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1 **Photooxidation-Induced Changes in Optical, Electrochemical and Photochemical**
2 **Properties of Humic Substances**

3

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29 **Abstract**

30 Three dissolved humic substances (HS), two aquatic fulvic acids and one soil humic acid
31 were irradiated to examine the resulting changes in HS redox and photochemical properties, the
32 relationship between these changes, and their relationship to changes in the optical properties.
33 For all HS, irradiation caused photooxidation as shown by decreasing electron donating
34 capacities. This was accompanied by decreases in specific UV absorbance and increases in the
35 E2/E3 ratio (254 nm absorbance divided by 365 nm). In contrast, photooxidation had little effect
36 on the samples' electron accepting capacities. The coupled changes in optical and redox
37 properties for the different HS suggest that phenols are an important determinant of aquatic HS
38 optical properties and that quinones may play a more important role in soil HS. Apparent
39 quantum yields of H₂O₂, •OH, and triplet HS decreased with photooxidation, thus demonstrating
40 selective destruction of HS photosensitizing chromophores. In contrast, singlet oxygen (¹O₂)
41 quantum yields increased, which is ascribed to either decreased ¹O₂ quenching within the HS
42 microenvironment or the presence of a pool of photostable sensitizers. The photochemical
43 properties show clear trends with SUVA and E2/E3, but the trends differ substantially between
44 aquatic and soil HS. Importantly, photooxidation produces a relationship between the ¹O₂
45 quantum yield and E2/E3 that differs distinctly from that observed with untreated HS. This
46 suggests that there may be watershed-specific correlations between HS chemical and optical
47 properties that reflect the dominant processes controlling the HS character.

48

49 **Introduction**

50 Dissolved organic matter (DOM) is a ubiquitous component of natural surface waters
51 produced by transformation of plant and plankton-derived precursor molecules. It comprises

52 moderately hydrophobic aromatic polyelectrolytes of variable molecular weight (100's to 1000's
53 of g/mol) (1,2) and plays an important role in the biogeochemistry of aquatic environments. For
54 example, microorganisms use DOM as a source of C and N and as an electron shuttle in
55 anaerobic respiration (3). DOM also plays important roles in pollutant dynamics, for instance by
56 sorbing organic contaminants and chelating trace and heavy metals (4,5). Absorption of sunlight
57 by aromatic chromophores in DOM (1,6) leads to formation of reactive oxygen species (ROS)
58 including singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot\text{OH}$) (7,8).
59 DOM triplet states ($^3\text{DOM}^*$) are both important precursor species for many of these ROS and
60 strong oxidants in DOM-sensitized photoreactions (7-9). Together, these photooxidants play a
61 critical role in the redox speciation of trace metals (10-12), transformation rates of organic
62 contaminants (9,13-15), and solar inactivation of pathogens (16).

63 Absorption of sunlight also leads to DOM photobleaching (destruction of chromophores),
64 photooxidation, and production of low molecular weight organic compounds and inorganic
65 species such as CO and CO_2 (17-23). These processes may involve the loss of specific
66 functional moieties and lead to changes in the physicochemical and optical properties of DOM.
67 For example, lignin phenols disappear rapidly in the early stages of photooxidation (24-26).
68 Other studies have used FT-ICR-MS and ^{13}C NMR spectroscopy to show that DOM loses
69 aromatic groups during photooxidation (27,28). Concomitant changes in DOM optical
70 properties are consistent with a loss of DOM aromaticity (% aromatic C by ^{13}C NMR), including
71 decreases in specific UV absorbance (SUVA, absorbance per mg-C) and fluorescence intensity,
72 and increases in spectral slope and the E2/E3 ratio (ratio of the absorbance at 254 to 365 nm)
73 (29-35).

74 The influence of photooxidation on DOM photochemistry remains poorly investigated
75 and understood. Substantial effects seem plausible given that photooxidation changes E2/E3 and
76 SUVA values and that these parameters correlate with quantum yields of $^1\text{O}_2$ and CO
77 photoproduction (36-41). Zhang *et al.* reported that prolonged irradiation decreased quantum
78 yields for CO production (38). To our knowledge, however, no systematic study exists of how
79 irradiation affects photooxidant quantum yields. Cavani *et al.* reported that $^1\text{O}_2$ production rates
80 from peat humic acid were unaffected by eight hours of irradiation at 365 nm (42). In contrast,
81 Andrews *et al.* reported that H_2O_2 quantum yields for various aquatic samples decreased with
82 increasing irradiation using simulated sunlight (43). The results of these two studies are,
83 however, difficult to compare since they not only involve different ROS but also used different
84 samples, methods, irradiation times, and assessment endpoints (i.e., production rates versus
85 quantum yields). Furthermore, the observed trends were not related to the extent of DOM
86 oxidation, which was, until recently, difficult to quantify due to the lack of an appropriate
87 method. The introduction of mediated electrochemical oxidation and reduction (MER and MEO,
88 respectively) now allows reliable quantification of DOM redox state in terms of electron
89 donating and accepting capacities (EDC and EAC) (44,45). The EDC and EAC of DOM have
90 been ascribed to phenol and quinone moieties, respectively, which are also chromophores
91 believed to play an important role in DOM photochemistry (8,9,46-49).

92 The objective of this study was to systematically investigate the effects of photooxidation
93 on DOM optical, electrochemical and photochemical properties. Studying these changes
94 simultaneously is expected to provide insights into relationships between DOM aromaticity,
95 redox-state, and photoreactivity and to improve understanding of the DOM photobleaching
96 process. Experiments were conducted with three dissolved humic substances (HS): two aquatic

97 fulvic acids (FAs) and, for contrast, a soil humic acid (HA). Changes in the absorption spectra
98 and apparent quantum yields for the photooxidants $^1\text{O}_2$, H_2O_2 , $\cdot\text{OH}$, and triplet HS ($^3\text{HS}^*$) were
99 measured as a function of irradiation time. The extent of photooxidation was quantified by
100 monitoring changes in EDC and EAC. Spectroscopic data were also used to examine whether
101 correlations between optical and photochemical properties for photooxidized HS are consistent
102 with reported correlations for native DOM isolates (36,37).

103

104 **Materials & Methods**

105 *Materials.* Nordic Aquatic Fulvic Acid (NAFA), Suwannee River Fulvic Acid (SRFA),
106 and Elliot Soil Humic Acid (ESHA) standards were obtained from the International Humic
107 Substances Society (IHSS, www.humicsubstances.org) and used as received. Details for other
108 materials can be found in the *Supporting Information*.

109 *Solutions for Irradiation.* All solutions were prepared with Nanopure water (Barnstead)
110 with resistivity $>18.2 \text{ M}\Omega \text{ cm}$. The photooxidation experiments were conducted at high HS
111 concentrations (250 mg/L) to ensure the availability of sufficient HS for subsequent analyses.
112 The solutions were prepared by dissolving 25 mg of solid HS isolate in 50 mL of H_2O followed
113 by addition of 1 M NaOH to adjust the pH to 8.0. After pH stabilization ($> 30 \text{ min}$), solutions
114 were stirred overnight at room temperature and subsequently diluted to a total volume of 100
115 mL. The pH was readjusted to 7.0, followed by filtration (0.22 μm) to remove particulate
116 material and sterilize the samples, which were stored for six days at 4 $^\circ\text{C}$ before use in
117 experiments.

118 *Irradiation Procedure.* Aliquots of each HS solution (20 to 25 mL) were transferred to
119 18 mm diameter quartz tubes containing a magnetic stir bar. The tubes were capped with septa

120 fitted to allow air sparging of the solutions during irradiation. Sample tubes were placed below
121 the lamp at approximately 30° from horizontal and immersed in a recirculating water bath at 25
122 °C. A Suntest solar simulator was used at a nominal setting of 700 W m⁻². The total photon flux
123 (300 to 700 nm) was 1.4x10⁻⁴ Es L⁻¹ s⁻¹, as estimated from *p*-nitroanisole/pyridine actinometry
124 (50). Samples were irradiated for a total of 59 h in periods of 11 or 12 h with continuous stirring
125 and sparging with synthetic air. The air bubbles were confined to the center of the sparged
126 solutions and, because the solutions were optically thick, likely had minimal effect on the
127 radiation delivery.

128 Solution volumes (determined gravimetrically) and pH were measured at the beginning
129 and end of each irradiation period. After each period, small amounts of water were added to
130 replace evaporative losses (always < 0.2 mL) and small volumes of 1 M NaOH were added to re-
131 adjust the pH to 7.0 (the pH never fell below 6.5). At selected intervals, aliquots of solution
132 were removed for analysis and experimentation. To allow for intra-HS redox equilibration, these
133 were stored at 4 °C for at least 3 d before conducting electrochemical and photochemical
134 experiments. All experiments and analyses were conducted within approximately 3 weeks.
135 Duplicate and triplicate photochemical and electrochemical measurements were highly
136 reproducible, indicating that there were no post-irradiation chemical alterations of the HS as
137 detected by our methods.

138 *Optical Properties and Absorbed Energy.* Absorbance spectra were collected in 1 cm
139 quartz cuvettes on a Cary 100 spectrophotometer (Varian) using 1 nm slits and phosphate buffer
140 as a blank. Prior to measuring absorbance spectra, HS solutions were diluted in 5 mM phosphate
141 buffer (pH 7.0) by a factor of three (SRFA and NAFA) or ten (ESHA) to ensure that
142 measurements fell into the linear range of the instrument. Optical parameters, including the

143 E2/E3 ratio and specific UV absorbance at 280 nm (SUVA₂₈₀) were calculated from the
144 measured spectra as detailed in the *Supporting Information*.

145 Absorption and lamp emission spectra were used to determine the energy absorbed
146 between 300 and 500 nm during irradiation (details in *Supporting Information*). This wavelength
147 range was chosen for its importance to HS photochemistry (20,34,43,47,51-56). The conclusions
148 drawn in this study are based on relative changes and change little by setting the long wavelength
149 cutoff to 400 or 450 nm.

150 *Electrochemical Measurements.* EDC and EAC values were quantified according to
151 Aeschbacher *et al.* (44,45,57) in a glovebox under N₂ (O₂ < 0.1 ppm; 25 ± 1 °C, M. Braun Ltd.,
152 Germany). Anoxic buffer solutions were used as described previously (44,45,57). HS solutions
153 (3.2 ml of each) from the photooxidation experiments were made anoxic by purging with argon
154 for 20 min prior to transfer to the glovebox. Detailed methods are provided as *Supporting*
155 *Information*.

156 *Photochemical Experiments.* Photooxidant quantum yields were determined using two
157 merry-go-round reactors containing mercury vapor lamps having emission maxima at 365 nm
158 (58-60). All quantum yields are therefore reported for excitation at 365 nm. Samples from
159 photooxidation experiments were diluted prior to measuring the production of ¹O₂, H₂O₂, •OH,
160 and triplet HS (³HS*). The transient species ¹O₂, •OH, and ³HS* were quantified using the probes
161 furfuryl alcohol (FFA), terephthalate (TPA), and 2,4,6-trimethylphenol (TMP), respectively
162 (36,59,61). Experimental details and the calculation of apparent quantum yields are provided as
163 *Supporting Information*. Production rates of H₂O₂ were quantified using the Amplex Red assay
164 (Invitrogen) (62). Analytical details and quantum yield calculations are provided as *Supporting*
165 *Information*.

166 **Results and Discussion**

167 *Optical Properties.* Irradiation caused photobleaching in all the HS. Figure 1 shows the
168 fraction of absorbance remaining and absolute changes in HS absorption coefficients after 59 h
169 (absorption spectra provided as *Supporting Information*). Consistent with previous reports,
170 larger absolute absorbance losses occurred at UV wavelengths, and higher percent losses
171 occurred in the visible. These changes were accompanied by decreases in SUVA₂₈₀ and
172 increases in E2/E3 (Fig. 2) (29,34,35,63). Large differences were observed between the soil-
173 derived ESHA and the two aquatic FA in both the magnitude of the optical property changes and
174 the type of changes observed. Specifically, SRFA and NAFA both showed extensive
175 photobleaching (Fig. 1) and clear changes in SUVA₂₈₀ and E2/E3 that were approximately linear
176 with the amount of energy absorbed (Fig. 2). In contrast, much less photobleaching occurred for
177 ESHA even though it absorbed approximately twice the 300 to 500 nm energy (Fig. 1), and the
178 changes in SUVA₂₈₀ and E2/E3 were much smaller and non-linear. These data suggest that
179 ESHA was more resistant than the aquatic FAs to photooxidation. However, it is possible that
180 this difference in resistance was exaggerated by inner-filtering in the ESHA solution. A
181 substantially higher fraction of the energy absorbed by ESHA lay in the visible (ca. 90%) than
182 for the aquatic FAs (ca. 70%), and visible irradiation is known to cause much less efficient
183 photobleaching (34,35,64). Thus, the difference between the photobleaching efficiencies for
184 ESHA and the aquatic FAs is probably not as large as implied by these data.

185 The difference in magnitude of the optical changes notwithstanding, all samples displayed
186 loss of SUVA₂₈₀ and increases in E2/E3 with photobleaching. Both changes indicate loss of
187 aromatic groups (65,66) and decreases in molecular weight (66,67). Indeed, decreases in
188 molecular weight were observed by size-exclusion chromatography (*Supporting Information*)

189 accompanied by small (ca. 10%) losses of organic-C after 59 h of irradiation (*Supporting*
190 *Information*). The molecular weight dependence of E2/E3 is believed to derive from an
191 increased probability of electronic interactions between chromophores in larger DOM molecules.
192 Specifically, intramolecular charge transfer (CT) complexes involving electron-donating groups
193 (e.g., phenols) and electron-accepting groups (e.g., aromatic ketones and quinones) produces
194 broad, featureless absorbance in the near-UV and visible (i.e., above approximately 370 nm) (68-
195 70). Thus, observed increases in E2/E3 with irradiation suggest that CT complexes are being
196 destroyed, probably by both decreasing DOM molecular weight and photooxidation of donor
197 and/or acceptor moieties in DOM.

198 *Redox Properties and Relationships to Optical Properties.* In the CT model for DOM
199 optical properties, substituted phenols are suspected electron donors, and aromatic ketones and
200 possibly quinones are suspected acceptors (68-70). Phenols and quinones are also major
201 determinants of DOM electrochemical properties. For example, a diverse set of DOM showed a
202 strong correlation between EDC and phenol contents (44) (operationally defined as 2x the
203 titrated charge between pH 8 and 10 (71)). The EDC of DOM also varies with E_h and pH in
204 ways comparable to low molecular weight phenols (44). Finally, the EAC of DOM correlates
205 well with its % aromaticity (44), and DOM accepts electrons over a range of reduction potentials
206 consistent with quinones as major electron acceptors (45).

207 Figure 3 presents the changes in EDC and EAC with irradiation (values tabulated as
208 *Supporting Information*). The EDC values of all HS samples decreased monotonically with
209 increasing irradiation, with smaller decreases for ESHA that mirror the smaller changes observed
210 in its optical properties compared to the aquatic FAs. To our knowledge, this is the first direct
211 demonstration of DOM photooxidation as quantified by EDC loss. In comparison, all HS

212 samples showed small changes in EAC. This finding strongly suggests that there was no direct
213 relation between the changes in electron donating and accepting moieties, and, hence, that
214 photooxidation did not convert electron donating phenols and hydroquinones into electron
215 accepting quinone moieties. Instead, the fact that decreases in EDC were not accompanied by
216 similar increases in EAC suggests that photooxidation irreversibly destroyed phenolic moieties.
217 The reason for the small changes in EAC is unknown. They could reflect either a resistance to
218 oxidation by the relevant moieties or a pseudo-steady state resulting from their loss and
219 formation at approximately equal rates.

220 A comparison of Figs. 2 and 3 shows that the optical properties directly reflect the extent
221 of photooxidation and suggests that the optical properties depend on the amount and nature of
222 redox active moieties. To assess this, we reanalyzed the trends in SUVA₂₈₀ and E2/E3 as a
223 function of EDC and EAC (Figure 4). For the aquatic FAs, SUVA₂₈₀ displays a linear
224 relationship to EDC ($r^2 = 0.904$). For ESHA, there is no apparent relationship, possibly due to
225 the much smaller changes in EDC with irradiation. Stronger correlations of SUVA₂₈₀ with EDC
226 for the aquatic FAs than for soil HA are consistent with prior reports that aromatic-C and EDC
227 are well-correlated for diverse aquatic HS samples but not for different soil-derived HS (44).
228 Given that phenolic moieties constitute much more of the aromatic-C in the aquatic FAs than
229 ESHA (71,72) (*Supporting Information*), it appears likely that phenols are a major determinant
230 of both EDC and SUVA₂₈₀ in aquatic HS. In contrast, SUVA₂₈₀ appears to vary more with EAC
231 for ESHA (Fig. 4), which could imply that quinones are notable contributors to SUVA₂₈₀ in soil
232 HA. However, this conclusion is tempered by the small and irregular changes in EAC with
233 photooxidation.

234 To examine the relationship of E2/E3 to EDC and EAC, a larger data set was analyzed
235 that included various untreated aquatic and soil HS (44) in addition to the photooxidized
236 samples; missing E2/E3 ratios were measured. For aquatic HS, E2/E3 shows an inverse
237 relationship to EDC, whereas for the soil HA these data are more scattered (Fig. 4).
238 Furthermore, E2/E3 appears to be more sensitive to changes in EDC (steeper slope) for the
239 photooxidized aquatic FAs than for untreated HS isolates. These observations suggest that (i) for
240 aquatic HS, E2/E3 depends strongly on phenol content and (ii) that this dependence is
241 pronounced in samples undergoing photooxidation. For soil HS, on the other hand, E2/E3 seems
242 to be more dependent on EAC (Fig. 4), whereas this relationship is a bit more scattered for
243 aquatic HS, and there is no obvious relationship for the photooxidized FAs. These differences
244 suggest that quinones contribute more to CT absorbance in soil HS than in aquatic HS.
245 Furthermore, in the aquatic FAs undergoing photooxidation, the extent of CT absorbance seems
246 to be independent of quinones. This conclusion is consistent with recent evidence that quinones
247 are not major determinants of CT absorbance in aquatic HS (46,70).

248 *Photooxidant Quantum Yields.* We further investigated the effect of irradiation on $^1\text{O}_2$,
249 H_2O_2 , and $\cdot\text{OH}$ quantum yields (Φ), defined as the fraction of absorbed photons producing
250 photooxidant. For $^3\text{HS}^*$, there is no method to quantify all of the $^3\text{HS}^*$ formed. Instead, a proxy
251 for $\Phi_{^3\text{HS}^*}$ is used, the quantum yield coefficient, f_{TMP} (M^{-1}), which is the rate constant for TMP
252 loss divided by the rate of light absorption (61). Although photochemical reaction rates are
253 sometimes quantified as carbon-normalized rate constants (provided as *Supporting Information*),
254 Φ is a more fundamental parameter with broader general applicability to photochemical
255 modeling. Figure 5 shows how $\Phi_{^1\text{O}_2}$, $\Phi_{\text{H}_2\text{O}_2}$, Φ_{OH} and f_{TMP} vary with irradiation. For all HS,
256 the quantum yields of H_2O_2 , $\cdot\text{OH}$, and $^3\text{HS}^*$ decreased, but, in stark contrast, $\Phi_{^1\text{O}_2}$ increased.

257 Attributing physical causes to these trends requires recognition that the quantum yields
258 are “apparent” because they may be affected by a variety of secondary phenomena. For instance,
259 $\Phi_{\text{H}_2\text{O}_2}$ is a function of the primary quantum yield of O_2^- and the relative rates of uncatalyzed
260 versus HS-catalyzed O_2^- dismutation (36). Because uncatalyzed dismutation is apparently the
261 dominant process during SRFA photolysis under conditions similar to those used here (73),
262 decreases in $\Phi_{\text{H}_2\text{O}_2}$ likely reflect lower O_2^- production efficiencies rather than lower dismutation
263 rates. It has recently been suggested that the excited state HS precursor to O_2^- is a long-lived
264 charge-separated species created by electron transfer from singlet excited state donors to ground
265 state acceptor moieties (74). The observed EDC loss is consistent with this model, as loss of
266 donors would reduce the yield of charge-separated species and decrease $\Phi_{\text{H}_2\text{O}_2}$.

267 In the case of Φ_{OH} , the TPA probe detects both free $\cdot\text{OH}$ and other, lower energy
268 hydroxylating species (59). Here, we do not distinguish between these hydroxylating species.
269 However, control experiments were conducted with catalase to assess the contribution of H_2O_2 -
270 dependent, Fenton-like hydroxylation (59). Irradiation did not significantly alter the fraction of
271 hydroxylation occurring through H_2O_2 -dependent pathways, which was approximately 50% for
272 ESHA and 20% for SRFA, in good agreement with a previous report (59). For NAFA, there was
273 essentially no TPA hydroxylation via H_2O_2 . Thus, only a fraction of the decrease in Φ_{OH} can be
274 ascribed to the decrease in $\Phi_{\text{H}_2\text{O}_2}$. The remainder must be due to lower quantum yields of
275 excited state oxidants, which are of unknown character, because the mechanism of $\cdot\text{OH}$
276 production by DOM is not established.

277 The parameter used to assess $^3\text{HS}^*$ formation, f_{TMP} , depends on both the primary quantum
278 yield of $^3\text{HS}^*$ and the rate constant for reaction between $^3\text{HS}^*$ and TMP (61). Notably, HS do not
279 inhibit TMP oxidation (58). Decreases in f_{TMP} thus reflect either a general decrease in $^3\text{HS}^*$

280 precursor chromophores or a decrease in a specific $^3\text{HS}^*$ pool that reacts rapidly with TMP. In
281 either case, the observed decreases in f_{TMP} indicate that photooxidant production efficiency
282 decreases with irradiation, in agreement with the Φ_{OH} results. For TMP oxidation, the $^3\text{HS}^*$
283 precursors are believed to be mainly aromatic ketones and possibly quinones (46,61). The
284 present data provide divergent evidence for the role of quinones. For instance, the aquatic FAs
285 have much lower EAC and f_{TMP} than ESHA, consistent with excited state quinones as oxidants of
286 TMP. However, this conclusion does not seem to be compatible with the fact that decreases in
287 f_{TMP} were not paralleled by comparable decreases in EAC.

288 Distinct from the other quantum yields, $\Phi_{1\text{O}_2}$ increased with irradiation. Note that the
289 FFA probe detects $^1\text{O}_2$ in the bulk aqueous phase that has escaped the HS microenvironment
290 without being quenched therein (75). It is generally accepted that $^1\text{O}_2$ is produced by energy
291 transfer from $^3\text{HS}^*$ to O_2 . Reports by Halladja *et al.* (76) and Sharpless (47) indicate that a high
292 degree of overlap exists between the $^3\text{HS}^*$ pools that produce $^1\text{O}_2$ and those that oxidize TMP. If
293 true, a simple way to reconcile the increases in $\Phi_{1\text{O}_2}$ with the decreases in f_{TMP} is to hypothesize
294 that irradiation decreases the efficiency of $^1\text{O}_2$ quenching by HS. Thus, even if the yield of $^3\text{HS}^*$
295 decreases, higher $^1\text{O}_2$ yields could be observed if $^1\text{O}_2$ is less effectively quenched within the
296 DOM microenvironment. The loss of EDC is consistent with this hypothesis because quenching
297 of $^1\text{O}_2$ by HS probably occurs by an electron transfer mechanism (77), which is expected to
298 become less effective as electron donors in HS are destroyed. Also consistent with this view is a
299 recent report that wastewater DOM $\Phi_{1\text{O}_2}$ increases with chemical oxidation by both ozone and
300 chlorine, which destroy electron rich groups selectively and non-selectively, respectively (41).
301 An alternative explanation for the increases in $\Phi_{1\text{O}_2}$ is that there may be two classes of $^1\text{O}_2$
302 sensitizer (e.g., aromatic ketones and quinones), and that only one of these (Class 1) strongly

303 determines HS optical properties. The destruction of Class 1 sensitizers would decrease
304 absorbance, but the other class of sensitizer would continue producing $^1\text{O}_2$. Hence, the results
305 can also be accommodated by a model in which aromatic ketones are Class 1 sensitizers, and
306 quinones are the other Class. This is consistent with negligible EAC loss accompanied by large
307 changes in E2/E3 in the aquatic FAs, where the loss of aromatic ketones would be expected to
308 greatly alter the optical properties (see preceding section) (46,70). The hypothesis is also
309 consistent with increasing $\Phi_{1\text{O}_2}$ in the absence of large E2/E3 changes for ESHA; here, quinones
310 may be contributing more to both the photochemical and optical properties (see preceding
311 section) as other sensitizing chromophores are destroyed.

312 *Relationship of $\Phi_{\text{H}_2\text{O}_2}$ and $\Phi_{1\text{O}_2}$ to Optical and Redox Properties.* Previous reports have
313 demonstrated correlations of E2/E3 with $\Phi_{\text{H}_2\text{O}_2}$ and $\Phi_{1\text{O}_2}$ (36,37). Sharpless and co-workers
314 have argued that these originate from the influence of CT interactions on both the optical
315 properties and photochemistry of DOM (36). Because photooxidation is an important natural
316 DOM transformation process, we explored whether our results conform to the previously
317 reported correlations. We further investigated relationships between photochemical and
318 electrochemical properties. Figure 6 shows $\Phi_{1\text{O}_2}$ and $\Phi_{\text{H}_2\text{O}_2}$ as a function of both E2/E3 and
319 EDC for samples undergoing photooxidation. Related plots involving f_{TMP} and $\Phi_{\text{H}_2\text{O}_2}$ and EAC
320 are provided as *Supporting Information*. We did not construct plots using SUVA_{280} , as it
321 correlates inversely and strongly with E2/E3 (*Supporting Information*). Such plots would simply
322 display the reverse of the trends in Figure 6. Although the directions of the trends were the same
323 for all samples, $\Phi_{\text{H}_2\text{O}_2}$ and $\Phi_{1\text{O}_2}$ vary much more with both E2/E3 and EDC for ESHA than for
324 the aquatic FAs.

325 Figure 6 also presents the results of Dalrymple *et al.*, who studied HS and DOM isolates
326 (36), and Peterson *et al.*, who studied whole water from Lake Superior and its tributaries (37).
327 Peterson *et al.*'s results are shown as the reported linear trend because the data include many
328 samples with E2/E3 values well above the range used in Figure 6. For the aquatic FAs
329 undergoing photooxidation, the directions of the trends in $\Phi_{\text{H}_2\text{O}_2}$ and $\Phi_{1\text{O}_2}$ with E2/E3 agree with
330 previous reports (36,37). The $\Phi_{\text{H}_2\text{O}_2}$ results display good quantitative agreement with those of
331 Dalrymple *et al.* (36), suggesting that E2/E3 may be a robust predictor of $\Phi_{\text{H}_2\text{O}_2}$. However, $\Phi_{1\text{O}_2}$
332 is less sensitive to changes in E2/E3 for the photooxidized FAs than for untreated DOM isolates.
333 Notably, the relationship between $\Phi_{1\text{O}_2}$ and E2/E3 for photooxidized FAs resembles that of the
334 Lake Superior samples much more than that of the untreated DOM. This suggests that
335 photobleaching is a major control on DOM optical properties and photochemistry in Lake
336 Superior. Furthermore, it indicates that DOM photooxidation or other dominant DOM
337 degradation processes in particular ecosystems may lead to watershed-specific correlations
338 between optical and photochemical properties.

339 With EDC, a weak decreasing trend in $\Phi_{1\text{O}_2}$ is observed, while $\Phi_{\text{H}_2\text{O}_2}$ increases sharply,
340 albeit with different slopes for aquatic HS and soil HS (Fig. 6). The trends can be explained in
341 terms of photophysical concepts expounded previously (36,47,74). For example, the decrease in
342 $\Phi_{1\text{O}_2}$ with EDC could reflect lower $^3\text{HS}^*$ yields in samples where higher concentrations of
343 electron donating groups engage electron accepting photosensitizers in CT complexes, thus
344 producing absorbance that does not lead to $^3\text{HS}^*$ (36,47). Conversely, the increase in $\Phi_{\text{H}_2\text{O}_2}$ with
345 EDC could result from increased formation of charge-separated excited-state precursors to H_2O_2
346 (74) as the content of electron donors increases. As shown in the *Supporting Information*, $\Phi_{1\text{O}_2}$
347 for the photooxidized FAs and several IHSS aquatic HS and DOM isolates follow similar inverse

348 relationships to both EDC and EAC, suggesting that DOM with higher concentrations of redox
349 active moieties sensitize $^1\text{O}_2$ production less efficiently. This point is also illustrated by plotting
350 $\Phi_{1\text{O}_2}$ versus the total redox activity (EDC + EAC) for all samples in this study plus others for
351 which $\Phi_{1\text{O}_2}$, EDC, and EAC are available (*Supporting Information*).

352 *Environmental Implications.* Aquatic HS have much higher EDC than soil HS, while the
353 opposite is true for EAC (44). The present results show that irradiation destroys EDC but alters
354 EAC very little. The lack of EAC creation, even while EDC is destroyed, argues against
355 photochemical oxidation of donor groups to acceptor groups. Rather, the accumulated data point
356 to irreversible oxidation, the target of which may be phenols. Additionally, the photochemical
357 results suggest that aromatic ketones and other possible $^3\text{DOM}^*$ precursors such as flavones and
358 chromones are rapidly destroyed by photooxidation.

359 These results predict a decrease in all photooxidant quantum yields except $^1\text{O}_2$ in natural
360 systems where DOM undergoes photooxidation. The direct implication of this finding is that
361 constant quantum yields for HS-derived photooxidants cannot be assumed over the course of
362 days of solar exposure. Based on the energy absorbed in our photooxidation experiments, we
363 estimate that the changes observed here would occur with approximately 240 h of summer solar
364 noon irradiation at 41°N in the upper first cm of a typical inland water (5 mg OC of aquatic
365 DOM from terrestrial sources). However, this estimate is confounded some by uncertainties,
366 such as the extent to which inner-filtering altered the observed photobleaching efficiencies in
367 these experiments and the extent to which storage of our solutions at high concentration for 3 d
368 after irradiation may have fostered intermolecular redox reactions that would not be observed in
369 natural waters. Nonetheless, the contrast between the photochemical trends for photooxidized and
370 untreated HS and DOM (Fig. 6) suggests that variability in photochemical properties of natural

371 waters may arise from multiple processes – mixing of different DOM sources, or photooxidation
372 of a single DOM source – each of which can uniquely alter the optical-photochemical
373 correlations. It is also apparent that compositional differences between aquatic and soil HS lead
374 to very different controls on the optical behavior and hence very different correlations between
375 the optical and photochemical properties. Further efforts to relate the optical and photochemical
376 properties to DOM structure will be needed to provide a sound basis for understanding DOM
377 source-dependent differences in behavior.

378

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383

384 **Supporting Information Available**

385 Chemicals used, absorption spectra; optical properties; calculations of absorbed energy;
386 electrochemistry details; photochemistry experimental details and calculations; TOC method and
387 results; SEC-OCD results; phenol and aromatic C content of HS; E2/E3 and SUVA₂₈₀
388 relationship to aromaticity; tabulated EDC and EAC; carbon-normalized rates and rate constants
389 for photochemical experiments; f_{TMP} and Φ_{OH} versus E2/E3 and EDC; photooxidant quantum
390 yields versus EAC; correlation between E2/E3 and SUVA₂₈₀; Φ_{IO_2} versus EDC and EAC and
391 versus (EDC + EAC)

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398 **Figure Captions**

399

400 **Figure 1:** Change in absorbance with irradiation time: (a) $f_{A,59h}$, fraction of absorbance
401 remaining at 59 h; (b) Δa_{59h} , change in absorbance at 59 h (base-10 absorption coefficient).

402

403 **Figure 2:** Changes in (a) $SUVA_{280}$ ($L\ mg^{-1}\ m^{-1}$) and (b) E2/E3 versus energy absorbed from
404 300 to 500 nm: (●) SRFA; (◇) NAFA; (▲) ESHA.

405

406 **Figure 3:** Change in EDC and EAC with irradiation time. Samples taken at 0, 11, 35, and 59 h.

407

408 **Figure 4:** $SUVA_{280}$ ($L\ mg^{-1}\ m^{-1}$) (top) and E2/E3 (bottom) versus EDC (pH 7, 0.73 V) and
409 EAC (pH 7, -0.49 V): (●) SRFA; (◇) NAFA; (▲) ESHA. For E2/E3, data are also included
410 for IHSS isolates: aquatic (x); soil (○) (redox data from Aeschbacher *et al.* (44)).

411

412 **Figure 5:** Changes in photooxidant quantum yields with irradiation time. Error bars represent
413 standard deviation of duplicate or triplicate measurements. (●) SRFA; (◇) NAFA; (▲) ESHA.

414

415 **Figure 6:** Quantum yields of 1O_2 and H_2O_2 versus E2/E3 and EDC. (●) SRFA; (◇) NAFA;
416 (▲) ESHA; (■) data from Dalrymple *et al.* (36); (---) trend reported by Peterson *et al.* (37).

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Figure 1

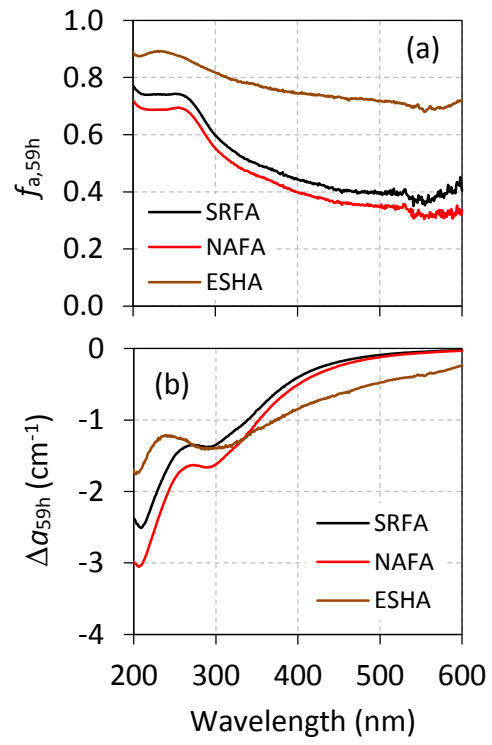
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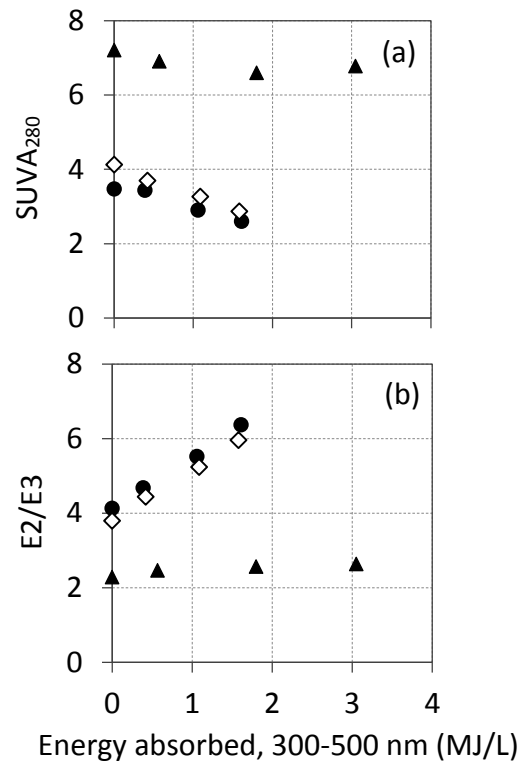


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Figure 2

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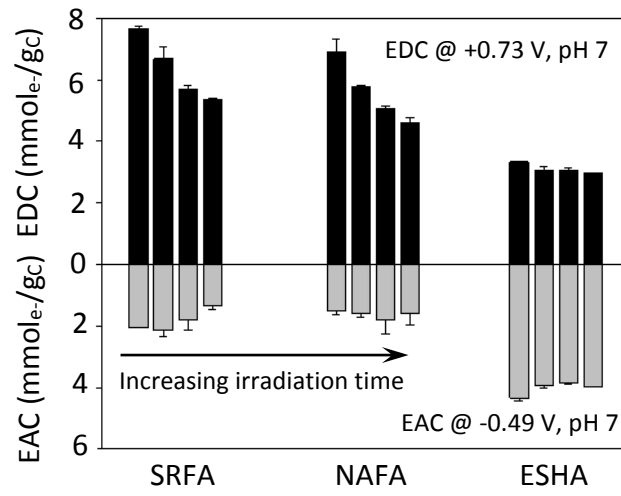
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Figure 3

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Figure 4

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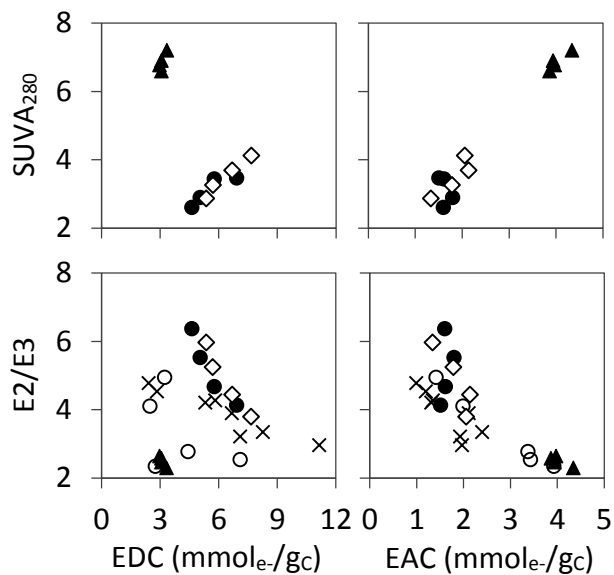
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Figure 5

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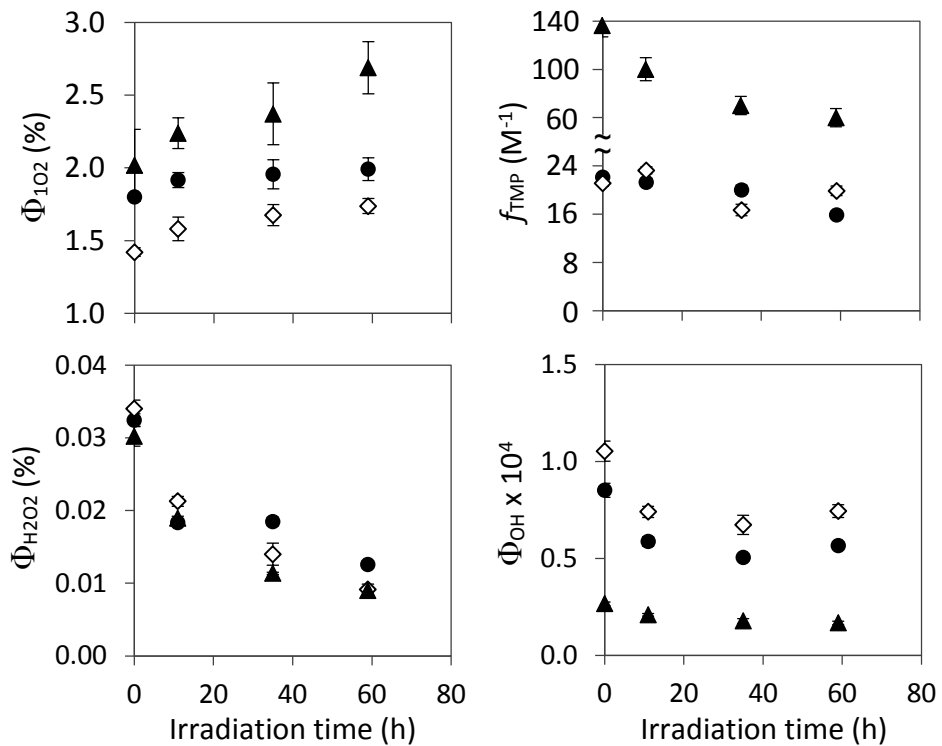
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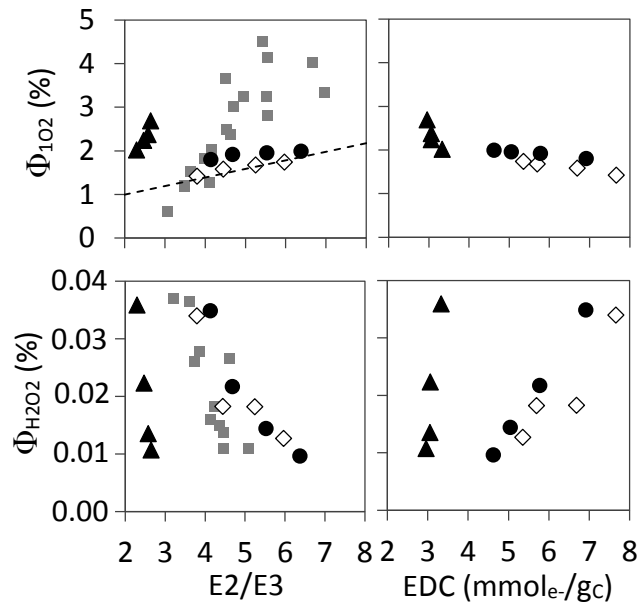
Figure 6

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