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Biological and photochemical transformations of amino acids and lignin phenols in riverine dissolved organic matter

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Abstract The photo- and bio-degradation of dissolved organic matter (DOM) in water from the Broad River were investigated in laboratory experiments using a solar simulator to control the intensity and exposure of samples to irradiation. The water samples included a natural assemblage of microorganisms, and the daily exposure of samples to irradiation was varied to distinguish the relative contributions of photochemical and biological degradation. Concentrations of dissolved organic carbon (DOC) and specific components of DOM, including chromophoric DOM (CDOM), lignin phenols and amino acids, were monitored to investigate the reactivity and predominant pathway of degradation of these DOM components. Biodegradation was primarily responsible for the overall remineralization of DOC and losses of the amino acid component of DOM, whereas photodegradation was primarily responsible for losses of the chromophoric and lignin phenol components of DOM. The rates of photodegradation of lignin phenols were strongly influenced by the presence of methoxy groups on the aryl ring. Syringyl (S) phenols have two methoxy substitutions, vanillyl (V) phenols have one methoxy substitution, and *p*-hydroxy (P) phenols are not substituted with methoxy groups. Photochemical decay constants were highest for S phenols, lowest for P phenols and followed a consistent pattern (S > V > P) in the experiments. The carbon-normalized yields of amino acids and lignin phenols were found to be useful molecular indicators of the highly reactive (i.e. labile) components of biodegradable and photodegradable DOM, respectively.

Keywords Biodegradation · Photodegradation · DOM · Amino acids · Lignin phenols

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Introduction

The enzymatic prowess of microorganisms has long been recognized as a key factor in the decomposition of detrital organic matter (Alexander 1965). Enzymes are the gatekeepers of biodegradation as well as biosynthesis, and it is generally held that microorganisms have the potential to degrade any biosynthesized organic molecule. However, it is also well recognized that the rates of biodegradation of biomolecules are highly variable depending on inherent biochemical properties and environmental conditions. Lignins are very complex macromolecules comprised of phenylpropane subunits linked by

a variety of carbon–carbon and ether bonds, and they are resistant to biodegradation relative to other macromolecules, such as polysaccharides and proteins (Sarkanen and Ludwig 1971). Likewise, environmental conditions, such as the availability of molecular oxygen and pH, also have a major influence on pathways and rates of biodegradation (Benner et al. 1984, 1985). Overall, biodegradation tends to be chemically selective during early stages of organic matter decomposition resulting in the gradual enrichment of resistant molecules in the remaining organic detritus (Hedges and Prahl 1993).

Photodegradation of organic molecules by solar radiation is also recognized as an important process in the decomposition of detrital organic matter (Mopper and Kieber 2002). Organic molecules that absorb light in the ultraviolet region of the solar spectrum are often photoreactive. Aromatic molecules, such as lignins, absorb strongly in the ultraviolet and are highly susceptible to photodegradation, whereas most aliphatic molecules are not very susceptible to photodegradation (Opsahl and Benner 1998; Schmitt-Kopplin et al. 1998; Osburn et al. 2001). Photodegradation is chemically selective and compared with biodegradation, the diversity of organic molecules subject to photodegradation is relatively narrow.

Biodegradation and photodegradation are now considered to be the major processes mediating the decomposition and cycling of dissolved organic matter (DOM) in aquatic environments. Much of what we know about these processes comes from experiments conducted under conditions that isolate these processes. These experiments have demonstrated that photodegradation can have a substantial impact on subsequent biodegradation through the production of biologically labile substrates, the removal of biologically labile substrates, and the production of substrates that resist biodegradation (Mopper and Kieber 2002). In the present study, biodegradation and photodegradation co-occur, and the effect of each process is highlighted by varying the exposure time to photodegradation. Concentrations and compositions of lignin phenols, amino acids, dissolved organic carbon (DOC) and chromophoric DOM (CDOM) were measured to investigate the relative roles of these processes and the overall extent of decomposition of DOM in experiments with surface waters from the Broad River.

Materials and methods

Sample collection and incubation

Water samples were collected in acid-washed and rinsed 12-l carboys on February 18 and May 8, 2002, from the Broad River about 2 km above the confluence of the Broad and Saluda rivers in Columbia, South Carolina. The monthly average water gage height near the collection site was 16.046 m during February 2002 and 15.941 m during May, 2002. Water samples were transported to the laboratory and filtered through combusted (3 h at 450°C) Whatman GF/F filters (0.7 μm nominal pore size) within 2 h of collection. Filtration removed most particles and eukaryotic organisms, but the filtrate included an active assemblage of heterotrophic bacteria that have been shown to be the dominant decomposers of organic matter in similar aquatic environments (Benner et al. 1986). On each sampling date, filtered water (600 ml) was placed in each of four replicate 1-l quartz Erlenmeyer flasks and exposed to irradiation (400 W m^{-2}) in a solar simulator (Suntest XLS). The solar simulator was equipped with a xenon lamp and ultraviolet filter with a low wavelength cutoff at ~285 nm. The spectrum of the lamp closely resembles that of natural sunlight, and the irradiation level chosen is representative of solar radiation reaching the surface of the Broad River in summer. Flasks were placed in a water bath connected to a temperature-controlled recirculating chiller maintained at 20 ± 0.5°C throughout the incubation period.

The water samples collected in February were exposed to 20 h of light in the solar simulator every day. This treatment resulted in an irradiation dose of 257 MJ m^{-2} during the 9-day incubation period. The extended daily exposure to simulated sunlight resulted in extensive photochemical transformations during the relatively short incubation period. In contrast, the water samples collected in May were exposed to 5 h of light in the solar simulator every day. This treatment was more representative of the natural light exposure of surface waters in the Broad River. Ideally, water samples collected on the same date would have been used in these experiments, but this would have required two solar simulators. Therefore, the rates of bio- and photo-degradation measured in these experiments are not directly comparable, but the relative roles of these processes

in the decomposition of DOM are unlikely to vary substantially.

A sample flask was removed periodically from the solar simulator and the total volume (600 ml) was used for chemical and spectrophotometric analyses of dissolved organic matter (DOM) and chromophoric DOM (CDOM). During the February experiment a flask was removed from the solar simulator on days 2, 4, 7 and 9 and aliquots of the sample were taken for measurements of dissolved organic carbon (DOC), lignin phenols, total hydrolysable amino acids, and absorption at 350 nm. During the May experiment a flask was removed from the solar simulator on days 7, 14, 34 and 42 for the same chemical and optical analyses. These sampling times were designed to keep the irradiation dose between sampling periods constant in the two experiments while providing an extended period of biodegradation between sampling dates in May.

Optical and chemical characterization of DOM

Concentrations of DOC were measured by high temperature combustion using a Shimadzu TOC-V analyzer and corrected for the instrument blank (Benner and Strom 1993). The absorption of water samples at 350 nm was determined using a 5 cm quartz cuvette and a dual beam Shimadzu 1601 spectrophotometer. Milli-Q Plus UV treated water was used as a blank. Absorption coefficients (a_{350} m^{-1}) were calculated as $a_{350} = (A \times \ln 10)\ C^{-1}$, where A is the absorbance at 350 nm and C is the pathlength (m) of the cuvette.

Dissolved lignin was isolated from water samples (500 ml) by solid phase extraction (Louchouarn et al. 2000; Hernes and Benner 2003). Water samples were acidified to pH 2.5 with HCl and passed through cleaned and primed C18 extraction cartridges (Varian MegaBond Elut) at a flow rate of 50 ml min^{-1}. Dissolved lignin was eluted from the cartridges in 50 ml of methanol and dried in a Savant SpeedVac system. Lignin was analyzed using the CuO oxidation method, and lignin oxidation products were separated and quantified using a Hewlett Packard 5890 gas chromatograph with a DB5-MS capillary column and a Hewlett Packard 5972 mass selective detector using selected ion monitoring (Hernes and Benner 2003). Eleven lignin phenols were measured: *p*-hydroxybenzaldehyde (PAL), *p*-hydroxyacetophenone (PON), *p*-hydroxybenzoic acid (PAD), vanillin (VAL), acetovanillone (VON), vanillic acid (VAD), syringaldehyde (SAD), acetosyringone (SON), syringic acid (SAD), *p*-coumaric acid (CAD), and ferulic acid (FAD).

Water samples (100 µl) were dried under a stream of nitrogen gas in a laminar flow hood and subjected to a microwave-assisted, vapor-phase hydrolysis with 6 M HCl at 150°C for 32.5 min for the analysis of amino acids (Kaiser and Benner 2005). Following hydrolysis samples were neutralized and separated as o-phthaldialdehyde derivatives. Total hydrolysable amino acids (THAA) were determined with an Agilent 1100 high-performance liquid chromatograph system using a Licrosphere RP18 (4 × 250 cm, 5 µm) column (Davis and Benner 2005). Free and combined amino acids are measured together using this method. Fifteen amino acids were measured: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), threonine (Thr), arginine (Arg), β-alanine (BALA), alanine (Ala), tyrosine (Tyr), γ-aminobutyric acid (GABA), valine (Val), phenylalanine (Phe), L-isoleucine (L-Ile), leucine (Leu), and lysine (Lys). During acid hydrolysis, asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, and they are included in the reported measurements of Asp and Glu.

Results

Concentrations of DOC and lignin phenols ($\sum_6$) and CDOM absorption (a_{350}) were higher in February than in May (Table 1). River discharge (i.e. gage height) was also higher in February (16.046 m) than in May (15.941 m). The concentration of lignin phenols in February was nearly double the concentration in May, whereas CDOM absorption was only 25% higher in February (Table 1). These results indicate that other CDOM components besides lignin were important chromophores in these water samples. Concentrations of dissolved total hydrolysable amino acids were nearly identical on the two sampling dates.

The decomposition of DOM in these experiments is compared as the percentages of initial concentrations of chemical and optical parameters remaining versus time or irradiation dose. The rates of decomposition of DOC and amino acids were similar between the experiments, but the percentages of DOC

Table 1 Optical and chemical parameters during decomposition experiments with water from the Broad River

Days	Irradiance dose (MJ m^{-2})	Light: Dark	DOC (μmol l^{-1})	a_{350} (m^{-1})	a^*_{350} L (mmol C)$^{-1}$ m^{-1}	$\sum_6$ (μg l^{-1})	λ_6 (mg/100 mg OC)	$a_{350}/\sum_6$	THAA (nmol l^{-1})	THAA (% DOC)
February										
0	0		231	7.908	34.3	10.30	0.373	0.77	1,193	1.94
2	58	5.0	212	5.100	24.1	6.44	0.253	0.79	932	1.54
4	115	5.0	nd	3.727	nd	4.35	0.123	0.86	753	nd
7	199	4.7	nd	2.437	nd	2.20	0.080	1.11	nd	nd
9	257	4.7	202	1.838	9.1	1.26	0.052	1.46	nd	nd
May										
0	0		197	6.326	32.1	5.82	0.246	1.09	1,198	2.31
7	56	0.3	166	4.358	26.3	2.86	0.144	1.52	702	1.47
14	109	0.3	160	3.382	21.1	1.28	0.066	2.65	565	1.17
34	242	0.3	140	1.815	12.9	0.84	0.050	2.15	527	1.14
42	307	0.3	134	1.470	11.0	0.48	0.030	3.04	491	1.11

Incubations were maintained at 20°C and periodically exposed to simulated sunlight at an irradiance of 400 W m^{-2}. The ratio of light-to-dark exposure (L/D) is shown during each incubation period. The CDOM absorption coefficient (a_{350}), the DOC-normalized absorption coefficient (a^*_{350}) and concentrations of DOC, total hydrolysable amino acids (THAA) and six major lignin phenols ($\sum_6$) were measured

nd not determined

and amino acid removal were much greater in the May experiment (Fig. 1a, b). A 32% loss of DOC and 59% loss of amino acids were observed during the 42 days experiment in May compared with losses of 12 and 37%, respectively, during the 9 days experiment in February (Fig. 1a, b). In contrast, the rates of lignin phenol decomposition and CDOM (a_{350}) loss were much greater in the February experiment, whereas the percentages of decomposition were similar in the February and May experiments (Fig. 1c, d). In February there was a 77% loss of a_{350} and 88% loss of lignin phenols during the 9 days experiment, and in May there was a 77% loss of a_{350} and 92% loss of lignin phenols during the 42 days experiment (Fig. 1c, d).

Losses of a_{350} and lignin phenols declined exponentially when plotted versus the irradiation dose received during the February and May experiments (Fig. 2a, b). The coefficients of determination were highly significant ($p < 0.01$), and the simple exponential decay constants for a_{350} and lignin phenols were similar between experiments. Declines in a_{350} and lignin phenols were linearly correlated ($p < 0.01$) during both experiments (Fig. 3). Lignin phenol losses were greater relative to a_{350} losses during the February experiment compared with the May experiment. Extrapolation of regression lines in both experiments indicated CDOM (a_{350}) persists after the complete removal of lignin phenols ($\sum_6$).

Changes in the composition of lignin phenols were observed during the decomposition experiments. The CuO oxidation of lignin produces a suite of three *p*-hydroxy phenols (P), three vanillyl phenols (V), three syringyl phenols (S), and two cinnamyl phenols (Table 2). The P/V, S/V, and C/V ratios in Broad River water were lower in February (0.41, 0.48, 0.06, respectively) than in May (0.47, 0.65, 0.12, respectively). The P/V ratios increased in both experiments during decomposition (Fig. 4a), whereas the S/V ratios decreased in both experiments (Fig. 4b). The C/V ratio in the February experiment decreased during decomposition, but there was no significant trend in C/V ratios during the May experiment (Table 2). As with the loss of lignin phenols, changes in lignin composition were more strongly related to irradiation exposure than to time of incubation.

The initial acid:aldehyde (ad:al) ratios in each of the major lignin phenol families (P, V, S) were 1.87 (PAD:PAL), 2.42 (VAD:VAL), and 1.14 (SAD:SAL) in the February samples and 2.52 (PAD:PAL), 1.60 (VAD:VAL), and 1.19 (SAD:SAL) in the May samples (Table 2). Values varied during the experiments, but

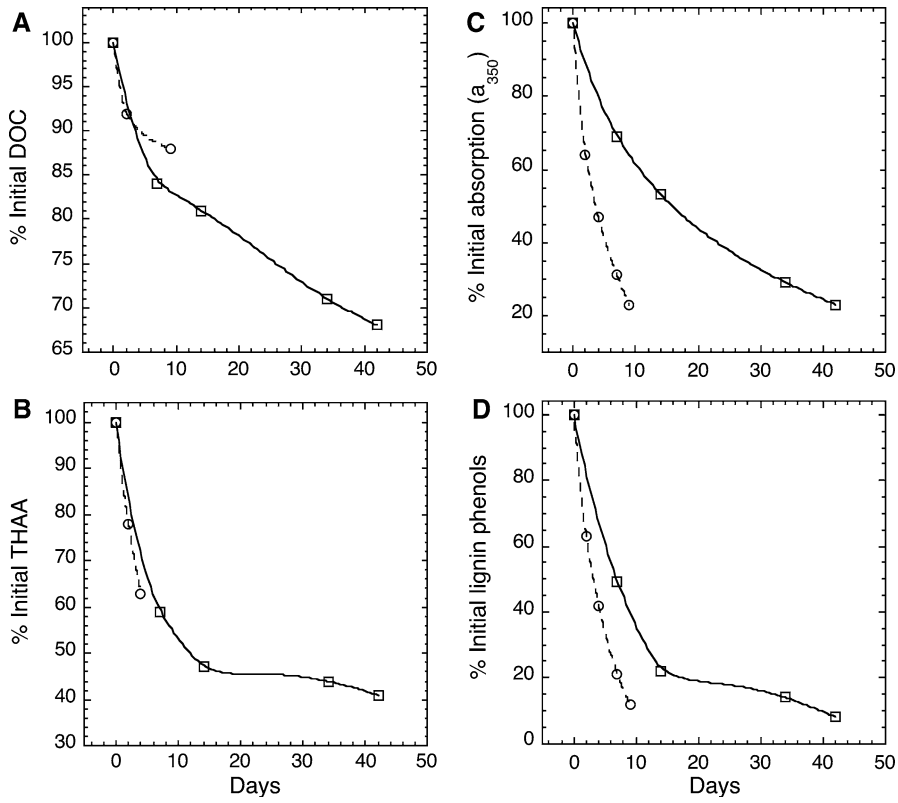

Fig. 1 The loss of DOC (**a**), total hydrolysable amino acids (**b**), CDOM absorption at 350 nm (**c**), and lignin phenols ($\sum_6$) (**d**) during decomposition experiments with Broad River water collected in February (*open circles* and *dashed line*) and May (*open squares* and *solid line*). The percentages of initial concentrations of variables are plotted versus days of incubation

there was a general trend towards increasing ad:al ratios with increasing decomposition. The final values were 2.06 (PAD:PAL), 2.52 (VAD:VAL), and 0.82 (SAD:SAL) in the February experiment and 4.07 (PAD:PAL), 2.01 (VAD:VAL), and 1.97 (SAD:SAL) in the May experiment (Table 2).

Simple exponential decay constants were calculated for the 11 phenols produced during CuO oxidation of lignin and 3,5-dihydroxybenzoic acid (Table 2). The decay constants are expressed per unit irradiance dose rather than time because photodegradation is the primary mechanism for the decomposition of these compounds. In February the average decay constants for the P, V and S phenol families were −0.0042, −0.0077 and −0.0090, respectively, and in May the decay constants were −0.0043, −0.0065 and −0.0100, respectively. The decay constants for these phenol families followed a consistent pattern with S > V > P. Within the cinnamyl phenols, decay constants for ferulic acid were greater than those for *p*-coumaric acid. The decay constants for 3,5-dihydroxybenzoic acid were low and similar to those for the P phenols.

The amino acid compositions of DOM were similar in the February and May river water samples (Fig. 5a, b). Gly was the most abundant amino acid (23 mol%), followed by Ala, Asp, Glu, Ser, Thr and Val. The eight other amino acids analyzed were ≤5 mol% each. Changes in amino acid composition were observed during decomposition, and those compositional changes were also similar in the February and May experiments (Fig. 5a, b). The mol% compositions of Gly, Asp, and the non-protein amino acids *β*-alanine and *γ*-aminobutyric acid increased during decomposition, whereas the mol% compositions of the other amino acids either decreased or were unchanged.

The decomposition rates of lignin phenols were much greater than those of DOC in the February and May experiments. The DOC-normalized yields of lignin phenols (Λ_6) declined rapidly during the first 110 MJ m^{-2} irradiation dose in both experiments (Fig. 6). About 65% of the initial lignin phenols in river water were photodecomposed during this period. The decline in Λ_6 was more gradual as the irradiation dose increased from 110 to 300 MJ m^{-2},

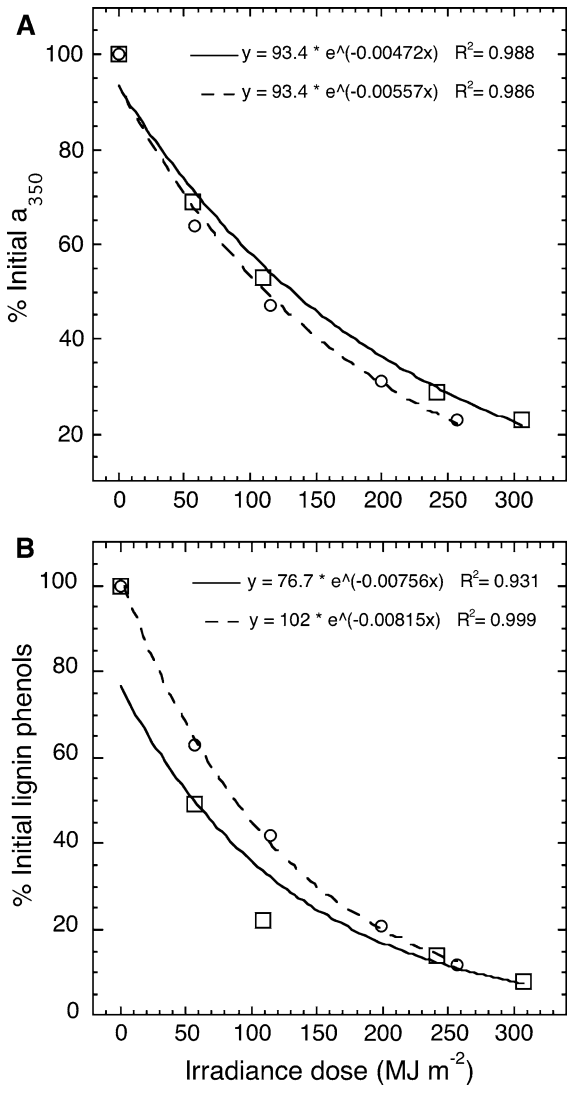

Fig. 2 The loss of CDOM absorption at 350 nm (**a**) and lignin phenols ($\sum_6$) (**b**) during decomposition experiments with Broad River water collected in February (*open circles* and *dashed line*) and May (*open squares* and *solid line*). The percentages of initial concentrations of variables are plotted versus irradiation dose

indicating a shift in the photoreactivity of the remaining lignin.

During the May experiment the incubations received ~5 h of irradiation each day and the experiment lasted for 42 days. Microbial degradation of DOC and amino acids was much more extensive than during the February experiment which lasted 9 days. The DOC-normalized yields of amino acids decreased rapidly during the first 14 days of incubation followed by more gradual declines in yields from

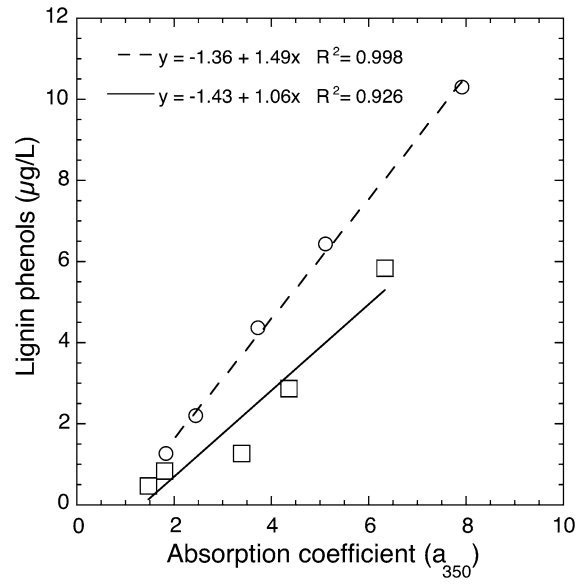

Fig. 3 The relationships between lignin phenol ($\sum_6$) concentrations and CDOM concentrations during decomposition experiments with Broad River water collected in February (*open circles* and *dashed line*) and May (*open squares* and *solid line*)

14 to 42 days (Fig. 7). The photodegradation of lignin phenols and decrease in yields followed a very similar pattern (Fig. 7).

Discussion

Photo- and bio-degradation of riverine DOM

Most studies of the decomposition of DOM have been conducted under dark conditions to determine the rates and extent of biodegradation or under natural/simulated solar radiation without microbes to determine the rates and extent of photodegradation. These studies have been particularly useful for understanding the mechanisms and relative contributions of biological and photochemical processes to DOM decomposition (Miller and Zepp 1995; Moran and Zepp 1997; Obernosterer and Benner 2004), but they provide an incomplete assessment of the interplay and combined effects of photochemical and biological processes, which naturally co-occur in surface waters. In the present study, experiments with Broad River water included active microbial populations and exposure to simulated solar radiation, and they varied in duration and daily exposure to light.

Table 2 Concentrations of lignin phenols and 3,5-dihydroxybenzoic acid (DiOHBA) during decomposition experiments with water from the Broad River

Days	Irradiance dose (MJ m^{-2})	PAL (ng l^{-1})	PON (ng l^{-1})	PAD (ng l^{-1})	VAL (ng l^{-1})	VON (ng l^{-1})	VAD (ng l^{-1})	SAL (ng l^{-1})	SON (ng l^{-1})	SAD (ng l^{-1})	CAD (ng l^{-1})	FAD (ng l^{-1})	DiOHBA (ng l^{-1})
February													
0	0	701	769	1,309	1,835	1,568	3,450	1,220	1,067	1,160	197	214	1,854
2	58	522	634	921	1,100	832	2,757	662	449	643	127	117	1,169
4	115	470	599	865	948	534	1,660	532	278	408	111	86	992
7	199	240	392	568	305	257	1,035	231	133	243	69	64	670
9	257	200	308	413	225	162	567	129	71	106	34	18	428
k		−0.0051	−0.0036	−0.0043	−0.0085	−0.0087	−0.0070	−0.0085	−0.0101	−0.0087	−0.0063	−0.0084	−0.0053
r^2		0.969	0.967	0.976	0.968	0.997	0.983	0.987	0.989	0.982	0.952	0.902	0.979
May													
0	0	355	407	895	1,037	834	1,665	740	672	876	194	230	1,608
7	56	190	249	539	487	357	1,111	310	227	373	81	99	872
14	109	94	141	353	214	152	575	92	73	171	43	58	686
34	242	102	137	270	207	114	321	79	43	81	21	18	396
42	307	51	114	209	113	71	227	19	16	38	12	7	363
k		−0.0052	−0.0036	−0.0043	−0.0062	−0.0072	−0.0064	−0.0103	−0.0110	−0.0095	−0.0084	−0.0107	−0.0046
r^2		0.817	0.779	0.911	0.837	0.877	0.968	0.890	0.925	0.965	0.957	0.991	0.927

Exponential decay constants (k; m^2 MJ^{-1}) and coefficients of determination (r^2) are shown

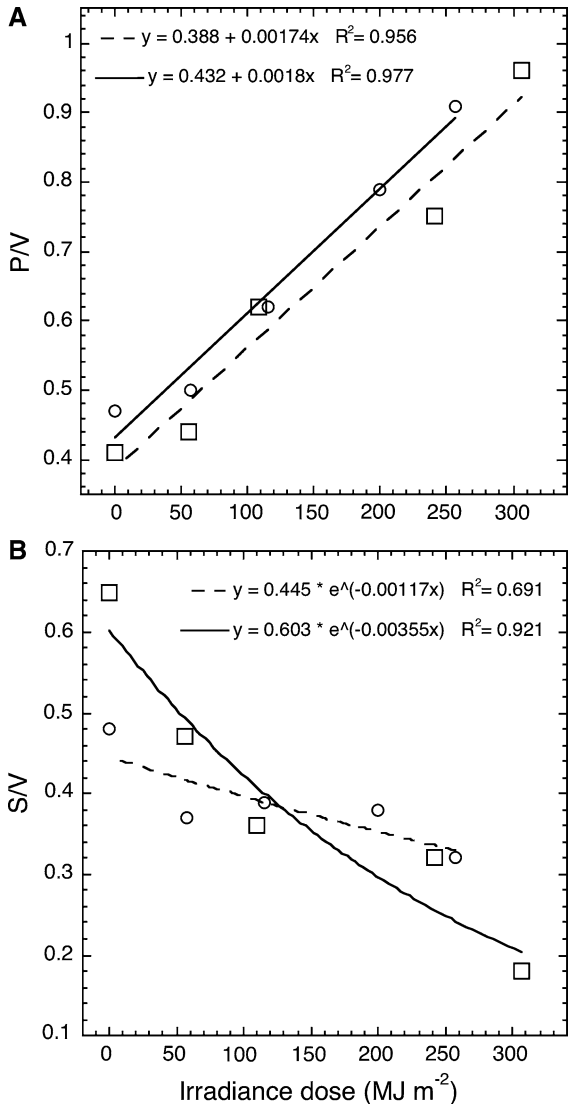

Fig. 4 Changes in the ratios of **a** *p*-hydroxy and vanillyl phenols (P/V), and **b** syringyl and vanillyl phenols (S/V) during decomposition experiments with Broad River water collected in February (*open circles* and *dashed line*) and May (*open squares* and *solid line*)

Biological processes were largely responsible for the decomposition of DOC and total hydrolysable amino acids because the loss of these components was primarily dependent on the duration of experiments (i.e. dark exposure) rather than exposure to simulated solar radiation. Unlike DOC and amino acid losses, the decomposition of lignin phenols and loss of CDOM (a_{350}) was mediated primarily by photochemical processes because the loss of these components was dependent on exposure to simulated solar radiation rather than the duration of experiments. Overall, these experiments demonstrated that biological and photochemical processes preferentially degrade different chemical components of DOM.

The DOC remineralized during 1–2 week bioassay experiments (i.e. dark conditions) has been described as labile DOC (Sondergaard and Middelboe 1995). These authors synthesized bioassay data from a wide variety of aquatic environments and found that on average about 17% of DOC was labile. A seasonal study of the bioreactivity of DOC in the Sacramento and San Joaquin rivers indicated 10–39% was labile during 2-week bioassays (Stepanauskas et al. 2005). Exposure of river water to simulated solar radiation for 4 h had varying impacts on DOM bioreactivity, with exposure during summer and fall sometimes enhancing bioreactivity and DOM lability and exposure during winter and spring reducing bioreactivity (Stepanauskas et al. 2005). Longer (5–8 weeks) bioassays with water from several rivers in Georgia (USA) indicated that only about 6% of the DOC was labile (Moran et al. 1999). Exposure of these waters to simulated solar radiation for 7 h typically enhanced bioreactivity and DOC lability. A total of 19% of Broad River DOC was remineralized during the initial 2-weeks of the May experiment, which included 5 h of simulated solar radiation each day. The low percentage of labile DOC in Georgia rivers (Moran et al. 1999) and the similarities of piedmont rivers in Georgia and South Carolina, indicate that daily irradiation enhanced the overall remineralization of Broad River DOC in the present study. Photochemical processes clearly played a predominant role in CDOM bleaching and the degradation of dissolved lignin.

Total hydrolysable amino acids have been identified as bioreactive components of riverine (Volk et al. 1997), lake (Weiss and Simon 1999) and seawater DOM (Amon et al. 2001). Amino acids typically comprise about 1–3% of the DOC in rivers, but they can account for as much as 5–10% of bioavailable DOC (Benner 2003). In February and May amino acids accounted for 1.9 and 2.3%, respectively, of the DOC in the Broad River. Amino acids accounted for 7.2% of the labile DOC that was degraded during the first 14 days of the May decomposition experiment. A total of 15 amino acids were measured in Broad River DOM, and the pattern of amino acid composition during decomposition was similar to that observed

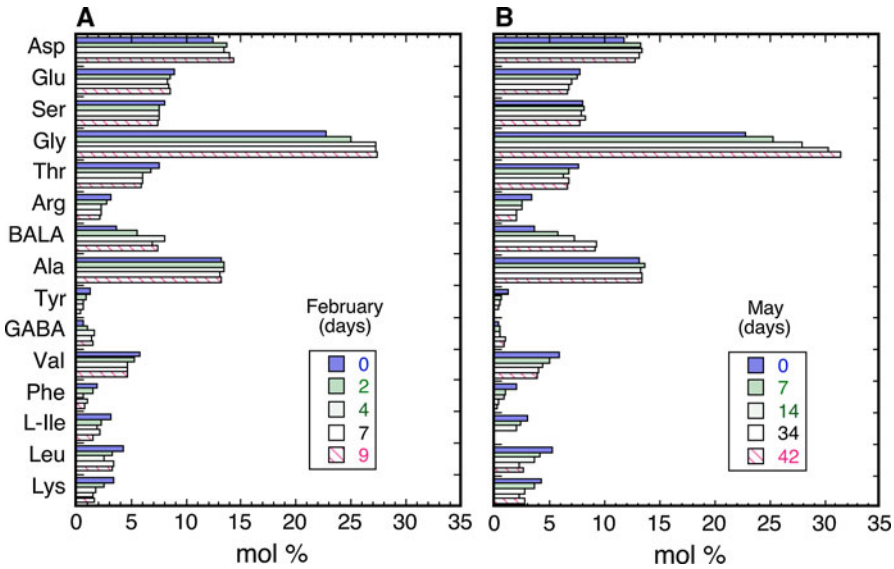

Fig. 5 Changes in amino acid compositions during decomposition experiments with Broad River water collected in February (**a**) and May (**b**)

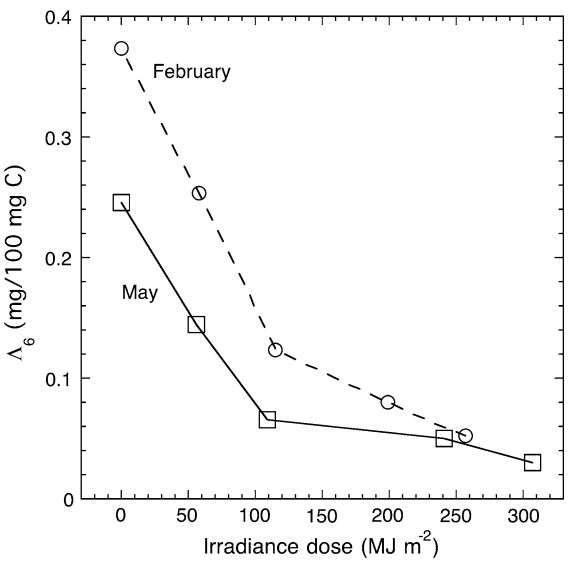

Fig. 6 Changes in carbon-normalized lignin phenol yields (Λ_6) during decomposition experiments with Broad River water collected in February (*open circles* and *dashed line*) and May (*open squares* and *solid line*)

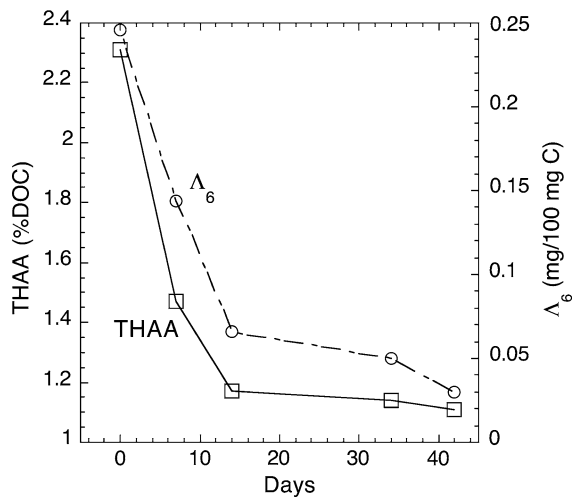

Fig. 7 Changes in carbon-normalized amino acid (THAA) and lignin phenol (Λ_6) yields during decomposition experiments with Broad River water collected in May

previously due to biodegradation (Hedges et al. 1994; Volk et al. 1997). Glycine was the most abundant amino acid, and it increased in relative abundance during decomposition accounting for 31 mol% of amino acids at the end of the May experiment. The aromatic amino acids phenylalanine and tyrosine accounted for only a few mol% of the initial amino acids in DOM and were nearly completely removed during decomposition. These amino acids absorb ultraviolet light and are components of CDOM. It is possible that photodegradation played a role in the loss of these amino acids (Reitner et al. 2001).

Dissolved lignin in rivers is susceptible to microbial degradation, but rates of photodegradation of lignin are typically several-fold higher than rates of biodegradation (Opsahl and Benner 1998; Hernes and Benner 2003). Therefore, biodegradation accounted for a minor fraction of the observed lignin degradation during the February experiment, which received 20 h of irradiation each day. Biodegradation removed lignin phenols from Mississippi River water at a rate of 0.31% day^{-1} during dark incubations lasting

28 days (Opsahl and Benner 1998). Assuming a similar rate of biodegradation during the 42 days May experiment with Broad River water, we estimate biodegradation removed 13% of the lignin. This indicates photodegradation was responsible for the removal of 79% of the lignin during the May experiment. The strong correlation between lignin phenols (Σ_6) and CDOM (a_{350}) throughout the decomposition of Broad River DOM indicated that the aromatic structures of lignin were degraded.

Photodegradation and structure–reactivity relationships among CDOM components

A large number of compounds with diverse chemical structures contribute to CDOM, and it seems likely these compounds would exhibit varying susceptibilities to photodegradation. The photochemical decay coefficients for the six syringyl and vanillyl lignin phenols represented by Σ_6 were greater than those for CDOM (a_{350}), indicating some components of CDOM were more resistant to decomposition than these six lignin phenols. Likewise, the a_{350}:Σ_6 ratio increased steadily during both experiments (Table 1). It is possible that microorganisms released CDOM during the experiments and thereby contributed to increasing a_{350}:Σ_6 ratios, but it appears that the primary driver of the a_{350}:Σ_6 ratio is the preferential photodegradation of lignin phenols relative to other CDOM components.

The CuO oxidation procedure used in this study yielded five other lignin phenols in addition to the six syringyl and vanillyl phenols. The CuO oxidation products, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid and *p*-coumaric acid, can have non-lignin sources, so *p*-hydroxy phenols and cinnamyl phenols are not normally used as indicators of terrigenous organic carbon in the ocean (Hedges and Parker 1976; Hedges et al. 1988; Hernes and Benner 2002). The amino acid tyrosine appears to be the primary non-lignin source of these compounds, as it is converted into these phenols with varying efficiencies during CuO oxidation (Hedges 1975; Hedges and Parker 1976; Hernes and Benner 2002). We directly measured tyrosine concentrations in Broad River water and calculated that the conversion of Tyr to *p*-hydroxybenzaldehyde and *p*-coumaric acid could account for as much as 67 and 13%, respectively, of the measured concentrations of these phenols assuming all of the tyrosine in river water was recovered in methanol extracts from the solid phase extraction columns used for isolating lignin from river water samples. However, the recovery of dissolved organic nitrogen from river water using this solid-phase extraction method is quite low (<40%), so it is likely that the majority (>75 and >95%, respectively) of the measured *p*-hydroxybenzaldehyde and *p*-coumaric acid in this study was derived from lignin. The conversion of tyrosine to *p*-hydroxybenzoic acid during CuO oxidation is very inefficient (Hedges 1975), so we assume this phenol is predominantly derived from lignin.

The rates of photodegradation of lignin phenols appear to be influenced by the presence of methoxy groups on the aryl ring. Syringyl (S) phenols have methoxy substitutions on carbon atoms 3 and 5, vanillyl (V) phenols have methoxy substitutions on carbon atom 3, and *p*-hydroxy (P) phenols are not substituted with methoxy groups. Photochemical decay constants were highest for S phenols and lowest for P phenols and followed a consistent pattern (S > V > P) in the two experiments. On average, P and V phenol decay constants were 45 and 75%, respectively, of S phenol decay constants. Of the two cinnamyl (C) phenols, ferulic acid, has a methoxy substitution on carbon atom 3 and had an average decay constant similar to S phenols. The other C phenol, *p*-coumaric acid, has no methoxy substitutions and had an average decay constant similar to V phenols. The phenol, 3,5-dihydroxybenzoic acid, is not substituted with methoxy groups and appears to be derived from soils (Dickens et al. 2007). It exhibited decay constants similar to P phenols. Among the P phenols, *p*-hydroxyacetophenone has no known source other than lignin, and it exhibited the lowest decay constant among all phenols analyzed in this study.

Previous studies of the photodegradation of DOM and natural lignin in natural waters have not investigated the photoreactivity of P phenols. However, two previous studies with Mississippi River water found that S phenols were more photoreactive than V phenols (Opsahl and Benner 1998; Hernes and Benner 2003), indicating methoxy substitution of lignin phenols enhances their photoreactivity. The present study with Broad River water samples confirms this structure–reactivity relationship and extends it to the P phenols, which have no methoxy

substitutions. Studies of the photodegradation of lignin model compounds also found that rates of photodegradation increased dramatically with increasing methoxy substitution of phenols (McNally et al. 2005). In contrast, a recent study with water from the Congo River found that V phenols were more susceptible to photodegradation than S phenols (Spencer et al. 2009). These authors suggested that a small percentage of lignin was resistant to photodegradation and enriched in S phenols. The photoresistant lignin could be part of a molecular complex that reduces the exposure of lignin phenols to ultraviolet light and sensitized photodegradation. It is also possible the photosensitizer is depleted or no longer active.

Lignin phenols and amino acids as indicators of photo- and bio-labile DOM

In the present study the concept of labile DOM is extended to include the photo-labile as well as bio-labile components. The daily simulated solar irradiance in the May experiment (5 h day^{-1}) better represented natural sunlight exposure in the Broad River than the high daily exposure during the February experiment (20 h day^{-1}), so the following discussion focuses on the May experiment. During the initial 14 days of the experiment, 19% of the DOC, 53% of the amino acids and 78% of the lignin phenols were removed and are described as the labile components of DOM. Amino acids accounted for ~7% of the labile DOC and were removed primarily by biodegradation. Lignin phenols accounted for ~1% of labile DOC and were removed primarily by photodegradation.

The decomposition experiments conducted in the present study required considerable time and resources and are not amenable for the analysis of photo-labile and bio-labile DOM in a large number of samples. It would be particularly helpful to have a much simpler approach for estimating the amount and distribution of labile DOC in aquatic environments. Amino acids have been shown to be very useful molecular indicators of bio-labile DOM (Benner 2003; Davis and Benner 2007). The percentage of DOC as amino acids, referred to herein as the C-normalized yield of amino acids, is indicative of the relative bioavailability of DOM with higher yields indicating greater amounts of bioavailable DOM. A quantitative approach for using C-normalized yield of amino acids in marine DOM as indicators of labile and semi-labile DOC was recently developed and calibrated using standard bioassay experiments (Davis and Benner 2007). Semi-labile DOC is operationally defined as carbon that is utilized on time scales of months to years. Using this approach, measurements of the concentrations of DOC and amino acids can be used to calculate the concentrations of labile and semi-labile DOC in seawater samples. The rapid decline of the C-normalized yield of amino acids during the first 14 days of the May experiment was indicative of bio-labile DOC, and the slow decline of amino acid yields during the last 28 days was indicative of the biodegradation of semi-labile DOC (Fig. 7). The shift between labile and semi-labile DOC decomposition occurred at an amino acid yield of 1.2% DOC, which is within the range of yields (1.1–1.6% DOC) estimated for semi-labile DOC in marine waters (Davis and Benner 2007). It appears that C-normalized yields of amino acids that exceed values of 1.2% DOC in Broad River water are generally indicative of the presence of bio-labile DOM.

Lignin phenols are highly susceptible to photodegradation and results from the May decomposition experiment demonstrated the potential of using lignin phenols as molecular indicators of photo-labile DOC. The C-normalized yield of lignin phenols (Λ_6) declined rapidly during the first 14 days of the May experiment indicating the degradation of photo-labile DOC, and the slow decline of lignin phenol yields during the last 28 days were indicative of the photodegradation of semi-labile DOC (Fig. 7). The shift between labile and semi-labile DOC photodecomposition occurred at a C-normalized lignin phenol yield of 0.07 mg lignin phenols 100 mg C^{-1}, indicating that yields greater than this are indicative of photo-labile DOC in Broad River water.

This study demonstrated the potential for using lignin phenols and amino acids as qualitative indicators of photo-labile and bio-labile DOM, but considerably more research is needed to develop this approach for making quantitative estimates of photo-labile and bio-labile DOC in the Broad River. Yields of lignin phenols and amino acids in fresh DOM that has not been significantly bio- and photo-degraded are needed as well as estimates of the yields of these compounds in refractory DOM (Davis and Benner

2007). Additional decomposition experiments are also needed for calibration and to investigate seasonal variability in the composition and reactivity of DOM. However, the use of specific chemical components of DOM as indicators of reactivity is a promising research direction for the assessing the quality of DOM and the quantity of labile and semi-labile DOC in freshwater as well as marine systems.

Implications of process-selective degradation

In many aquatic systems with DOM derived from vascular plants, the combined effects of photo- and bio-degradation have been shown to remineralize more DOC than either of these processes working in isolation (Miller and Moran 1997; Mopper and Kieber 2002; Obernosterer and Benner 2004). As demonstrated in the present study, biological and photochemical processes preferentially degrade different components of DOM. Photochemical processes preferentially remove CDOM, which is rich in aromatic molecules such as lignin that are predominantly of plant origin. These results are consistent with previous observations indicating that photochemical transformations of phytoplankton-derived DOM generally do not lead to enhanced remineralization of DOC and in some cases impede overall remineralization (Benner and Biddanda 1998; Obernosterer et al. 1999; Tranvik and Bertilsson 2001; Obernosterer and Benner 2004).

Lignin is about 4–6‰ depleted in ^{13}C relative to carbohydrates in plant material, and during biodegradation carbohydrates are preferentially degraded leaving a detrital material that is depleted in ^{13}C and enriched in lignin (Benner et al. 1987). In contrast, the photodegradation of plant material preferentially removes lignin leaving a detrital material that is enriched in carbohydrates and by inference ^{13}C (Opsahl and Benner 1993). Likewise, the photodegradation of riverine DOC preferentially removes lignin (Opsahl and Benner 1998; Hernes and Benner 2003; Spencer et al. 2009), whereas the biodegradation of riverine DOC preferentially removes carbohydrates and amino acids (Volk et al. 1997; Weiss and Simon 1999). During photodegradation of riverine DOC, the preferential removal of lignin leads to the gradual enrichment of ^{13}C in the remaining DOC (Opsahl and Zepp 2001; Spencer et al. 2009). These studies have demonstrated that changes in the chemical composition and ^{13}C content of organic matter track each other during biological and photochemical decomposition processes.

The decomposition of DOM by biological and photochemical processes can alter its stable C isotopic composition and thereby complicate the utility of C isotopes for distinguishing organic matter origin. Opsahl and Zepp (2001) conducted decomposition experiments that included microbes and the combined effects of biodegradation and photodegradation during DOM exposure to natural sunlight for 16–21 days. They observed lignin losses of 62–79% and ^{13}C enrichments in DOM of 0.8–1.6‰ in experiments with water from the Altamaha and Satilla rivers. Spencer et al. (2009) conducted 57-day photodegradation studies in a solar simulator with mercuric chloride-poisoned water from the Congo River. They observed a lignin loss of 98% and a 3.1‰ enrichment of ^{13}C in DOM. These studies are not directly comparable, but it seems likely that the smaller C isotopic shift in DOM observed by Opsahl and Zepp (2001) is due largely to the combined effects of bio- and photo-degradation, which remove ^{13}C-enriched carbohydrates and amino acids as well as ^{13}C-depleted lignin and other aromatics (Osburn et al. 2001).

Lignin phenols and stable C isotopic compositions of DOM are commonly used to estimate contributions of terrigenous DOC to the ocean (Druffel et al. 1992; Hedges et al. 1997; Opsahl and Benner 1997; Raymond and Bauer 2001). The observed influence of photodegradation on lignin phenol yields and C isotopic compositions of riverine DOM indicates the potential to underestimate terrigenous C in seawater using these approaches. However, biodegradation is a continuous process that will offset the relatively rapid reduction in lignin phenol yields and selective enrichment of ^{13}C in DOM due to photodegradation. Surface waters in ocean margins that receive substantial inputs of continental runoff are regions where the selective photodegradation of lignin phenols is most likely to influence estimates of terrigenous DOC (Amon and Benner 1996; Vodacek et al. 1997). The combined effects of photodegradation and biodegradation should be considered when evaluating the fidelity of lignin phenols and stable C isotopes as tracers of terrigenous DOC in the ocean.

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