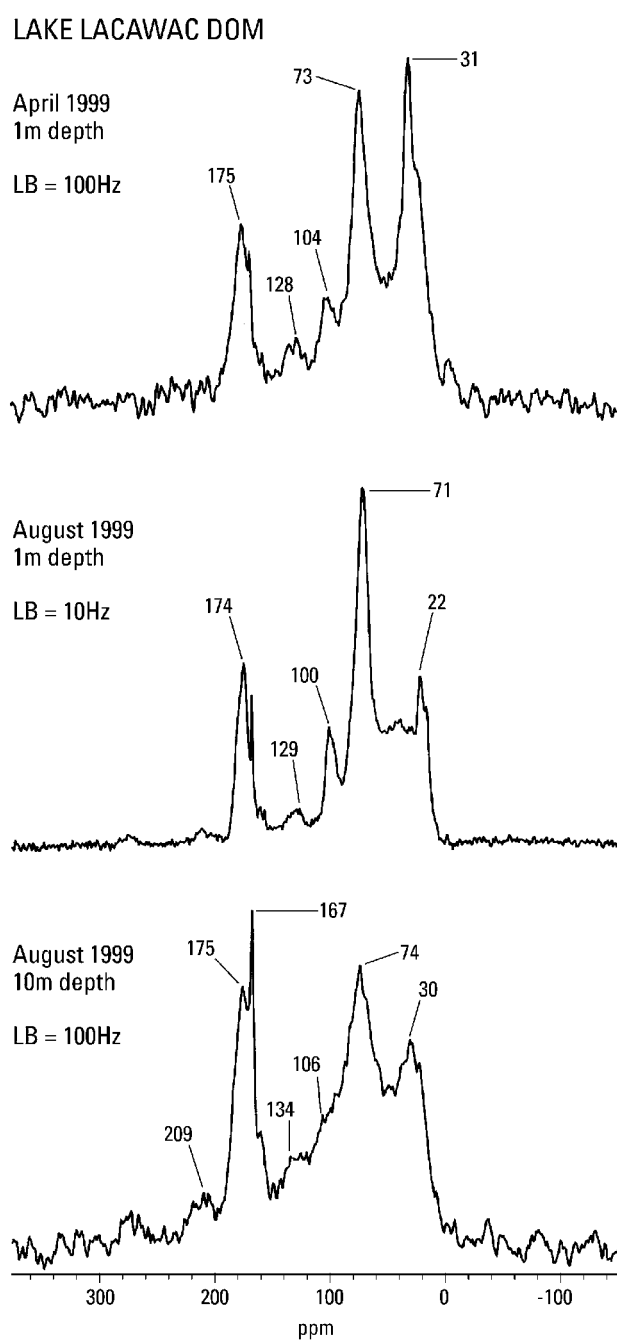


Figure 8. Solid state CP/MAS ^{13}C -NMR spectra for Lake Lacawac DOM during 1999. The April 1999 samples were taken during spring mixing when optical and chemical conditions were homogeneous. The August 1999 samples were collected at the same time as the other optical and chemical parameters reported. LB = line broadening.

Table 3. Peak areas (ppm) as percent of total carbon for CP/MAS ^{13}C -NMR spectra of Lake Lacawac samples from 1999. See Table 2 for peak assignments

DOM Sample	C2 (220-180)	C1 (180-160)	AR (160-110)	AL3 (110-90)	AL2 (90-60)	AL1 (60-0)
4-Apr-99	5.7	10.9	9.9	8.7	26.3	38.4
4-Aug-99 (1 m)	4.0	12.6	6.8	9.0	34.8	32.9
4-Aug-99 (10 m)	7.8	16.4	12.9	10.7	25.1	27.1

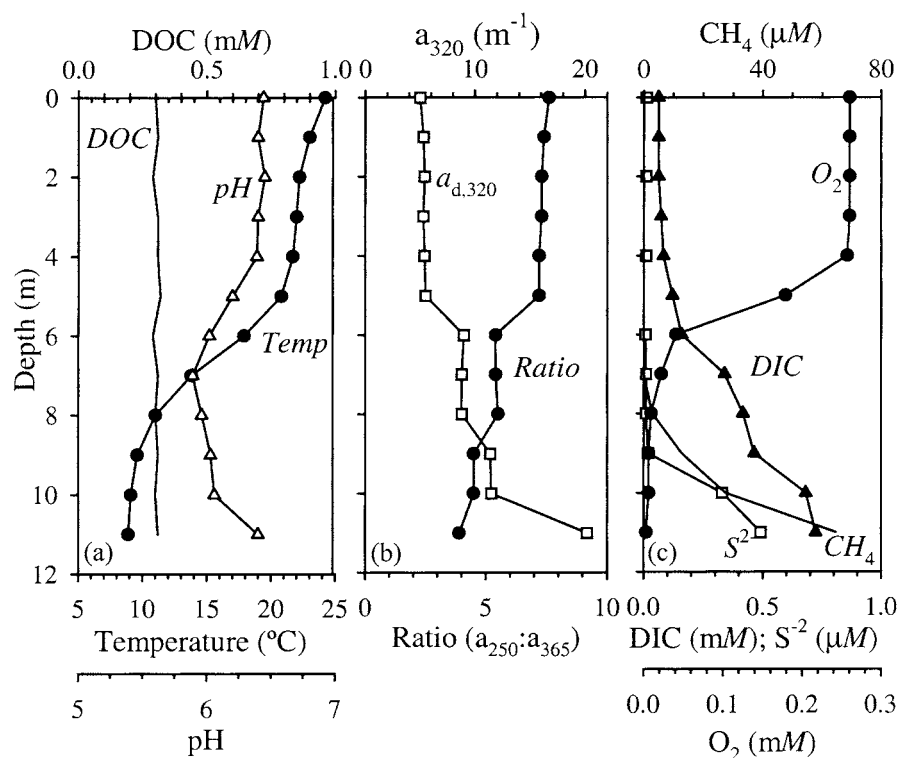


Figure 9. Chemical and optical profiles of Lake Lacawac on 2 September 1999 showing differences between the epilimnion and hypolimnion. (a) DOC concentration, pH, and temperature, (b) UV dissolved absorbance at 320 nm and the optical index of molecular weight, (c) methane, sulfide, oxygen, and total DIC.

Discussion

Solar photochemical oxidation of DOM was apparent in both our experimental exposure of humic-rich bog water and in our measurements of surface waters from both Lake Lacawac and Lake Giles. The optical change of the bog DOM (29% loss of $a_{d,320}$) was nearly twice that of the loss of DOC (16%), and several times greater than the enrichment of ^{13}C (4%). However, the 36% loss of aromatic C was similar to the loss of $a_{d,320}$. We note that the losses of dissolved absorbance and DOC we observed in the bog DOM experiments were similar in magnitude to other experimental exposures of freshwater DOM (Opsahl & Benner 1998; Morris & Hargreaves 1997; Hongve 1994). Moreover, Molot and Dillon (1997) have calculated a mean color loss coefficient (in estimated Hazen units) from several lakes and streams in central Ontario, Canada, that was approximately 2.5 times greater than the mean DOC loss coefficient. The strong UV absorbance of the bog DOM and high DOC concentration coupled with a relatively short exposure period (7 day), may therefore have resulted in photooxidation of only a small portion of the total photoreactive material.

The 29% loss in $a_{d,320}$ we observed for the bog DOM suggests that photobleaching is not entirely due to photochemical oxidation of the entire chromophore to DIC, though the loss of carboxylic acid carbons as a result of photochemical decarboxylation reactions also suggests photooxidation of DOC to DIC. We observed a loss of $716\ \mu\text{M}$ DOC in the bog water exposure, and assuming this loss of C was due to its photochemical oxidation to CO_2 , we used the 1:1 molar relationship between photochemical consumption of DOC and O_2 reported by Amon and Benner (1996) to estimate a photochemical consumption of $716\ \mu\text{M}$ O_2 in the bog DOM exposure. This amount of oxygen would have depleted our air-equilibrated bog DOM solution (estimated at $250\ \mu\text{M}$ O_2) in about 2.5 days. Therefore, we suggest that DOC photooxidation to DIC in our bog DOM exposure was inhibited by oxygen depletion, even given the gas permeability of the polyethylene bags.

Because $a_{250}:a_{365}$ did decrease by 19%, and the aromatic C decreased by 36%, it may be that high molecular weight (HMW) DOM was photodegraded to LMW DOM that was less UV absorptive. Alternatively, it is possible that chromophores responsible for loss of UV absorbance are not the same as those responsible for oxidation of DOC to DIC. Kieber et al. (1990) studied photobleaching of marine CDOM and photoproduction of LMW carbonyl compounds. They found that abiotic oxygen consumption did not cause the observed photobleaching, or carbonyl production in their samples. Because degradation of humic structures would likely liberate LMW compounds like carbonyls, our NMR evidence for loss of aromaticity is reasonable, even

given the lesser amount of DOM photooxidation measured as DOC loss and inferred from our stable isotope data. In addition, Kulovaara et al. (1996) obtained a similar loss of aromaticity in artificial UV irradiations of Nordic humic and fulvic acids. Thus, the changes in bulk DOM chemistry indicated by ^{13}C -NMR and stable isotopes may only account for a portion of the change in optical properties.

Evidence for seasonal photobleaching of DOM in the lakes is readily apparent from measurements of UV absorbance (Figure 5), and our chemical data suggest contemporaneous photodegradation. For both lakes we observed increases in $a_{250}:a_{365}$ and decreases in DOC concentration that suggest photodegradation of HMW DOM to LMW DOM and DIC. Moreover, we observed an increase in $\delta^{13}\text{C}$ of the surface water DOM in both lakes that develop during progressive exposure to high summer UVR levels.

The most likely mechanism that caused the change in $\delta^{13}\text{C}$ we observed in both the bog DOM exposures and in the seasonal sampling of lakes would be the degradation of HMW DOM to LMW compounds and their potential oxidation to DIC or CO. Photochemical decarboxylation is a likely process and has been reported for Suwannee humic and fulvic acids (Miles & Brezonik 1981), soil fulvic acids (Chen 1978), and for aldehydes and ketones (Norris Type I and II reactions, Calvert & Pitts 1969). Our ^{13}C -NMR spectra for the bog exposure showed depletion of aromatic C, similar to a study of synthetic lignin photooxidation by Vähätalo et al. (1999). The spectrum of the exposed bog DOM also shows losses of carboxyl carbons and ketone carbons.

The observed differences in the bog DOM $\delta^{13}\text{C}$ that we attribute to photooxidation may result from an isotope effect produced by removal of some component of the bulk DOM. Lignin-derived compounds have been identified as both a major component of terrestrially derived DOM and as photoreactive moieties (Thurman 1985; Opsahl & Benner 1998; Castellan et al. 1987; Ertel 1990). Therefore, these compounds are likely candidates for photooxidation in the bog water samples. Castellan et al. (1987) showed that in the laboratory α C-O bond cleavage is the primary photochemical step for degradation of lignin by lamp UVR. Moreover, Kieber et al. (1990) calculated that C-C bonds that can bind component groups of lignin together are susceptible to direct photolysis by UV-B radiation. While our data do not provide insight into such reaction pathways, similar mechanisms most likely occur in the bog DOM. By mass balance, the isotopic composition of the photooxidized compounds like DIC must be light in order to shift $\delta^{13}\text{C}$ to heavier values, as we observed in the epilimnion of Lacawac and Giles.

Depletion of lignin-derived moieties provides a convincing mechanism for the changes in $\delta^{13}\text{C}$ we observed in the lakes, even though the aromatic signal we observed in the ^{13}C -NMR spectra were lower than in the bog samples.

^{13}C -NMR spectra clearly indicate loss of aromatic C in both the bog water exposure and the Lake Lacawac surface samples from early April to early August. Again, assuming the aliphatic carbon peak at 0–60 ppm in the bog spectra remains constant, loss of carboxylic acid carbons at 160–180 ppm is still consistent with photooxidation of LMW C to CO_2 and loss of low molecular weight carboxylic acids. Loss of aromaticity is also supported by the increase in the $a_{250}:a_{365}$ ratio observed in the surface waters of both lakes. The loss of DOC we observed in the lakes may be due to the mineralization of LMW photoproducts (e.g., aldehydes) to DIC (Miller & Zepp 1995; Kieber et al. 1990). The change in the $\delta^{13}\text{C}$ of the remaining DOM would thus be due to depletion of the aforementioned lignin-derived moieties, or to depletion of other aromatic compounds originating from plants, such as tannins and flavonoids. Benner et al. (1987) measured the stable carbon isotope ratio of lignin, cellulose, and hemicellulose from tissues of several C_3 plant species. The bulk signatures of their oak and maple samples were measured between -27.5 and -27.2‰ , respectively, which is consistent with our bog and lake water DOM measurements (-28‰), and they found that lignin was depleted in ^{13}C by 4–7‰ relative to whole plant tissue. Schulten and Gleixner (1999) and Gleixner and Schmidt (1998) have reported similar values of lignin for a variety of lake DOM and Goñi and Eglinton (1996) have reported lignin $\delta^{13}\text{C}$ values that were lighter than the bulk values for vascular plant materials. Schmidt and Gleixner (1998) point out that kinetic isotope effects during catabolic synthesis of secondary plant compounds produce isotopic discriminations in plant products resulting in isotopically-lighter (i.e., containing more of the ^{12}C isotope) compounds such as lignin. Removal of aromatics such as lignin-derived moieties by photooxidation may therefore shift $\delta^{13}\text{C}$ of bulk DOM to heavier isotopic values. Since lignin is composed of many aromatic compounds, this result would be consistent with our ^{13}C -NMR results, which demonstrated a relative loss of aromatic carbon.

A simple mass balance calculation based on the observed 18% loss of DOC in the bog exposures shows how lignin removal shifts $\delta^{13}\text{C}$ based on our experimental data (Table 2):

$$\delta^{13}\text{C}_{\text{init}} = 0.16\delta^{13}\text{C}_{\text{min}} + 0.84\delta^{13}\text{C}_{\text{rem}} \quad (1)$$

where the subscripts $_{\text{init}}$, $_{\text{min}}$, and $_{\text{rem}}$ refer to the stable carbon isotope value of the initial bog water, the bog DOM mineralized (lost), and the bog DOM remaining and 0.18 and 0.82 are the proportions. Rearranging and substituting measured values from Table 2 and Figure 4:

$$\delta^{13}\text{C}_{\text{min}} = \frac{-28\text{‰} - (0.84^* - 27.1\text{‰})}{0.16} \quad (2)$$

The solution yields a $\delta^{13}\text{C}_{\text{min}}$ of -33‰ , a reasonable value for DIC produced from isotopically light substrates like lignin.

The carbon stable isotope data collected for Lake Lacawac in August of 1999 provides some insight into the validity of photooxidation driving the seasonal patterns in $\delta^{13}\text{C}$. Lakes are not static systems and DOM is constantly being added from the watershed and from primary and secondary production within the lake. We might expect to see a change in the stable isotope ratio of the DOM to increasingly reflect autochthonous production during the relatively dry summers of 1998 and 1999 in northeastern Pennsylvania. One way to examine this is to measure the $\delta^{13}\text{C}$ of POM, which is likely dominated by algal production. In Lake Lacawac, $\delta^{13}\text{C}$ of POM ranged between -30‰ and -33‰ ; values previously reported for freshwater algae (Jones et al. 1998, 1999; France 1996; Bunn & Boon 1993; Fry & Sherr 1984). $\delta^{13}\text{C}$ of the DOM was near -26‰ for the epilimnion and -25‰ for the hypolimnion. While the isotopic shift implies photooxidation in the surface waters, the precursor of the DOM that was photooxidized is less clear. The strong dissolved UV absorbance of Lake Lacawac, however, suggests that this material was terrestrial in origin. Thus, it appears that the DOM in Lake Lacawac is dominated by allochthonous DOM, even during periods of restricted watershed input (e.g., dry summers), and uncoupled from $\delta^{13}\text{C}$ of the POM.

While this rationale may explain the $\delta^{13}\text{C}$ patterns in the epilimnion, it does not explain the $\delta^{13}\text{C}$ patterns in the hypolimnion. In August of both 1998 and 1999, we observed $\delta^{13}\text{C}$ of hypolimnion DOM to be isotopically heavier than the terrestrial signal. In August of 1999, the hypolimnion $a_{250}:a_{365}$ ratio was much lower than the epilimnion (2.9 vs. 5.1) and lower than the value in April (5.3) when the lake was mixed, suggesting the presence of HMW DOM. Furthermore, the DOC concentration remained relatively constant throughout the water column, around $305\text{ }\mu\text{M}$. These data suggest that DOM is being turned over in the hypolimnion, with possible microbial production of humic like substances (cf. Steinberg & Munster 1985).

The chemical data gained from ^{13}C -NMR spectra provide independent evidence to corroborate our stable isotope information (Table 3). Both the exposed bog DOM and Lacawac epilimnion spectra are lower in abundance of aromatic C than the initial bog DOM and Lacawac hypolimnion spectra. Moreover, the Lacawac hypolimnion DOM spectrum also shows an increase in aromatic C by 3% relative to the 10 April DOM spectrum. Because the hypolimnion DOM spectrum was markedly different from the initial bog DOM spectrum, in that it had 30% less aromatic C and about 50% more carboxyl C, we suggest that the hypolimnion DOM is not, therefore, fresh allochthonous input from the watershed. The increase in absorbance

we observed in the hypolimnion of Lake Lacawac during 1998 and 1999 most likely results from autochthonous production of HMW DOM, though we cannot rule out other sources of dissolved absorbance (e.g., chelated iron, Miles & Brezonik 1981).

The anaerobic conditions we observed in the bottom waters of Lacawac in September of 1999 (absence of O_2 , presence of H_2S and CH_4) suggest that many different microbial physiologies may be present in the hypolimnion that could both: (1) metabolize terrestrial DOM introduced during the spring and 'trapped' during summer stratification, and (2) produce isotopically heavy DOM (e.g., acetate reduction during methanogenesis, Herczeg 1988; Gelwicks et al. 1994). Waldron et al. (1998) have recently reported $\Delta^{13}C_{\text{substrate-methane}}$ for bacterial acetate fermentation methanogenesis and hypothesized a 5–6‰ enrichment of the substrate. The magnitude of this enrichment is very near what we have observed in the anaerobic hypolimnion of Lake Lacawac during the summer (3–4‰). Moreover, abundant dissolved inorganic N and P has been measured in the anaerobic hypolimnion of Lacawac during late summer (Moeller unpublished data) that might fuel anaerobic metabolism of HMW terrestrial DOM over the summer, thus removing lignin similarly to photodegradation. Sinking and senescence of the lake's algae could supply LMW DOM that may be metabolized by anaerobic microbes, that in turn produce HMW, UV-absorbing DOM (Tranvik 1993). Brophy and Carlson (1989) demonstrated that glucose added to seawater was transformed to higher molecular weight material when incubated with naturally occurring microbial populations. However, the formation of lignin-like structures, and their photo- and biolability, from such reactions is unknown for anaerobic conditions. Harvey and Boran (1985) and Kieber et al. (1997) were able to demonstrate fulvic acid production from lipid precursors in marine systems, but their experiments were performed in air with available sunlight; clearly, the Lacawac hypolimnion does not experience these conditions during summer stratification. Further analyses and experiments are planned to investigate this possibility.

Both humic Lake Lacawac and clear Lake Giles experienced enrichments in epilimnial $\delta^{13}C$ during summer stratification and then apparently returned to a homogeneous value upon fall lake mixing. This pattern is consistent with dissolved absorbance data collected simultaneously and suggests that photooxidation modified the DOM in surface waters. Optical indices of molecular weight corroborate this evidence and suggest the formation of LMW DOM from HMW DOM. The contrast we observed between the two lakes is distinct and occurs in the hypolimnia of these lakes. The increase in the $\delta^{13}C$ of DOM at 10 m in Lake Lacawac is attributed to anaerobic microbial metabolism of terrestrial DOM and 'trapping' of photooxidized

DOM during stratification. In Lake Giles, the decrease in $\delta^{13}\text{C}$ toward late June may be due to benthic moss growth and senescence. The $a_{250}:a_{365}$ index suggests HMW DOM in the hypolimnion of Lake Lacawac accumulates during summer stratification; the pattern is less pronounced in Lake Giles. Both lakes experience an increase in the UV absorbance of hypolimnial DOM during the summer.

This study has determined for the first time that stable isotopes and NMR can be used to elucidate photochemical and microbial mechanisms influencing the seasonal variation in the UV absorbance of DOM in temperate lakes. Furthermore, we have presented the first stable isotope data suggesting *in situ* photooxidation of DOM in lake ecosystems. This method has advantages over other methods (e.g., DIC production or oxygen consumption) in that it is a more direct measure of the change in DOM chemistry caused by UVR exposure. For example, Graneli et al. (1996) and Miller and Zepp (1995) have shown that DIC (as CO_2) is produced in sterilized humic water samples exposed to natural sunlight thereby inferring the photomineralization of DOC. However, other sources of DIC (such as bacterial respiration) complicate determining its source in natural waters. Compound-specific stable isotope analysis, which might also elucidate individual chromophores and specific bacterial physiologies that generate DOM, may yield more insight into the DOM sources and transformation and reaction pathways. We note that photooxidation should be a consideration in studies of stable isotope values of DOM in aquatic ecosystems. The 0.3–2‰ enrichment we observed in the epilimnion of our study lakes is on the same order of magnitude as observed for organic matter between trophic levels in many lake food web studies ($\sim 1\text{‰}$; cf. Fry & Sherr 1984). Also, photooxidation may play a factor in determining the stable isotope value of DOM in coastal ecosystems where terrestrial C mixes with marine C. We caution that the interpretation of carbon stable isotope ratios in aquatic ecosystems that receive appreciable UVR should include some consideration of the isotopic effects of photooxidation.

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