

# Photooxidation of wetland and riverine dissolved organic matter: altered copper complexation and organic composition

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**Abstract** In natural waters, the uptake of transition metals such as copper (Cu) by aquatic biota depends on the activity of the free cupric ion ( $\{Cu^{2+}\}$ ) rather than on total Cu concentration. Thus, an important ecological function of dissolved organic matter (DOM) in aquatic ecosystems is Cu–DOM complexation, which greatly decreases the  $\{Cu^{2+}\}$ . However, Cu bioavailability is greatly modified by source and environmental history of DOM because DOM affinity for Cu varies by orders of magnitude among DOM sources; moreover, DOM is photochemically unstable. During 72-h irradiation experiments at intensities approximating sunlight with DOM from a palustrine wetland and a third-order river, we investigated photooxidative effects on DOM complexation of Cu as well

as spectral and chemical changes in DOM that might explain altered Cu complexation. Irradiation decreased Cu complexation by riverine DOM, but unexpectedly increased Cu complexation by wetland DOM, resulting in 150% greater  $\{Cu^{2+}\}$  in riverine DOM at the same dissolved organic carbon concentrations. The specific ultraviolet absorption (*SUV<sub>a</sub>*) and humic substances tracked photochemical changes in the conditional stability constants of Cu–DOM complexes, suggesting that the aromaticity of DOM influences its affinity for Cu. Carbonyl concentration in  $^{13}C$  nuclear magnetic resonance spectra ( $^{13}C$ -NMR) covaried directly with Cu binding-site densities in DOM. However, no aspect of Cu–DOM complexation consistently covaried with fluorophores (i.e., the fluorescence index) or low molecular weight organic acids. Our results suggest that global increases in UV radiation will affect Cu–DOM complexation and subsequent Cu toxicity depending on light regime as well as DOM source.

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## Introduction

For aquatic biota, the speciation of a transition metal such as copper (Cu) determines its biologic

uptake as a nutrient and toxin because the most readily bioavailable Cu species is the free cupric ion ( $\text{Cu}^{2+}$ ). Cu complexation with dissolved organic matter (DOM) and inorganic solutes (e.g.,  $\text{CO}_3$  and OH) greatly decreases the chemical activity of the cupric ion ( $\{\text{Cu}^{2+}\}$ ). Waters having much different concentrations and types of DOM and inorganic solutes may have the same  $\{\text{Cu}^{2+}\}$  and same biologic effect regardless of total Cu concentration (Erickson et al., 1996). Total Cu cannot predict Cu bioavailability. The challenge is that we must directly measure, or become able to reliably predict  $\{\text{Cu}^{2+}\}$ , for a variety of natural waters subject to distinct and unmonitored environmental processes such as photooxidation.

At concentrations averaging  $6 \text{ mg l}^{-1}$  in freshwater systems worldwide (Wetzel, 2001), DOM can play a major role in determining Cu toxicity in natural waters (Meador, 1991; Playle et al., 1993; Erickson et al., 1996; MacRae et al., 1999; Richards et al., 2001). In recognition of its importance, DOM concentration has been included in water quality criteria for Cu (USEPA, 2003) in addition to inorganic solute chemistry. In that algorithm, all polyelectrolyte DOM macromolecules are considered to have the same Cu-complexation characteristics. However, the binding affinity of DOM for Cu varies by more than an order of magnitude among DOM sources (McKnight et al., 1983; Breault et al., 1996). Such distinctions in metal complexation significantly alter the biologic uptake of metals (Richards et al., 2001; De Schamphelaere et al., 2004; Schwartz et al., 2004). Differences in binding affinity may stem from physico-chemical distinctions in DOM originating from allochthonous sources (terrestrial, vascular plants) versus autochthonous biomass (algae, macrophytes, and bacteria; McKnight et al., 1997; McCallister et al., 2006).

Allochthonous DOM has a much higher ratio of aromatic:aliphatic carbon than aquatic DOM. Aromaticity is important because the electron-withdrawing character of aromatic moieties (aromatics) in DOM increases its molecular electronegativity and enhances complexation of positively charged  $\text{Cu}^{2+}$  (Leenheer et al., 1998). High inputs of lignin from vascular plants contribute AR to allochthonous DOM, but lignin is not present in algae and bacteria (McKnight

et al., 1997; McKnight & Aiken, 1998; Opsahl & Benner, 1998). The aromatics present in aquatic DOM originate from intracellular quinones in the chloroplasts and mitochondria of algae and bacteria (McKnight et al., 1997; McKnight & Aiken, 1998; Klapper et al., 2002). Compared to allochthonous DOM, autochthonous DOM contains fewer aromatic compounds and relatively more proteins and lipids, which decreases its carbon to nitrogen ratio (McCallister et al., 2006).

In addition to source-dependent differences in DOM, DOM is photochemically unstable under ambient sunlight (De Haan, 1993; Bourbonniere et al., 1997; Molot & Dillon, 1997). Interestingly, the origin of DOM—whether it originates from allochthonous versus autochthonous sources—strongly influences some photoreactions. For example, irradiation of allochthonous DOM or DOM from vascular macrophytes (Wetzel et al., 1995; Miller et al., 2002) produces low molecular weight (LMW) organic acids that microbes readily respire. However, photolysis does not produce these microbially labile compounds in solutions containing algal derived DOM (Thomas & Lara, 1995; Benner & Biddanda, 1998; Tranvik & Kokalj, 1998; Obernosterer et al., 2001). Similarly, solar irradiation of mixtures of allochthonous and autochthonous DOM removes 10–60% of its dissolved organic carbon (DOC) through oxidation to inorganic carbon (photomineralization) in the span of 2–16 days (Molot & Dillon, 1997; Xie et al., 2004). DOC is a readily analyzed component that comprises ~50% of whole DOM. However, DOM from pure cultures of algae is not photomineralized (Obernosterer & Benner, 2004).

In contrast, it appears that photobleaching (decreased absorbance of ultraviolet (UV) radiation and visible light) is independent of source. Irradiated DOM is commonly photobleached by 20–50% per unit residual DOC. Decreased absorbance of UV radiation indicates the loss of  $\text{sp}^2$  hybridized bonds (e.g., the double bonds of aromatics) in both allochthonous (Moran et al., 2000; Del Vecchio & Blough, 2002) and pure algal cultures of DOM (Obernosterer & Benner, 2004).

In natural waters, DOM is comprised of a combination of allochthonous and algal sources, thus a reasonable expectation is that both photobleaching (loss of aromaticity) and photomineralization

(loss of DOC) of DOM influence Cu complexation. Indeed, field studies under ambient sunlight show that transformation of DOM significantly can diminish its binding-site density for Cu in seawater (Moffett et al., 1990) and in estuarine waters (Shank et al., 2006). Such photooxidative loss of binding sites likely explains observed increases in  $\{Cu^{2+}\}$  in the euphotic zone of seawater, particularly during periods of stratification (Moffett, 1995). Controlled laboratory experiments also show that binding-site concentrations on strong Cu ligands in photooxidized DOM decrease significantly by 20% (Sander et al., 2005) to 90% (Shank et al., 2006). In addition to transformational loss of binding sites on irradiated DOM molecules, photomineralization that destroys DOC causes release of previously bound  $Cu^{2+}$  (Sunda & Hanson, 1979). Another potential mechanism is the release of the cuprous ion ( $Cu^+$ ) from photo-excited DOM after ligand-to-metal charge transfer followed by rapid reoxidation of  $Cu^+$  to  $Cu^{2+}$  (Moffett & Zika, 1987; Voelker et al., 2000). These studies clearly demonstrate that exposure history affects Cu–DOM complexation, but it is unclear how DOM source and photooxidation interact in dynamic natural systems to affect Cu speciation and bioavailability.

Our study objective was to test whether precursor organic material from hydrologically contrasting sources (i.e., wetland versus riverine DOM) alters photochemical effects on Cu complexation by DOM. To interpret changes in Cu–DOM complexation, we followed changes in other spectral and chemical DOM characteristics during irradiation. Although neither DOM source represented an end member in an allochthonous to autochthonous source gradient, photooxidation of the wetland and the riverine DOM had significant, sometimes opposite, effects on Cu complexation, demonstrating important uncertainties for Cu bioavailability.

## Methods

### DOM collection and residence time calculations

We collected DOM during spring runoff (16 and 17 June 1999) from two surface waters with

contrasting hydrology: the Laramie River (41.10° N, 106.01° W, 2,280 m; designated LRDOM), and Chimney Park wetland (41.05° N, 106.10° W, 2,715 m altitude; designated CPWDOM) in the Rocky Mountains of Wyoming, USA. The Laramie River is a third-order, cobble-bottom river that drains a mixed grassland (mainly *Poaceae*)/lodgepole pine (*Pinus contortus*) watershed. Chimney Park, a palustrine wetland, receives a mixture of organic input from the adjacent lodgepole pine forest, willows (*Salix* sp.), emergent sedges (*Carex* sp.), and submersed vascular macrophytes (*Myriophyllum* sp.) colonized by epiphytic microbial communities.

Filtered natural waters (0.2- $\mu$ m filters, Corning Costar) were passed through a  $H^+$  cation-exchange resin that removed  $\geq 95\%$  of all metals. After processing for approximately 8 h through a portable reverse osmosis system (PROS/2S, E.M. Purdue; manufacturer, Serkiz & Perdue, 1990) concentrates were stored in the dark at 4°C in acid-washed, ashed (500°C for 2 h), borosilicate bottles at circumneutral pH to prevent spontaneous hydrolysis at pH nearing or below 2. Monitoring of storage effects over the span of several months indicated no change in DOC concentration or the rate of DOC loss during irradiation. Previous comparisons of natural organic matter from raw filtered water, sorption onto XAD resin, and reverse osmosis show that reverse osmosis does not alter DOM structure or elemental composition (Maurice et al., 2002).

For Chimney Park wetland, we calculated the residence time based on water volume, channelized inflow and outflow, and assumed that groundwater inflow and outflow were approximately equal in this relatively flat basin (Taube, 2000). To calculate the transport period to the next major tributary Sand Creek, we calculated average channel volume for the reach of interest and obtained discharge rates for the Laramie River from the USGS gauging station (#06659500) located 2 Km downstream from our field site. Because it was a more conservative estimate, we used the June average discharge in calculations, which was slightly higher than the 1 day discharge at our field site.

## Chemical analyses

We analyzed DOC concentration by catalytic combustion (Shimadzu 5000A), anions by ion chromatography (Dionex DX-100), and cations, Ni, and Zn by flame atomic absorption spectrophotometry (Perkin-Elmer 372). Other trace metal concentrations were determined by graphite furnace atomic absorption spectrophotometry (Varian SpectrAA-600). pH, alkalinity and hardness were determined according to standard methods (APHA et al., 1995). We analyzed changes in dissolved O<sub>2</sub> concentration by Winkler titration.

## Irradiation of DOM

Photodegradation experiments were conducted at the University of Wyoming at radiation intensities simulating natural sunlight. Photoreactivity depends on absorbance of electromagnetic radiation rather than DOC concentration. Consequently, we normalized photoreactivity by diluting DOM concentrates to a standard absorbance of 0.33 at 350 nm in a 1-cm cell, an absorbance that is similar to that of other fresh waters (Miller & Zepp, 1995; Moran et al., 2000; Obernosterer & Benner, 2004). We irradiated triplicate DOM solutions under the full-spectrum 1.0-kW xenon lamp of a solar simulator (Atlas Suntest CPS+) for 2, 4, 8, 24, 48, and 72 h. Controls were unirradiated treatments wrapped in foil (designated as 0 h in tables and figures). To avoid confounding photochemical effects with thermal or evaporative changes, we irradiated DOM solutions sealed in quartz Hungate tubes (14.6 × 1.9 cm) in a continuous-circulation cooling bath (25°C). Radiation intensities were measured with a radiometer (International Light model 1400). For total UV detection (detector range: 250–400 nm), we used a broad band UV detector (SUD 240) with cosine correction quartz and Teflon diffusers (W#8186 and T#17733) with a total UV filter (SPS300). For UV-B detection (detector range: 265–332 nm), we replaced the total UV filter and a UVB-1 filter (#24246). UV-A intensity was calculated by subtraction of UV-B from total UV. The photosynthetically active radiation detector (SUD 033) had cosine correc-

tion quartz and Teflon diffusers (W#8186 and T#17769). Compared to noontime cloudless conditions recorded at the field sites on 23 July 1999 and 27 June 2001, radiation intensities (International Light 1400) within the solar simulator were 1.4× for UV-B (265–332 nm), 1.8× for UV-A (332–400 nm), and equal to photosynthetically active radiation (400–1,000 nm). In 1 day, surface water theoretically receives the equivalent of 7.6 h of noon sun (=12 h × 2 π<sup>-1</sup>) (Miller & Zepp, 1995). Therefore, 72 h exposures in the solar simulator equaled ~13.2 d of UV-B at our field sites.

## Cupric ion titrations and data analysis

We quantified changes in Cu–DOM complexation by analyzing results from potentiometric titrations of Cu(NO<sub>3</sub>)<sub>2</sub> into three, separately-irradiated solutions of natural DOM for each exposure period and unirradiated controls. Titrations were conducted at constant temperature (25 ± 0.8°C,  $\bar{x} \pm SD$ ) with an automated microburette, mV meter, and MTS Software (McIntosh Analytical Systems). We recorded {Cu<sup>2+</sup>} with an ion-selective electrode (Cu-ISE; Orion 9429) and a double-junction Ag/AgCl reference electrode (Orion 09-02) in solutions with an ionic strength of 0.01 mol l<sup>-1</sup> (as NaNO<sub>3</sub>). We standardized to DOC concentrations to 5 mg l<sup>-1</sup> in control and irradiated solutions. To compensate for the loss of DOC in irradiated solutions (≤19 and 22% DOC losses), we diluted DOM with decreasing amounts of 18 Mohm water. We maintained solution pH at 5.98 ± 0.02 ( $\bar{x} \pm SD$ ) during potentiometric titrations to avoid variation in Cu complexation due to changes in pH (e.g., formation of CuOH complexes). We calibrated the Cu-ISE with ethylenediamine (EN) titrations over a pCu<sup>2+</sup> range of 4.3–14 (pCu<sup>2+</sup> = -log [Cu<sup>2+</sup>]; Benedetti et al., 1995). The Nernstian response of the calibration for Cu(EN) was 28.17 mV/pCu<sup>2+</sup>. This was slightly less than the theoretical slope of 29.5 mV/pCu<sup>2+</sup> but it was consistent and reproducible. During titrations, we allowed each injection to equilibrate for 2–30 min until the rate of change in mV reading was <0.2 mV min<sup>-1</sup>. Among replicates of Cu–DOM

titrations,  $\{\text{Cu}^{2+}\}$  did not vary more than 2% at any titration point.

We based Cu–DOM complexation on two classes of ligands determined with the geochemical optimization program FITEQL 4.0 (Herbelin & Westall, 1999). The high-affinity ligand had a high conditional stability constant ( $K_1$ ) and low binding-site concentration ( $[L_1]$ ). The weaker ligand with a lower conditional stability constant ( $K_2$ ) had  $\sim 10\times$  greater binding-site density ( $[L_2]$ ) than  $[L_1]$ . We evaluated the fit of our model for exposure times 0 and 72 h by inputting organic and inorganic composition in the geochemical speciation program MINTQA2 3.11 (Allison et al., 1991) and comparing observed  $\{\text{Cu}^{2+}\}$  to calculated  $\{\text{Cu}^{2+}\}$ . For those calculations, we input conditional stability constants and ligand densities of Cu–DOM complexes, and all solute concentrations except for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$  (Table 1). We excluded those cations from calculations because several studies with humic substances (hydrophobic organics that comprise  $\sim 60\%$  of DOM; Perdue, 1985) demonstrate that alkali and alkaline earth metals do not effectively compete with Cu for binding sites (Tipping, 1993; Breault et al., 1996) because of greater affinity for Cu possibly due to diffuse-layer binding and specific-site binding of Cu (Hering & Morel, 1988; Alberts et al., 1992). Having excluded competition from these cations, our calculated  $\{\text{Cu}^{2+}\}$  were within 0.2–21% ( $\bar{x} \pm \text{SD} = 5.1 \pm 9.0$ ) of observed  $\{\text{Cu}^{2+}\}$  in DOM titration solutions.

### Alkalimetric titrations

For comparison of proton binding to Cu binding by DOM, we performed alkalimetric titrations to determine the acid dissociation constants of wetland and riverine DOM in controls and in treatments irradiated for 72 h. Under a  $\text{N}_2$  atmosphere in an anaerobic chamber,  $2 \text{ mol l}^{-1}$  analytical grade NaOH was titrated into DOM, in which the pH had been lowered to  $3.5 \pm 0.2$  ( $\bar{x} \pm \text{SD}$ ) with HCl. Our estimate of carboxyl groups may be low because many carboxyls on fulvic acid have  $\text{pK}_a$  values lower than pH 2 (Leenheer et al., 1995) and thus, were not included in our alkalimetric titrations that began at pH 3.5. Fulvic acids are

**Table 1** Chemistry of field site waters and waters used in Cu titrations

Waters	DOM [DOC] ( $\text{mg l}^{-1}$ )	pH	Alkalinity ( $\text{meq l}^{-1}$ )	Concentration $\mu\text{mol l}^{-1}$									
				$\text{Na}^+$	$\text{NO}_3^-$	$\text{Ca}^{2+}$	$\text{Mg}^{2+}$	$\text{K}^+$	$\text{Cl}^-$	$\text{SO}_4^{2-}$	Cu	Fe	
Site waters	LR	7.1 (0.2)	6.7 (0.1)	0.40 (0.02)	0.09 (<0.01)	0.01 (<0.01)	180 (0.7)	68 (0.3)	18 (0.2)	34 (0.01)	130 (0.5)	bdl	7.0 (0.06)
	CP	15.0 (0.9)	6.7 (0.1)	0.20 (0.01)	0.13 (<0.01)	bdl	606 (11)	116 (1.2)	15 (2.4)	34 (0.9)	24 (0.5)	bdl	3.8 (0.11)
Cu titration	LR	5.1 (0.6)	5.98 (0.03)	0.02 (<0.01)	1.035 (<0.01)	482 (2.0)	196 (1.6)	162 (0.9)	7.3 (0.08)	32 (3.5)	84 (8.1)	0.02 (<0.01)	2.65 (0.04)
waters	CP	5.1 (0.4)	5.98 (0.03)	0.02 (<0.01)	1.020 (<0.01)	586 (1.3)	224 (1.0)	161 (0.9)	7.0 (0.05)	43 (22.8)	37 (4.1)	0.03 (<0.01)	0.36(0.04)

The waters from the Laramie River (LR) and Chimney Park wetland (CP) field sites were filtered (0.2  $\mu\text{m}$ ) before analyses. Waters used in Cu titrations were natural DOM concentrates collected from field sites after filtration and proton cation exchange. In titration waters, we normalized DOM concentrates to a dissolved organic carbon concentration ([DOC]) of  $5.1 \text{ mg l}^{-1}$ . Values represent DOM prior to irradiation for  $\bar{x} \pm \text{SD}$  ( $n = 3$  analytical aliquots). The following analytes were below detection limits (bdl parentheses contain detection limits in  $\mu\text{mol l}^{-1}$ ): Cd (0.002), Cu (0.01),  $\text{F}^-$  (0.74), Ni (0.54), Pb (0.002),  $\text{PO}_4^{3-}$  (2.48), and Zn (0.092).

soluble at all levels of pH and comprise the majority of humic substances (McKnight & Aiken, 1998). We determined the acid dissociation constants of DOM with FITEQL. We assign functional groups based on the  $pK$  associations described by Perdue (1985) for “carboxyl” groups here having a  $pK_1$  value of  $\sim 5.8$  and “phenolic” groups with a  $pK_2$  of  $\sim 9.3$ . These designations are operational, and are not meant to represent actual entities.

### Spectroscopic and chromatographic analyses

To investigate the nature of biogeochemical changes in DOM throughout a time series of exposures, we analyzed changes in the specific ultraviolet absorbance ( $SUV_a = 2.303 \times \text{absorbance}_{254 \text{ nm}} \text{ pathlength}^{-1}$  reported as  $\text{m}^{-1} \text{ L mg}^{-1}$ ) (Weishaar et al., 2003) with a diode array spectrophotometer (Hewlett-Packard 8452A). The fluorescence index (emission ratio 450:500 nm at excitation 370 nm) (McKnight et al., 2001) was collected using a Perkin-Elmer spectrofluorometer (LS-5). We determined the percentage of humic substances in DOM by their isolation on Amberlite XAD-8 resin (Aiken et al., 1992). We measured concentrations of LMW organic acids (acetic, citric, lactic, malic, oxalic, and succinic) with ion chromatography (Dionex ICE II). Carbon group determinations were performed by natural abundance  $^{13}\text{C}$  nuclear magnetic resonance ( $^{13}\text{C}$ -NMR) spectroscopy of controls and 72 h exposures with a Varian Inova-400 NMR spectrometer, configured for solid-state NMR spectroscopy. Cross polarization-magic angle spinning (CP/MAS)  $^{13}\text{C}$  spectra were measured at 100.63 MHz using a 4-mm MAS probe. The spinning rate was 14.0 KHz with a contact time of 5.0 ms and a pulse delay of 3.0 s. Radiofrequency fields of 71.5 KHz were used for cross-polarization, corresponding to a proton 90-degree pulse width of 3.5  $\mu\text{s}$ . High power proton decoupling was performed using a decoupling field strength corresponding to 80 KHz. Gaussian line-broadening, matched to the decay of the signal, corresponding to 250–500 Hz was applied prior to Fourier transformation of the time domain data. The chemical shifts were calibrated to an external sample of hexamethylbenzene (methyl carbon at

17.35 ppm). Interpretations of the spectra are derived from Wershaw (1992).

### Results

Site water chemistries, residence time, and transport period

Site waters had circumneutral pH, and aside from Fe, all other transition metals were below detection limits (Table 1). Initial differences in site waters were removed through proton–cation exchange, such that the DOM concentrated by reverse osmosis then diluted to a standard DOC concentration of  $5 \text{ mg l}^{-1}$  (labeled as Cu titration waters) had virtually identical carbonate and nitrate concentrations with slight variation in other cations and anions. The Fe concentration was higher in CPWDOM than in LRDOM. All other transition metals and phosphate in Cu titration waters were below detection limits. Residence time of CPWDOM was 33 days. The Laramie River had an average discharge rate of  $13 \text{ m s}^{-1}$ . At that rate, the water transport period from our field site to the next tributary Sand Creek, was approximately 40 h. That period encompassed 1–2 days of daylight,  $\sim 7.6$ – $15.2 \text{ h}$  in the solar simulator.

### Cu–DOM complexation

Irradiation had divergent effects on Cu complexation by the riverine versus the wetland DOM. Photooxidation rapidly removed binding sites in LRDOM, but slowly produced them in CPWDOM (Table 2, Fig. 1). For LRDOM, at 8 h of irradiation ( $\sim 1$  day of insolation at our field sites) total binding-site densities decreased significantly (ANOVA,  $[L_1]$ ,  $P = 0.03$ ;  $[L_2]$ ,  $P = 0.01$ ), and thereafter remained relatively constant. At 72 h of irradiation, 41% and 45% of  $[L_1]$  and  $[L_2]$  were destroyed, resulting in lower Cu complexation because irradiation of LRDOM did not alter LRDOM binding affinity (i.e., unchanged  $K_1$  and  $K_2$ ). At equal DOC concentrations of  $5 \text{ mg C l}^{-1}$  across the entire titration range,  $\{\text{Cu}^{2+}\}$  was  $83 \pm 4\%$  higher (mean of percentage difference between each

**Table 2** Photochemical changes in Cu–DOM complexation, chemistry, DOM spectroscopy, and H<sup>+</sup>–DOM complexation of LRDOM and CPWDOM

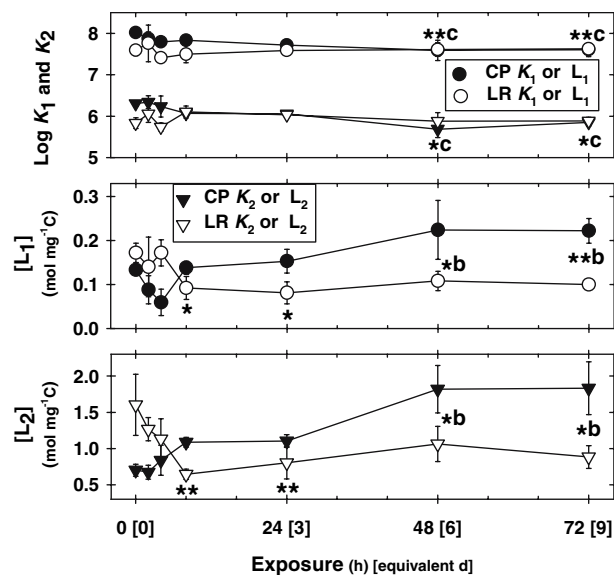
	LRDOM			CPWDOM		
	0 h	72 h	Δ (%)	0 h	72 h	Δ (%)
Log $K_1$	7.59 (0.13)	7.63 (0.08)	+10	8.02 (0.09)	7.61 (0.17)**	–61
Log $K_2$	5.83 (0.13)	5.88 (0.09)	+12	6.31 (0.07)	5.85 (0.09)*	–65
[ $L_1$ ] ( $\mu\text{mol mg C}^{-1}$ )	0.17 (0.02)	0.10 (0.01)*	–41	0.13 (<0.01)	0.22 (0.03)*	+69
[ $L_2$ ] ( $\mu\text{mol mg C}^{-1}$ )	1.60 (0.42)	0.88 (0.16)**	–45	0.70 (0.08)	1.83 (0.36)**	+161
[DOC] ( $\text{mg l}^{-1}$ )	32.17 (1.53)	25.01 (0.98)**	–22	43.32 (1.85)	35.33 (0.33)**	–19
FI	1.31 (<0.01)	1.04 (<0.01)***	–23	1.31 (0.01)	1.15 (0.01)***	–7
H S (%)	89.1 (2.6)	89.6 (8.7)	+1	88.5 (2.0)	83.7* (1.8)	–6
SUV <sub>a</sub> ( $\text{m}^{-1}/\text{mg}^{-1}$ )	3.74 (0.16)	3.75 (0.10)	+0.3	3.31 (0.05)	2.65** (0.02)	–18
[OA] ( $\text{mmol l}^{-1}$ )	0.94 (0.06)	1.58 (0.02)**	+68	1.33 (0.01)	1.44 (0.02)	+8
O <sub>2</sub> ( $\mu\text{mol l}^{-1}$ )	296.9 (22.1)	62.5 (17.7)***	–79	282.8 (2.2)	75.2 (0.2)***	–73
p $K_1$	9.04	9.27	–41	9.48	9.14	+18
p $K_2$	5.22	5.42	–34	5.49	4.98	+123
Carboxylic ( $\mu\text{mol mg C}^{-1}$ )	2.29	4.52	+97	2.79	4.42	+58
Phenolic ( $\mu\text{mol mg C}^{-1}$ )	4.14	7.07	+71	4.08	4.79	+17

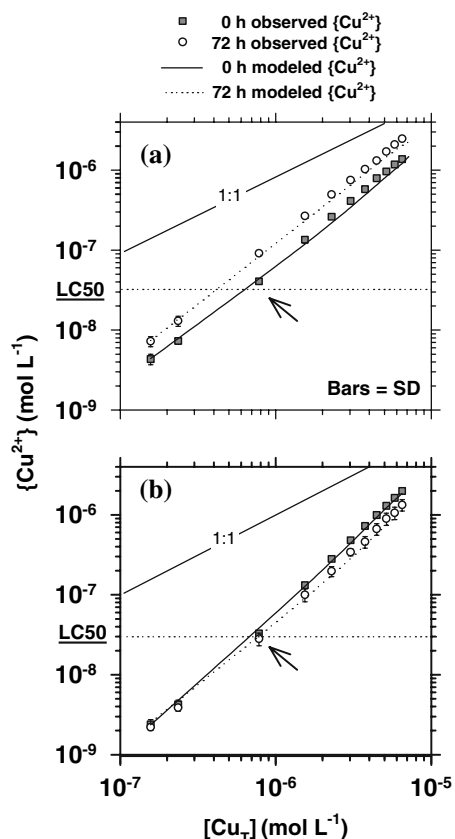
Averages ( $\pm$ SD) of analytical replicates for controls (0 h) and longest irradiation period (72 h). Values for intermediate exposures are shown in Figs 1 and 3.  $\Delta$  values are the positive or negative percent changes. FI = the fluorescence index; HS = humic substances; [OA] = sum of LMW organic acids; p $K_1$  and p $K_2$  = acid dissociation constants for H<sup>+</sup>–DOM; other abbreviations see text. Compared to unirradiated controls, \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$

titration point  $\pm$  SE) in 72-h exposures than in the unirradiated LRDOM controls (Fig. 2a). When we calculate the effect of DOC loss as lost complexation capacity due to the 22% decrease in DOC during photomineralization, {Cu<sup>2+</sup>} would have increased by  $126 \pm 5\%$  ( $\bar{x} \pm$  SE) compared to unirradiated controls (Table 2, Fig. 3).

In contrast to rapid loss of complexation capacity in LRDOM, photooxidation of CPWDOM slowly increased [ $L_1$ ] by 69% (ANOVA,  $P = 0.04$ ) and [ $L_2$ ] by 161% per mg DOC (ANOVA,  $P < 0.001$ ) (Table 2, Fig. 1). Production of binding sites became significantly greater than control densities only after 48 h of irradiation. The irradiation also decreased the binding

**Fig. 1** Time series of photochemical changes in the conditional stability constants (Log  $K_1$  and Log  $K_2$ ) and binding-site concentrations ([ $L_1$ ] and [ $L_2$ ]) for a two-ligand model of Cu–DOM complexation ( $\bar{x} \pm$  SD,  $n = 3$  analytical replicates). Unassigned asterisks indicate significant changes to both DOM types. We assign lower-case letters to changes in (c) CPWDOM alone or (b) both DOM types. \* $P \leq 0.05$ ; \*\* $P \leq 0.01$





**Fig. 2** Relationship between observed cupric ion activity ( $\{Cu^{2+}\}$ ) and total Cu concentration ( $[Cu_T]$ ) in DOM titration solutions that contained  $5\text{ mg l}^{-1}$  DOC from DOM irradiated for 0 or 72 h and collected from (a) LRDOM or (b) CPWDOM ( $\bar{x} \pm SD$ ,  $n = 3$  analytical replicates). Diagonal line shows theoretical 1:1 relationship between  $[Cu_T]$  and  $\{Cu^{2+}\}$  in the absence of DOM or inorganic ligands. Lines superimposed over symbols show fit of our modeled values for  $\{Cu^{2+}\}$ . Horizontal dashed line shows  $\{Cu^{2+}\}$  causing 50% mortality in larval fathead minnows (Erickson et al., 1996). Arrows point out  $\{Cu^{2+}\}$  changes in relation to this line before and after irradiation

affinity of CPWDOM (ANOVA,  $P < 0.01$ ). Before photodegradation, CPWDOM had greater binding affinity for Cu than did LRDOM ( $t$ -tests of analytical replicates,  $K_1$ :  $P = 0.01$ ;  $K_2$ :  $P = 0.04$ ). However, at 72 h of irradiation, binding affinities of both DOM types became virtually identical ( $t$ -tests,  $K_1$ :  $P = 0.88$ ;  $K_2$ :  $P = 0.90$ ). Despite the decreased binding affinity of CPWDOM, photochemical increases in  $[L_1]$  and  $[L_2]$  increased Cu complexation. Consequently, across all titration points,  $\{Cu^{2+}\}$  was  $26 \pm 3\%$  ( $\bar{x} \pm SE$ )

lower in irradiated CPWDOM treatments than in unirradiated controls in titration solutions containing  $5\text{ mg DOC l}^{-1}$  (Fig. 2b). Calculating the impact of the 19% loss of DOC on Cu complexation (Table 2), the DOC loss would mostly offset photooxidative production of new sites in CPWDOM. Thus, we calculate that irradiation of CPWDOM would have little overall impact on  $\{Cu^{2+}\}$  averaged across all titration points ( $-13 \pm 3\%$ ;  $\bar{x} \pm SE$ ).

#### DOM chemistry and photolytic changes

Several spectroscopic and chromatographic metrics reveal biogeochemical information about DOM. Humic substances and  $SUV_a$  provide information about bulk chemical characteristics of DOM. Humic substances have high aromatic content (McKnight & Aiken, 1998) and  $SUV_a$  is a direct correlate of aromaticity (Weishaar et al., 2003). The fluorescence index is a measure of the chemical characteristics of quinone moieties in the humic fraction (Klapper et al., 2002) and may distinguish allochthonous ( $\sim 1.4$ ) from autochthonous DOM ( $\sim 1.9$ ; McKnight et al., 2001). LMW organic acids indicate concentrations of aliphatic organics that weakly bind Cu.  $^{13}\text{C}$ -NMR spectra categorize the carbon of DOM macromolecules into functional groups such as aromatic and carboxylate moieties (Wershaw, 1992).

Prior to irradiation, DOM samples were similar in their humic fraction, fluorescence index values, and  $SUV_a$  values (Table 2, Fig. 3). Both samples were saturated with respect to oxygen. Although alkalimetric titrations indicated that phenolic and carboxylic group concentrations were similar between the riverine and wetland DOM, the binding affinity of CPDOM for protons was slightly greater than that of LRDOM. Carbohydrate carbons, ester carbons and amines (AL-I region of  $^{13}\text{C}$ -NMR spectra) dominated the carbon composition of both DOM types (Table 3, Fig. 4). Other aspects of DOM composition differed markedly. LRDOM had 25% higher absorbance at 350 nm per mg DOC (3 analytical replicates, paired  $t$ -tests,  $P = 0.01$ ) and lower total concentrations of LMW organic acids (acetic, citric, lactic, malic, oxalic, and succinic;  $P = 0.03$ ).

**Fig. 3** Time series of photochemical changes in DOM spectroscopy, and chemistry ( $\bar{x} \pm SD$ ,  $n = 3$  analytical replicates).

Abbreviations:

HS = humic substances,

Fl. Index = fluorescence

index. Unassigned

asterisks indicate

significant changes to

both DOM types. We

assign lower-case letters

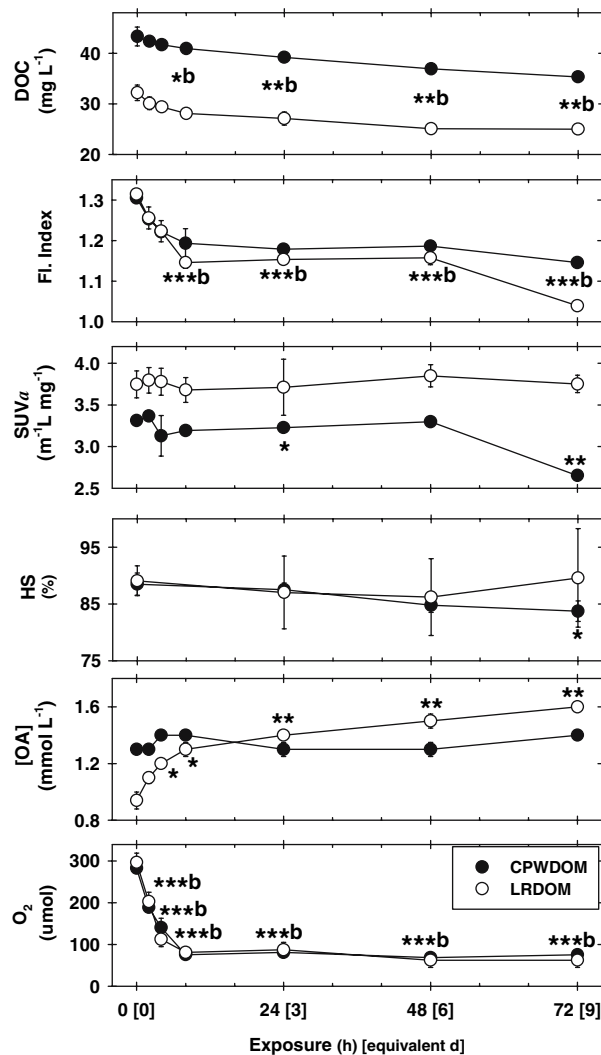
to changes in (c)

CPWDOM alone or (b)

both DOM types.

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ;

\*\*\* $P \leq 0.001$



The aromatic proportion (AR region of <sup>13</sup>C-NMR spectra) of the wetland DOM was about a third of the riverine DOM aromaticity.

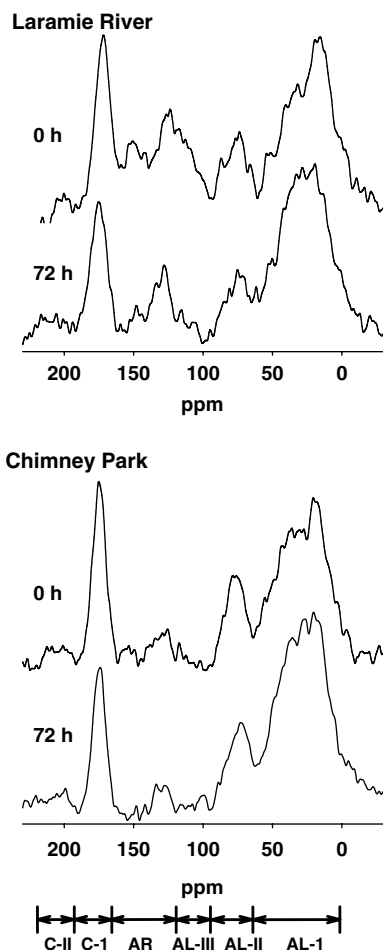
Some of these compositional similarities and differences were maintained after irradiation, others were not. Irradiation of both DOM types induced similar, significant declines in their DOC concentrations (ANOVA, DOC: LRDOM  $P = 0.01$ ; CPWDOM  $P = 0.04$ ; Table 2, Fig. 3), preserving pre-irradiation differences. Similarly, fluorescence indices decreased, becoming significantly different from controls within 8 h ( $P < 0.001$ ). At 8 h of irradiation, approximately

75% of dissolved O<sub>2</sub> concentrations were consumed, then remained at low oxidic levels in all treatments irradiated for 8–72 h. The lack of continuous oxygen input during laboratory irradiation of sealed vials possibly impeded photooxidation (Fig. 3). However, none of the treatments became anoxic and oxygen concentrations remained steady at low levels in all treatments irradiated for 8–72 h, indicating that oxygen was not the limiting factor. Nonetheless, if oxygen was limiting, then our findings are probably conservative compared to open, well-oxygenated systems.

**Table 3** Photochemical changes in structure from  $^{13}\text{C}$ -NMR spectra for LRDOM and CPWDOM

$^{13}\text{C}$ -NMR spectral regions	Description of structural groups	LRDOM			CPWDOM		
		0 h (%)	72 h (%)	Rel $\Delta$ (%)	0 h (%)	72 h (%)	Rel $\Delta$ (%)
AL-I, 0–60 ppm	Carbohydrate carbons, ester carbons and amines	41.14	54.56	+33	57.48	58.98	+3
AL-II, 60–90 ppm	Carbohydrate carbons, aliphatic alcohols and ethers	10.58	12.37	+17	13.29	13.63	+3
AL-III, 90–110 ppm	Anomeric carbohydrates, some aromatics and alkenes	3.83	1.69	-56	1.93	1.78	-8
AR, 110–160 ppm	Aromatic and olefin carbons	21.72	12.63	-42	7.53	4.67	-38
C-I, 160–190 ppm	Carboxyl carbons in carboxyl, ester, and amide groups	18.73	15.87	-15	17.67	15.34	-13
C-II, 190–210 ppm	Carbonyls in ketones and aldehydes	4.0	2.88	-28	2.11	5.60	+165
AR/AL-I	Ratio of aromatics to aliphatics	0.53	0.23	-56	0.13	0.08	-38
C-II/AR	Ratio of carbonyls to aromatics	0.18	0.23	+28	0.28	1.20	+328

Data are presented for controls (0 h) and longest irradiation period (72 h). Values are percentages of total carbon in each structural group.  $\Delta$  values (Rel  $\Delta$ ) are the relative differences in the percentages of structural groups before and after irradiation



**Fig. 4**  $^{13}\text{C}$ -NMR spectra of Chimney Park wetland DOM and Laramie River DOM irradiated for 0 h and 72 h. Table 3 defines regions designated across the bottom

In contrast to source-independent changes, neither the humic fraction nor  $\text{SUVA}$  values changed during irradiation of LRDOM, whereas, in CPWDOM both decreased significantly, becoming different from unirradiated controls after 72 h (ANOVA,  $P < 0.04$ ). Conversely, irradiation eradicated previously great differences in the concentration of LMW organic acids between the two DOM sources by producing 68% higher concentrations in LRDOM (ANOVA,  $P < 0.001$ ) while failing to change the total concentration or the relative proportion of any of the organic acids in CPWDOM. The first- ( $\text{p}K_1$ ;  $\text{p}K = -\log K$ ) and second-acid dissociation coefficients ( $\text{p}K_2$ ) of alkalimetric titrations increased in LRDOM, but decreased in CPWDOM (Table 2), resulting in slightly lower affinities for protons in irradiated CPWDOM. According to this operational assessment, irradiation increased carboxylic content more in LRDOM (+97%) than in CPWDOM (+58%) effecting similar binding-site densities for protons.

With regard to structural groups of carbon molecules, the end result of irradiation was similar proportions of carbohydrates in both DOM sources because it had little effect on the carbohydrate fractions in CPWDOM but greatly modified carbohydrate fractions in LRDOM (increased AL-I and AL-II, decreased AL-III regions of  $^{13}\text{C}$ -NMR spectra; Table 3, Fig. 4). Similar decreases in aromaticity and carboxylic carbons (AR and C-I regions) during irradiation

maintained the higher ratio of AR to aliphatic moieties (A1-I) in LRDOM than in CPWDOM. Irradiation had opposite effects on the proportions of carbonyls in ketones and aldehydes associated with aromatics (C-II region), decreasing them by 28% in LRDOM and increasing them by 165% in CPWDOM. As a consequence, the ratio of carbonyl binding sites to aromatic structures (C-II/AR regions) showed little change in LRDOM (+22%), but increased by 328% in CPWDOM after irradiation, such that CPWDOM had a 5-fold greater ratio of C-II/AR groups and twice as many C-II groups compared to LRDOM.

## Discussion

### Source and photooxidative effects on Cu–DOM complexation

Over 20 years ago, McKnight et al. (1983) demonstrated that the binding affinities and complexation capacities of the fulvic acid fraction of DOM for Cu vary among sources (Table 4). In addition to source-dependent distinctions, Cu complexation by humic substances changes seasonally (Breault et al., 1996). Those studies, and data presented here (Table 2), demonstrate that conditional stability constants can vary by more than an order of magnitude and binding-site

densities by 200–300% among DOM sources. The biotic ligand model (USEPA, 2003), the current basis of water quality criteria for Cu, is an extension of the Windermere Humic Aqueous Model developed by Tipping (1994). In that model, the high-affinity and low-affinity ligands bracket the conditional stability constants of other two ligand models presented here. Binding-site densities are greater than those of other models.

Photooxidation rates and prior irradiation of DOM undoubtedly produce the array of Cu-binding patterns observed in various time-series studies of Cu–DOM complexation (Table 5). The pattern of photooxidative changes in LRDOM (Fig. 1) was similar to the rapid decrease in site densities with unchanged binding affinity reported for the strong Cu ligand in DOM from the upper reach of Cape Fear estuary in North Carolina, USA (salinity = 23 psu). In that study, Shank et al. (2006) developed a one-ligand model with a fixed binding affinity. They acknowledged that other ligands were present, though beyond their scope of research. In a second experiment, binding site destruction was gradual in DOM collected from a more saline portion of the estuary ( $S = 23$  psu). During irradiation of DOM from Lake Hayes in New Zealand, binding-site densities in a strong, one-ligand Cu model also declined gradually (Table 4). However, that change was accompanied by a relatively rapid

**Table 4** Cu-organic complexation parameters from other studies

Organic type/field source	Collection date	Log $K_1$	Log $K_2$	$[L_1]$ ( $\mu\text{mol mg C}^{-1}$ )	$[L_2]$ ( $\mu\text{mol mg C}^{-1}$ )	Ref.
HS/Blackstone River	12/28/93	7.7	5.7	0.23	1.1	Breault et al. (1996)
	8/24/94	7.2	5.4	0.26	1.3	
Nashua River	1/03/94	7.0	5.4	0.25	1.1	McKnight et al. (1983)
	8/25/94	7.3	5.8	0.24	1.1	
Williams River	8/03/94	8.1	6.1	0.13	0.8	
HS/Ohio River	n.r.	8.3	6.6	0.12	0.65	
Brainard Lake	n.r.	7.0	5.4	0.38	1.20	
Yuma Canal	n.r.	8.5	6.0	0.12	0.59	
Fulvic acid (modeled)	n.a.	<b>9.64</b>	3.26	1.42*	2.37*	Biotic Ligand Model (BLM)
Humic acid (modeled)	n.a.	<b>8.55</b>	4.02	0.99*	1.65*	Tipping (1994), USEPA (2003)

Conditional stability constants were fixed in the BLM (bold font), meaning that only binding-site concentrations were allowed to vary (Tipping, 1994; USEPA, 2003). Much of the BLM is based on early work by Tipping (1994). The BLM model assigns all DOC to either fulvic or humic acids in a 9:1 ratio. \*In the BLM, the subset of bi-dentate Cu sites, shown here, was calculated by decreasing the reported proton-binding sites by a proximity factor of 40% (fulvic acid) and 50% (humic acid) (Tipping, 1994). n.r. = not reported, n.a. = not applicable because theoretically modeled

**Table 5** Time-series experiments of the effect of DOM irradiation on Cu complexation

Organic type/field source	Irradiation (d)	Log $K_1$	$[L_1](\mu\text{mol mg C}^{-1})$	Ref.
DOM/Cape Fear, Exp 1	0.0	<b>13.5</b>	0.020	Shank et al. (2006)
	1.5	<b>13.5</b>	0.011	
	14.5	<b>13.5</b>	0.001	
Exp 2	0.0	<b>13.5</b>	0.012	Sander et al. (2005)
	1.5	<b>13.5</b>	0.009	
	14.5	<b>13.5</b>	0.001	
DOM/Lake Hayes	0.0 h†	16.3	0.0038	Sander et al. (2005)
	30.0 h†	16.7	0.0036	
	175.0 h†	16.8	0.0029	

These studies characterized only the strong Cu ligand while acknowledging the presence of other weak Cu ligands. Bold font indicates that conditional stability constants were fixed, meaning that only binding-site concentrations were allowed to vary (Shank et al., 2006). †Sander et al. (2005) did not convert exposure in their solar simulator to equivalent days of ambient insolation

increase in binding affinity (Sander et al., 2005). We observed yet another pattern of slow photo-oxidative increases in binding-site density concomitant with decreased binding affinity in CPWDOM.

#### Ecological implications of Cu–DOM complexation

Considering the disparate patterns of Cu–DOM complexation reported here and in the literature, it is not surprising that DOM from different sources varies widely in its protection of aquatic organisms against metal uptake and toxicity. For example, the 50% effective concentrations (EC50) for immobilization of *Daphnia magna* ranged from 50.6  $\mu\text{g Cu l}^{-1}$  to 129  $\mu\text{g Cu l}^{-1}$  in the presence of 2 mg DOC  $\text{l}^{-1}$  from 4 different sources of DOM (De Schamphelaere et al., 2004). Comparing the protective effect of 5 mg DOC  $\text{l}^{-1}$  from 18 sources against 65  $\mu\text{g Cu l}^{-1}$ , Schwartz et al. (2004) observed that the time to 50% mortality for juvenile rainbow trout (*Oncorhynchus mykiss*) varied 4-fold from 24 h to 96 h. In another study with exposures containing 6.1 mg DOC  $\text{l}^{-1}$  and a mixture of six metals (Ag, Cd, Co, Cu, Hg, and Pb), DOM with the most allochthonous properties from Luther Marsh in Ontario induced the highest rainbow trout survival (96%). Under the same conditions, DOM with the most autochthonous characteristics from Sanctuary Pond was the least protective, increasing survival to only 63% (metals-only

exposures had 0% survivorship; Richards et al., 2001).

Because the availability of Cu to biological binding sites (e.g., fish gills) depends primarily on  $\{\text{Cu}^{2+}\}$  in the water, our findings have important implications for determining water quality criteria for metals. For Cu, criteria based on the recently-established biotic ligand model take into account the binding of Cu with biotic ligands (i.e., sensitive receptor tissue such as fish gills) and DOC concentrations in surface waters (e.g., USEPA, 2003). Larval fathead minnows (*Pimephales promelas*; Log  $K_{\text{Cu-minnow}} = 6.5$ ; Brooks et al., 2006) and the gills of adult fish (range Log  $K_{\text{Cu-gill}} = 7.2\text{--}8.4$ ) bind Cu about as strongly as DOM (Tables 2 and 4). Because the binding-site densities of fish are much lower (range = 0.002–0.06  $\mu\text{mol g}^{-1}$  wet weight) than that of DOM from many sources (Playle et al., 1993; MacRae et al., 1999; Taylor et al., 2003; Brooks et al., 2006) these biotic ligands have virtually no effect on bulk  $\{\text{Cu}^{2+}\}$  in the water column. For example, larval fish bound <0.2% of total Cu (based on MINTEQA2 calculations at circumneutral pH) and had no influence on bulk-water speciation of Cu at realistic biomass densities in exposure waters containing Cu concentrations near the LC50 (Brooks et al., 2006). In the biotic ligand model, all DOM has the same Cu-complexation characteristics regardless of DOM source, biodegradation, or the extent of sunlight exposure. However, our findings demonstrate important uncertainties.

Although prior to irradiation, average  $\{\text{Cu}^{2+}\}$  in titrations was not significantly different between the two DOM sources at the same DOC concentration, they responded quite differently to irradiation. After photodegradation for 72 h, Cu binding-site densities in LRDOM were half that of CPWDOM, but binding affinities were virtually identical. Thus, average  $\{\text{Cu}^{2+}\}$  had become  $148 \pm 16\%$  ( $\bar{x} \pm \text{SE}$ ) higher in LRDOM than in CPWDOM. This finding provides an overview of how photooxidation alters Cu–DOM complexation, but is difficult to evaluate biologically without reference to benchmark toxicity data. As a comparison, Erickson et al. (1996) reported 50% mortality (LC50) of larval fathead minnows (*Pimephales promelas*) as a function of  $\{\text{Cu}^{2+}\}$ . LC50 occurred at  $0.035 \mu\text{mol l}^{-1}$   $\{\text{Cu}^{2+}\}$  (horizontal, dashed line in Fig. 2). In the region of this biologically-relevant cupric activity, irradiation of LRDOM more than doubled  $\{\text{Cu}^{2+}\}$  from  $0.041 \mu\text{mol l}^{-1}$  in controls to  $0.091 \mu\text{mol l}^{-1}$ , increasing  $\{\text{Cu}^{2+}\}$  well above 50% mortality in fathead minnows. In contrast, irradiation of CPWDOM would probably have had little effect on Cu toxicity because  $\{\text{Cu}^{2+}\}$  decreased only slightly from  $0.033 \mu\text{mol l}^{-1}$  to  $0.028 \mu\text{mol l}^{-1}$ .

The potential impacts of these changes are environmentally realistic for our experimental system because the 33 days residence period in the Chimney Park wetland far exceeded the equivalent irradiation of our tests. LRDOM received 1–2 days of solar insolation before reaching the confluence with the next tributary, Sand Creek. Any significant geochemical or spectral changes that occurred in LRDOM did so within the first 8 h of irradiation in the solar simulator (~1 day of ambient sunlight) (Figs. 1 and 2). Thus, if we assume a conservative estimate of 1 day of sunlight exposure prior to dilution by Sand Creek waters, the changes we report in LRDOM could readily occur under these field conditions.

#### DOM source and transformation

Separating the causes of distinctions in Cu complexation by natural DOM from different sources with varied histories of environmental transformation is extremely challenging. Certainly, dia-

genetic processes contribute to compositional differences in DOM. Biodegradation pathways can alter fatty acids (Jandl et al., 2002), total organic carbon (Moore et al., 2003) and molecular composition (Certini et al., 2004) during residence in soils and wetlands. In addition, DOM differs in composition because of distinctions between allochthonous and autochthonous chemistry. In this study, fluorescence index values suggested that quinones in both unirradiated DOM sources derived from allochthonous sources. However, compared to LRDOM, 25% of the DOC in CPWDOM did not absorb UV radiation, did not fluoresce at an excitation wavelength of 370 nm, and thus, did not contribute to the fluorescence index. Another measure of autochthonous input is the ratio of structural aromatic to aliphatic carbon (AR/AL-I, Table 3). According to this ratio, LRDOM derived from a mixture of aquatic and terrestrial materials (reviewed in McKnight et al., 1997). CPWDOM had a lower AR/AL-I ratio similar to fulvic acid from Antarctic lakes that receive solely autochthonous inputs. In addition, lack of photoproduction of LMW organic acids in CPWDOM is consistent with this inert aspect of algal cultures (Obernosterer et al., 2001; Obernosterer & Benner, 2004). Thus, it is likely that a significant fraction of CPWDOM originated from autochthonous sources.

#### Photochemical dynamics and LMW compounds

Regardless of original source or environmental transformations prior to our collecting DOM, to elucidate causal mechanisms we evaluated how Cu-binding moieties functioned and changed during photooxidation. Other compositional changes in DOM indicate some critical links in understanding why photooxidation had opposite effects on Cu complexation by our two DOM types.

Molecular size determines the selective complexation of Cu versus protons on hydrogen-exchangeable binding sites. Small humic molecules ( $\leq 1,000$  Da) with less electrostatic potential dominate proton complexation. In contrast, large molecules ( $\geq 5,000$  Da) with greater electrostatic

potential preferentially bind Cu (Bartschat et al., 1992). Indeed, Leenheer et al. (1998) concluded that small aromatic molecules with functional groups directly attached to aromatic rings such as salicylates and phthalates preferentially bind protons and thus, are unimportant to metal binding because of proton competition with the metal for binding sites (see also review by Tipping, 2002). Here, neither carboxyls in alkalimetric titrations nor those in  $^{13}\text{C}$ -NMR spectra correlated with binding-site density in both DOM types (Tables 2 and 3). Alkalimetric titrations indicated that only about half of hydrogen-exchangeable, carboxyl groups complexed Cu. This high percentage of hydrogen-exchangeable carboxyl groups suggests that small molecules comprised much of the DOM. Moreover, we conclude that non-aromatic, carboxylic, LMW organic acids were not strong Cu ligands because their concentrations did not correlate with Cu complexation.

Photochemical production of LMW compounds likely explains why the fluorescence index did not correlate with Cu–DOM complexation. Given that our DOM samples contained high proportions of humic substances and that humic substances that bind metals fluoresce (Weber, 1988), the lack of correlation between the fluorescence index and Cu–DOM complexation was unexpected. However, as discussed above, the fluorescence index is not a universal measure of DOM composition. For example, the fluorescence index cannot predict humic substance concentration because not all humics fluoresce. The stability of the maximal emission peak ( $<2\%$  shift) and emission intensities at 450 nm ( $\leq 3\%$  decrease) indicate retention of the quinones that fluoresce at these wavelengths (Klapper et al., 2002). Emission intensities at 500 nm increased (+18% LRDOM; +10% CPWDOM), caused broader emission peaks, which are consistent with photooxidative cleavage of DOM into smaller molecular weight compounds (De Haan, 1993) that emit with greater intensity than high molecular weight fractions (Green et al., 1992). Essentially, these findings indicate that the fluorophores and their LMW products that generate the index do not reflect the mechanisms responsible for disparate changes in Cu–DOM complexation.

### Photochemical factors that tracked Cu complexation

The electron-withdrawing character of aromatic components in DOM such as quinones and lignin-derived polyphenols increases the overall molecular acidity of their electronegative substituents that bind Cu in multi-dentate complexes (Leenheer et al., 1998; Pasquarello et al., 2001). Consequently, it is logical that photochemical trends in these traits coincided with the strength of conditional stability constants during irradiation (Table 2). Irradiation of CPWDOM decreased  $K_1$  and  $K_2$ , humics,  $\text{SUVA}$  (Fig. 1), and aromaticity (Table 2). Conversely, in LRDOM  $K_1$  and  $K_2$ , humics, and  $\text{SUVA}$  were unchanged by photooxidation. Decreased aromaticity of LRDOM after photolysis, without concomitant change in  $\text{SUVA}$ , indicated that photomineralization removed DOC from AR, which conserved the ratio of aromatics to DOC concentration. In CPWDOM,  $\text{SUVA}$  decreased as aromaticity decreased during irradiation. Photobleaching outpaced DOC loss, indicating that irradiation converted aromatics to new organic compounds rather than removing them.

Strong binding sites in humic substances are mainly short-chain, oxygen-functional substituents on aromatics that may include ethers, esters, di-basic carboxylic acids, aldehydes, and ketones. Carbonyls in ketones and aldehydes (C-II region) correlated strongly with the lower (LRDOM) or higher (CPWDOM) trends in binding-site densities (Tables 2 and 3). This class of oxygen-functional carbons is located in the most downfield, unshielded region of  $^{13}\text{C}$ -NMR spectra, representing moieties that are highly electronegative, probably because of association with large aromatic molecules. Thus, it is logical that relatively small changes in these ligands could influence binding-site densities.

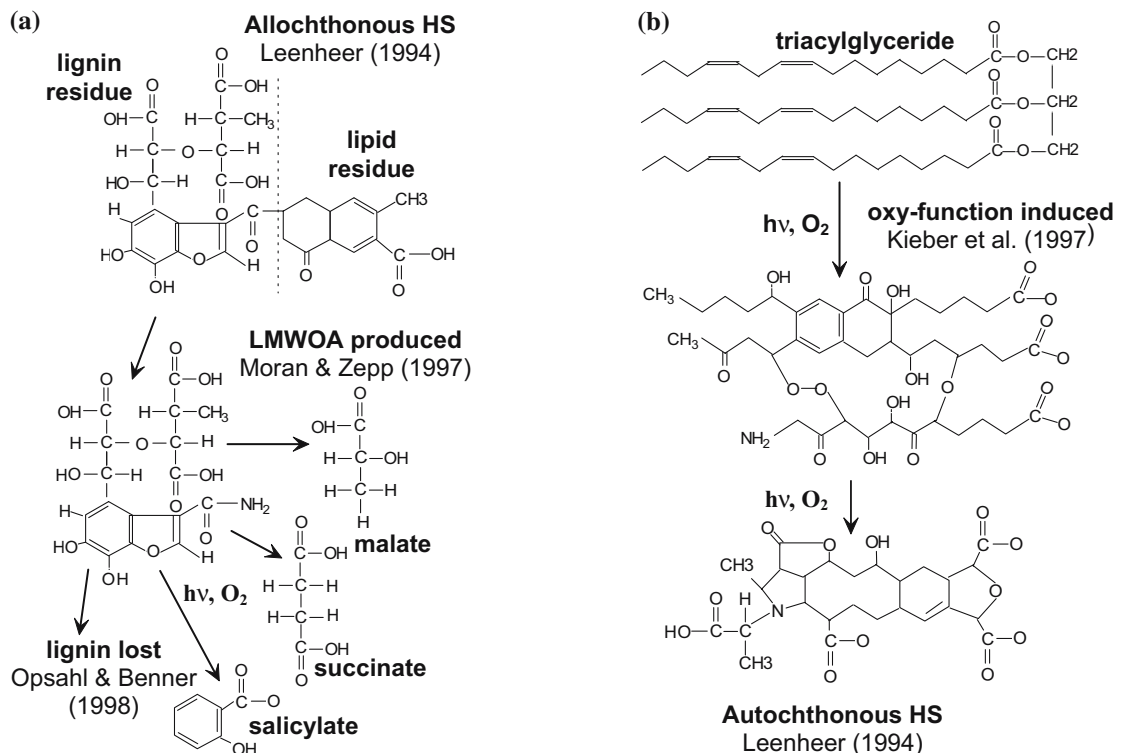
### Potential photochemical mechanisms that alter Cu–DOM complexation

Although the evidence is correlational, we theorize that the ratio of carbonyl to aromatic carbon groups (C-II/AR region, Table 2) largely explains trends in binding-site densities between the two

DOM sources after irradiation (Fig. 5). In LRDOM, we propose that photooxidation had no net effect on conditional stability constants or  $SUV_a$  because the relative balance in this ratio was maintained (e.g., a small 24% increase versus the 328% increase in CPWDOM). In LRDOM, it appears that photomineralization destroyed lignin-derived polyphenols (Opsahl & Benner, 1998) via photolytic cleavage of aromatic rings (Sun et al., 1998), which decreased aromaticity and DOC (Fig. 5a). Scission of ester substituents from lignin-derived polyphenols (Castellan et al., 1987) cleaved their carbonyl substituents in the C-II region, releasing them as LMW organic acids, which accounts for the doubling of LMW organic acids and large increases in AL-I and AL-II regions (Moran & Zepp, 1997).

In addition, photolytic decreases in binding-site densities in LRDOM might have occurred because of loss of nitrogenous ligands. At low Cu concentrations, high-affinity nitrogenous moieties dominate complexation (Croué et al., 2003), but comprise only 2–4% of DOM (McKnight et al., 1997; Croué et al., 2003). Model- (Langford et al., 1973) and natural-organic nitrogen are readily photomineralized (Bushaw et al., 1996). Thus, increased  $\{Cu^{2+}\}$  in the lowest total-Cu portion of LRDOM titrations (Fig. 2a) might be due to the 15% loss of amide carboxyls.

In CPWDOM, we hypothesize that photolysis decreased binding affinity via scission of the allochthonous fraction of lignin-derived polyphenols (Opsahl & Benner, 1998; Sun et al., 1998) (Fig. 5a). In algal-derived DOM containing



**Fig. 5** Modeled precursors and photooxidative ( $h\nu$ ,  $O_2$ ) changes in (a) allochthonous humic substances (HS; lignin and lipid components of Suwannee River fulvic acid, top molecule redrawn from Leenheer 1994) and (b) triacylglyceride of autochthonous HS. Photochemical changes in allochthonous DOM (a) include production of low molecular weight carboxylic acids (after Moran & Zepp, 1997), lignin phenols (after Opsahl & Benner, 1995), and

photochemical loss of lignin (Opsahl & Benner, 1998). Modeled photooxidation of autochthonous polyunsaturated lipids (b) yields increasingly oxidized alicyclic aldehydes (Harvey et al., 1983; Kieber et al., 1997) that result in the structure of fulvic acids from Soda Lake, Utah, USA (top two molecules of polyunsaturated fatty acid redrawn from Kieber et al. (1997), bottom molecule redrawn from Leenheer, 1994)

higher proportions of lipids (McKnight & Aiken, 1998; McCallister et al., 2006), we theorize that irradiation doubled the binding-site density of CPWDOM by polymerizing and photooxidizing polyunsaturated lipids from microbial communities (Fig. 5b). Harvey et al. (1983) proposed that polymerization and photooxidation of polyunsaturated lipids from phytoplankton were responsible for production of humic substances in marine environments. Kieber et al. (1997) showed that photooxidation of polyunsaturated fatty acids and triacylglycerides increased their oxygen functional group content and produced aliphatic aldehydes. Thus, lipid photooxidation might have caused the 165% increase in ketone and aldehyde carbonyls (C-II region), which greatly increased the ratio of carbonyl groups to aromaticity (Table 3). That is, the number of metal-binding substituents per aromatic moiety increased, producing binding-sites with weaker conditional stability constants in the residual CPWDOM. Unchanged complexation by irradiated CPWDOM at the onset of titration (i.e., lowest 3 titration points) suggests that net complexation by strong ligands, possibly nitrogenous compounds, was unaffected by photooxidation.

## Conclusion

Our study of the effects of photooxidation of DOM on Cu complexation indicate that: (1) carboxyl concentrations, the fluorescence index, and release of LMW organic acids are not general predictors of Cu–DOM complexation; (2) *SUV<sub>a</sub>* and humic substances consistently covaried with conditional stability constants within both DOM sources, and (3) small changes in carbonyls in ketone and aldehyde moieties appear to strongly influence binding-site densities.

Based on our findings, it is possible that current increases in UV radiation due to stratospheric ozone loss (McKenzie et al., 1999) will have greater impact on aquatic biota in waters dominated by inputs of allochthonous DOM, than in natural waters with low relative aromaticity and high primary productivity. Substantial, but divergent, photochemical effects on Cu–DOM com-

plexation indicate that both light regime and DOM source can strongly influence Cu bioavailability in aquatic ecosystems.

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