The role of reactive oxygen species in the degradation of lignin derived dissolved organic matter

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Abstract

Evidence suggests that reactive oxygen species (ROS) are important in transforming the chemical composition of the large pool of terrestrially-derived dissolved organic matter (DOM) exported from land to water annually. However, due to the challenges inherent in isolating the effects of individual ROS on DOM composition, the role of ROS in the photochemical alteration of DOM remains poorly characterized. In this work, terrestrial DOM was independently exposed to singlet oxygen (¹O₂), and superoxide (O₂^{•-} under controlled laboratory conditions. Using ultra-high resolution mass spectrometry to track molecular level alterations of DOM by ROS, these findings suggest exposure to ¹O₂ (generated using Rose Bengal and visible light) removed formulas with an O/C > 0.3, and primarily resulted in DOM comprised of formulas with higher oxygen content, while O₂^{•-} exposure (from KO₂ in DMSO) removed formulas with O/C < 0.3 and produced aliphatic formulas (H/C > 1.5). Comparison of DOM altered by ROS in this study to riverine and coastal DOM showed that (20-80%) overlap in formulas, providing evidence for the role of ROS in shaping the composition of DOM exported from rivers to oceans.

1. Introduction

20 Natural dissolved organic matter (DOM) is a dynamic pool of carbon on Earth
21 (Hedges, 1992), and is a critical intermediate in the global C cycle (Cole et al. 2007).
22 Rivers worldwide transport an estimated 0.4×10^{15} g of terrestrial dissolved organic
23 carbon (DOC) and approximately 0.2×10^{15} g of particulate organic carbon (POC)
24 annually to the oceans (Bianchi, 2011; Jaffe et al., 2013; Schlesinger and Melack, 1981),
25 roughly equivalent to the annual turnover of marine DOC (Williams and Druffel, 1987).
26 Studies of DOM carbon isotopes (Bauer, 2002; Lalonde et al., 2014), optical properties
27 (Blough and Del Vecchio, 2002) and lignin-phenol biomarkers (Opsahl and Benner,
28 1997, 1998) have suggested that terrestrial DOM disappears quickly once in oceans.
29 However, current understanding from rates of photochemical mineralization, bacterial
30 respiration, and salinity induced flocculation (Fox, 1983; Sholkovitz, 1976; Søndergaard
31 et al., 2003), suggest that these three major removal processes for terrestrial DOM likely
32 cannot account for all loss of this DOM in estuaries. Recent work suggests that upwards
33 of 50% of terrestrially-derived DOM may be partially oxidized or chemically altered by
34 sunlight during transit from land to coastal waters (i.e., partial degradation) (Cory et al.,
35 2014; Spencer et al., 2009), thereby masking identification of the origin of this C using
36 conventional isotopes and biomarker proxies (Blough and Del Vecchio, 2002; Helms et
37 al., 2008; Spencer et al., 2009; Waggoner et al., 2015).

38
39 Consistent with these recent studies showing that rates of photochemical
40 transformations are sufficient to process a large fraction of DOM export from land to
41 water, others have shown that photochemical alteration of terrestrial DOM results in
42 compounds resembling those found in marine waters (Chen et al., 2014; Cory et al.,

2010). Pathways for the photochemical alteration of terrestrial DOM may involve reactive oxygen species (ROS) such as singlet oxygen ($^1\text{O}_2$), hydroxyl radical ($^{\bullet}\text{OH}$), and superoxide ($\text{O}_2^{\bullet-}$), along with photo-activated DOM complexes (McNally et al., 2005; Page et al., 2013; Porcal et al., 2013; Scully et al., 2003; Ward et al., 2014). Photo-excited chromophoric DOM interacts with dissolved oxygen or water to produce reactive transient ROS and organic radicals, which in turn break down both the chromophoric DOM as well as the uncolored pool of DOM not accessible to direct photochemical degradation (Cory et al., 2009; Cory et al., 2010; Goldstone et al., 2002; Pullin et al., 2004; Scully et al., 2003). For example, it is well-established that DOM can react with $^1\text{O}_2$ (Cory et al., 2010) and $^{\bullet}\text{OH}$ in natural waters (Page et al., 2014). Furthermore, when DOM reacts with $^1\text{O}_2$ and $^{\bullet}\text{OH}$, evidence suggests that the primary products may be photochemically-modified DOM (i.e., partial photo-oxidation; (Cory et al., 2010; Cory et al., 2014; Waggoner et al., 2015), and not complete photo-oxidation of DOM to CO_2 (i.e., photo-mineralization, (Cory et al., 2010; Page et al., 2014; Southworth and Voelker, 2003). Further evidence in support of partial photo-oxidation of terrestrially-derived DOM by ROS includes the relatively high ratio of photochemical oxygen consumption to CO_2 production by DOM. Specifically, studies have shown that the molar ratio of photochemical O_2 consumption per CO_2 production by DOM is > 1 ; (Andrews et al., 2000; Cory et al., 2014; Miles and Brezonik, 1981; Ward and Cory, 2016), suggesting substantial oxygen incorporation into DOM. The role of ROS partial photo-oxidation of DOM is not well known due to the difficulty of isolating individual ROS and their reactions with DOM (Cory et al. 2009, 2010). At present, each ROS is expected to vary substantially in its reactivity with DOM.

For example, while $^1\text{O}_2$ is a fairly short-lived and selective oxidant, photochemical transformation of DOM by $^1\text{O}_2$ may be more important than other ROS due to higher $^1\text{O}_2$ concentrations in the hydrophobic interior of DOM (Grandbois et al., 2008; Latch and McNeill, 2006). Electrophilic $^1\text{O}_2$ can undergo cycloaddition with dienes and olefins abundant in DOM to form cyclic peroxides (Kearns, 1971). Non-cyclic allylic hydroperoxides can form upon $^1\text{O}_2$ reaction with olefins, as well as carbonyl containing compounds following olefin cleavage (Kearns, 1971). Previous work showed that alteration of DOM by $^1\text{O}_2$ led to production of O-containing aliphatic formulas (Cory et al. 2010).

Hydroxyl radical is an unselective oxidant and reacts at near diffusion rates with organic compounds and thus is expected to be an important oxidant of DOM (Goldstone et al., 2002). Hydroxyl radicals primarily react with DOM through addition to olefins or aromatic compounds, H abstraction, and electron transfer reactions (Walling, 1975; Wenk et al., 2011). Direct addition of $\cdot\text{OH}$ has been shown to result in both hydroxylation, and cleavage of side chain groups from compounds, with low molecular weight acids frequently observed following reaction of DOM with $\cdot\text{OH}$ (Goldstone et al., 2002; Walling, 1975). Electron transfer reactions associated with $\cdot\text{OH}$, while rare, are believed to yield organic radical cation species, which can then undergo dimerization reactions to generate new products (Walling, 1975). Consistent with the rapid and unselective oxidation of organics by $\cdot\text{OH}$, substantial alteration of lignin derived DOM upon exposure to $\cdot\text{OH}$ has been shown under laboratory conditions, resulting in the formation of condensed aromatics and molecules classified as carboxyl rich alicyclic molecules (CRAM) (Waggoner et al., 2015).

89 In contrast to $^1\text{O}_2$ and $\cdot\text{OH}$, $\text{O}_2^{\cdot-}$ is relatively long-lived, which may allow it to
90 react with a range of moieties within the DOM pool (Rose et al., 2010). Superoxide,
91 through nucleophilic substitution reactions, is capable of producing carboxylic acid
92 anions from esters, as well as dicarboxylic acids from unsaturated cyclic compounds
93 following ring cleavage (Lee-Ruff et al., 1976; Sawyer and Valentine, 1981). One
94 electron reduction of organic compounds is commonly observed with $\text{O}_2^{\cdot-}$, and reactions
95 with catechol like compounds, frequently present in DOM, result in radical anions that
96 can undergo ring cleavage and polymerization (Lee-Ruff et al., 1976; Sawyer and
97 Valentine, 1981). No study has investigated the effects of $\text{O}_2^{\cdot-}$ on DOM composition, but
98 given known reactions of organics with $\text{O}_2^{\cdot-}$ this ROS may contribute to oxidation and
99 oxygen incorporation into DOM.

100 Thus, despite strong evidence for the importance of ROS in transforming DOM
101 composition in sunlit surface waters, including much knowledge on the potential
102 reactivity of ROS with model compounds, few studies have characterized the effects of
103 individual ROS exposure on DOM composition. To address this knowledge gap, we
104 exposed lignin-derived DOM to $^1\text{O}_2$, and $\text{O}_2^{\cdot-}$ to isolate the molecular level alterations
105 specific to each ROS and then track those alterations using Fourier transform ion
106 cyclotron resonance mass spectrometry (FTICR-MS). By selectively exposing lignin to
107 each ROS under controlled conditions in the laboratory, we amplify the reactions of
108 interest to isolate their potential pathways for alteration of DOM. We combined this new
109 information with data obtained previously for $\cdot\text{OH}$ (Waggoner et al., 2015) to understand
110 the potential classes of terrestrial DOM that may be transformed by ROS, and to identity
111 potential products of these ROS-mediated transformations of DOM..

2. Materials and Methods

2.1 Lignin extraction

Alkali-soluble lignin, representing terrestrial DOM, was isolated from a brown rot fungal degraded wood sample, Atlantic white cedar (*Chamaecyparis thyoides*), collected from the Great Dismal Swamp in Virginia as previously described (Liu et al., 2011; Waggoner et al., 2015). The sample of wood was dried and ground to a fine powder prior to 24 hour stirring in 0.01 M NaOH. The resulting base extract was then filtered through a 0.7 μm glass fiber filter to remove any undissolved solids. This stock solution of lignin, approximately 400 ppm C, was subsequently used directly for treatment by $^1\text{O}_2$. For $\text{O}_2^{\cdot-}$ treatment, the base extract was cation exchanged with extensively washed Dowex 50Wx8 (H-form) to remove sodium prior to lyophilization.

2.2 Reaction with singlet oxygen

Singlet oxygen reactions were carried out in duplicate 500ml quartz round bottom flasks by adding the photosensitizer Rose Bengal (disodium salt, final concentration 1×10^{-5} M) to the base extracted lignin (pH 10) and bubbling with O_2 for approximately 20 minutes (Miller, 2005). The pH chosen was suggested to be more efficient for $^1\text{O}_2$ generation by Rose Bengal based on reaction rate kinetics when compared to reactions at neutral or slightly acidic pH, which show lignin and lignin model compounds preferentially react with excited state sensitizer over $^1\text{O}_2$ in non-basic conditions (Latch et al., 2003; McNally et al., 2005; Miller, 2005). A light control (no Rose Bengal photosensitizer) and dark control (foil wrapped) were also prepared for comparison. All solutions were placed in a Percival growth chamber equipped with T8 fluorescent lights (Philips F17T8/TL841), held at 25 °C and 100% light intensity for 48 hours of

photoirradiation. Light intensity was measured at $180 \mu\text{M M}^{-2} \text{s}^{-1}$ (400-700nm) using an LI-250A light meter over the duration of the experiment. Following irradiation, all samples were passed through cation exchange resin to remove salts that interfere with instrumental analysis. The resin was additionally rinsed with liquid chromatography-MS (LC-MS) grade methanol to elute any analyte sorbed to the resin.

The absorbance of the lignin + Rose Bengal solution was 1.6 times greater at 550 nm (peak absorbance of Rose Bengal) compared to the light control (lignin only; Fig. 1). Thus, in the lignin +Rose Bengal solution, there was more absorbance of the visible light by Rose Bengal than by lignin. In addition, the apparent quantum yield for $^1\text{O}_2$ by Rose Bengal at 550 nm (visible light) is ~ 0.7 (Haag et al., 1984), much higher than apparent quantum yields for ROS by lignin estimated at ~ 0.27 using model compounds (McNally et al., 2005). Together, these reaction conditions support selective reaction of lignin with $^1\text{O}_2$ because in the +Rose Bengal solution, the photochemical production of $^1\text{O}_2$ by Rose Bengal was likely much greater than photochemical production of other $^1\text{O}_2$ or other ROS by lignin. In addition, we verified photochemical production of $^1\text{O}_2$ from solutions containing only Rose Bengal and exposed to the same light as the lignin solution with and without Rose Bengal. Singlet oxygen was quantified from separate flasks containing Rose Bengal (at the same experimental concentration of $1 \times 10^{-5} \text{ M}$) and furfuryl alcohol (FFA; a probe for $^1\text{O}_2$). FFA concentration was added at $2 \times 10^{-4} \text{ M}$. Sub-samples for FFA degradation were collected at time points to verify photochemical production of $^1\text{O}_2$ from solutions of Rose Bengal exposed to the fluorescent bulbs used in the experiments (Haag and Hoigne, 1986). The $[\text{}^1\text{O}_2]_{\text{ss}}$ for the experiment was determined to be $1 \times 10^{-11} \text{ M}$. The concentration of $^1\text{O}_2$ was not measured in the lignin + Rose Bengal solutions because $^1\text{O}_2$

concentrations as measured by probes such as FFA may be influenced by reaction with
 and quenching by lignin, as demonstrated for lignin (Bentivenga et al., 1999; Bonini et
 al., 1998; McNally et al., 2005) and lignin model compounds (Crestini and D'Auria,
 1997; Crestini and D'Áuria, 1996). Further, it is difficult to speculate on differences in
 $[^1\text{O}_2]_{\text{ss}}$ in the lignin+ Rose Bengal solutions used in this study to the concentrations of
 $[^1\text{O}_2]_{\text{ss}}$ that terrestrial DOM may encounter in sunlit surface waters where DOM is the
 photochemical sensitizer for $^1\text{O}_2$ (i.e., in all sunlit surface waters (Andrews et al., 2000;
 Peterson et al., 2012)). This is because $^1\text{O}_2$ concentrations may vary several orders of
 magnitude from the hydrophobic interior of DOM to the more hydrophilic exterior (Latch
 and McNeill, 2006). Currently, there is no approach to selectively reproduce natural
 photochemical production of $^1\text{O}_2$ by DOM (or lignin) without introduction of other
 photochemically produced reactive oxygen species and intermediates by DOM. Thus,
 the goal of our experimental set-up was to maximize conditions for oxidation of lignin by
 $^1\text{O}_2$ by using Rose Bengal as the source of $^1\text{O}_2$ so that compared to controls, we may
 evaluate the potential effects of $^1\text{O}_2$ on lignin composition.

Figure 1. UV-Vis absorbance for lignin sample amended with (Red) and without (Black) the photosensitizer Rose Bengal, demonstrating prevalent absorbance by Rose Bengal in the visible light range.

2.3 Reaction with $O_2^{\cdot-}$

A stable $O_2^{\cdot-}$ solution was prepared following (Valentine and Curtis, 1975). Briefly, 0.30M dicyclohexyl-18-crown-6 was used to solubilize 0.15M KO_2 in dry DMSO. The concentration of $O_2^{\cdot-}$ has been demonstrated to be roughly equivalent to the concentration of KO_2 in solution (Valentine and Curtis, 1975). A lignin solution was prepared by dissolving 2.0 mg of lyophilized lignin extract in 8 ml of Milli-Q water. The reaction was carried out by adding 2.0 ml of 0.15M $O_2^{\cdot-}$ solution over the course of 30 minutes to 4 ml of lignin solution with rapid stirring. Freeze dried samples of base extracted lignin were also added to DMSO and exposed to the $O_2^{\cdot-}$ solution for a comparison of solvent effects on the reaction. Samples in which solid KO_2 was added directly to the lignin solution and a DMSO solution saturated with analyte were also analyzed as an additional control. Samples were passed through cation exchange resin as described above to remove inorganic salts that interfere with mass spec analysis, followed by a methanol rinse of the resin to recover sorbed analytes. This rinse was added to the aqueous/DMSO reaction medium.

2.4 TMAH thermochemolysis

All samples (+ ROS and controls) were subjected to tetramethylammonium hydroxide (TMAH) thermochemolysis to evaluate the state of lignin degradation compared to the initial lignin extract. Lignin phenol biomarkers, i.e., vanillic and syringic acids, were measured after TMAH thermochemolysis to assess the level of oxidative degradation following exposure to singlet oxygen. It is expected that as the level of

organic matter oxidation increases, the amount of acidic lignin phenols (vanillic and syringic acids) will increase relative to the corresponding aldehydes (vanillin and syringaldehyde) (Hedges and Ertel, 1982; Opsahl and Benner, 1998). Samples were dried and small amounts were placed in custom glass reaction tubes with TMAH (25% wt. in MeOH) as described by Filley et al. (2002). Briefly, methanol was removed under vacuum and all tubes were flame sealed prior to heating at 250 °C for 30 minutes. After returning to room temperature, the tubes were placed in liquid nitrogen to condense any gases formed before opening and extracting with ethyl acetate for GC-MS analysis.

2.5 FTICR-MS Analysis

Following $^1\text{O}_2$ and $\text{O}_2^{\bullet-}$ treatment, all samples were analyzed using a Bruker 12T Apex Qe FTICR-MS, housed in the College of Sciences Major Instrumentation Cluster at Old Dominion University, operated in negative electrospray ionization (ESI) mode. Samples were continually infused into an Apollo II ESI ion source at a rate of $120\ \mu\text{L}\ \text{hr}^{-1}$ using a syringe pump. ESI voltages were optimized for each sample to generate a stable ESI spray, with spray shield and capillary currents of 180-230 nA and 20-35 nA respectively. Ions were accumulated for 0.5-1.0s in a hexapole prior to being transferred to the ICR cell, where 300 transients were collected using a 4 MWord time domain and co-added. The resulting FID was zero-filled once and Sine-Bell apodized before undergoing fast Fourier transformation and a magnitude calculation using Bruker Daltonics Data Analysis software. Each sample was externally calibrated using a polyethylene glycol standard, and internally calibrated using fatty acids, dicarboxylic acids, and natural compounds belonging to CH_2 homologous series based on Sleighter and Hatcher (2008) prior to analysis. It is important to point out that FTICR-MS analysis

performed only observes molecules that charge in the negative ion electrospray mode. Such molecules would need to possess ionizable functional groups, such as oxygen containing functional groups commonly found in lignin. Any molecules lacking such groups would not be observed; however, it is likely that they would also not be part of the sample extract due to limited or no solubility.

Unique molecular formulas were assigned to peaks having a signal to noise (S/N) threshold of 3, using a MATLAB script written in house at Old Dominion University according to the criteria $^1\text{H}_{5-100} \text{ }^{16}\text{O}_{1-30} \text{ }^{14}\text{N}_{0-6} \text{ }^{32}\text{S}_{0-2} \text{ }^{34}\text{P}_{0-2}$, with subscripts denoting the range of atoms allowed for a single formula. Formulas were screened based on the rules described by Stubbins et al. (2010) for natural organic matter, and Kendrick mass defect homologous series (CH_2 , H_2 , CO_2 , and O_2) were used to select ambiguously assigned formulas (Kujawinski and Behn, 2006). All formulas assigned are within 1 ppm error of the measured m/z, with 94% of formulas having 0.5 ppm or lower error.

2.6 GC x GC TOF-MS Analysis

Lignin phenol biomarker analysis was performed using a LECO Pegasus 4D GC x GC TOF-MS following reaction with TMAH. Analyses were carried out with an autosampler (CTC Analytics) integrated to the GC system (Agilent Technologies, 6890N) fitted with a thermal modulation assembly. The columns used were a 30 m x 0.25 mm i.d. RTX-5 first dimension column, and 1 m x 0.1 mm i.d. RXI-17 second dimension column. Helium was used as a carrier gas and adjusted for constant flow throughout the run. The injector was operated in splitless mode and held at a constant 280°C throughout the run. The temperature program used consisted of an initial temperature of 40°C for 2 minutes then ramping to 280°C at 10°C min⁻¹ and holding for 10 min after a 1 µL sample

injection. A total modulation time of 4 seconds was used for the second dimension column consisting of a 1.30 second hot pulse and 0.70 second cold pulse. Peak areas were measured by the Pegasus software and identification of the products was via mass spectral comparisons to NIST library spectra.

3. Results

3.1 Chemical composition of Dismal Swamp lignin extract and dissolved organic matter

The molecular characteristics (elemental formulas from ESI-FTICR-MS data) of the alkali-soluble lignin extract are shown in Figure 2 as points on a van Krevelen diagram, generated by plotting the atomic H/C vs O/C ratios for the assigned formulas. Also depicted in Figure 2 is a van Krevelen diagram of dissolved organic matter from the Dismal Swamp for comparison to the lignin extract. Regions typically associated with lignin-like, carbohydrate-like, and tannin-like compounds have been added to van Krevelen diagrams, along with cutoff lines depicting where aromatic and condensed aromatic compounds are predicted to fall within the plot (Chen et al., 2014; Sleighter and Hatcher, 2008). For both the lignin extract and DOM from the Dismal Swamp, all formulas cluster within the region characteristic of lignin (Sleighter and Hatcher, 2007). Of the multitude of elemental formulas in the DOM (total number excluding isotopologues = 1873) approximately 58% correspond exactly to those observed in the lignin extract. One difference is the presence of formulas in the carbohydrate-like region for the lignin extract, formulas not observed in the DOM. This is most likely attributable to the more elevated levels of partly degraded cellulosic components in the wood as shown in solid-state ^{13}C NMR data (not shown).

Figure 2. Van Krevelen plots of CHO formulas for initial Dismal swamp DOM (A) and Dismal swamp lignin extract (B). Common regions associated with lignin (orange), carbohydrates (blue) and tannins (green) are displayed. Lines showing the cutoffs for aromatic compounds ($AI_{mod} \geq 0.5$) and condensed aromatic compounds ($AI_{mod} \geq 0.67$) are additionally shown.

3.2 Reaction with 1O_2

There were significant differences between 1O_2 -reacted vs. control samples in lignin phenols expected to be indicators of organic matter oxidation, as measured by TMAH thermochemolysis (i.e., vanillic and syringic acids; Fig. 3). Lignin extract exposed to elevated concentrations of 1O_2 (i.e., the + Rose Bengal treatment) showed substantially elevated (Ad/Al)_v ratio values compared to dark and light controls (i.e., no Rose Bengal). This result indicates greater degradation of lignin when exposed to 1O_2 (via addition of Rose Bengal) compared to the controls, given the expectation that oxidation of organic matter by 1O_2 increases the aldehyde to acid ratio of vanillyl compounds (Hedges and Ertel, 1982; Opsahl and Benner, 1998). There was no significant difference in the (Ad/Al)_v between the dark and light controls, providing further evidence for the Rose Bengal treatment as having substantially greater 1O_2 concentrations compared to the controls, and thus greater alteration of the lignin (Fig. 3).

Figure 3. Ratio of vanillic acid to vanillin (Ad/Al)_v, in dark control, light control, and replicate ¹O₂ exposed lignin DOM samples. TMAH thermochemolysis was performed in triplicate for all samples with error bars indicating standard deviation of measurements.

The molecular formulas generated from FTICR-MS analysis of the ¹O₂ exposed lignin extract resulted in 2617 assigned molecular formulas, 2297 being comprised of only C, H, and O atoms (~88%), as shown in the van Krevelen diagram (Fig. 4). Assigned formulas center around the region typically associated with lignin, with half of the newly produced formulas trending toward a higher O/C ratio, and plotting in an area commonly associated with tannin like compounds. The remaining new formulas largely plot in the higher H/C ratio portion of the lignin region, with a few formulas plotting in areas associated with aromatic compounds. A small decrease (9%) in the modified aromaticity index (AI_{mod}) for the CHO formulas in the lignin extract occurred as a result of ¹O₂ treatment, suggesting a slight decrease in the unsaturated formulas present.

Formulas assigned following ¹O₂ exposure were also used to investigate the potential pathways of organic matter alterations by ¹O₂ that might be occurring. A series of Kendrick mass defect analyses were performed on ¹O₂ exposed samples and controls using the following equations:

$$KM (COO) = IUPAC\ mass_{measured}[44.0000/43.98983] \quad (1)$$

$$KM (O_2) = IUPAC\ mass_{measured}[32.0000/31.98983] \quad (2)$$

$$KMD = [NM - KM] \quad (3)$$

where KM=Kendrick mass, NM= nominal mass, IUPAC mass = the measured exact mass of the peak based on the assigned formula. Kendrick mass defect plots are based on comparison of functional groups or atoms, such as COOH or O₂, vs. the nominal Kendrick mass. These plots identify the series of formulas differing by a selected mass corresponding to a monoatomic or diatomic atom or to functional group. For example, Kendrick mass defect plots showing a series of formulas differing from each other only by the number of oxygen atoms are distinguished by differences in m/z 32 corresponding to the mass of O or O₂. Likewise, formulas differing only in mass of m/z 44 corresponding to the mass of COOH functional group. Other trends, such as the addition of OH groups, characterized by a mass offset of m/z 18, can also be observed within the O₂ KMD plot. As shown in Figure 5 for the Kendrick mass defect plots for series of formulas differing in the number of O₂ or COOH groups, the plots define formulas separated horizontally with a slope of 0 to be part of a homologous series of formulas differing in the number of O atoms or COOH groups. The KMD series for O₂ shows a general increase in nominal mass over the starting base extract with an increase in the average number of formulas falling into a series (Fig. 5). This result is consistent with the addition of oxygen to the formulas present in the lignin extract prior to exposure to ¹O₂ resulting in an increase of overall nominal mass. The KMD for COOH also shows an increase in the average number of formulas per series. Furthermore, these formulas plot at the higher mass end of series, suggesting that additional carboxyl groups are being

formed due to the oxidation of organic matter by $^1\text{O}_2$. The increase in nominal mass
 observed for new compounds following $^1\text{O}_2$ exposure does not apply to the bulk lignin
 extract, which shows negligible change in the magnitude weighted average molecular
 mass.

Figure 4: Van Krevelen plots of CHO formulas produced following Dismal Swamp degraded lignin exposure to $^1\text{O}_2$ (A), and $\text{O}_2^{\bullet-}$ (B), with newly created formulas displayed in red and conserved formulas in black.

Figure 5. Kendrick mass defect plots for lignin following reaction with $^1\text{O}_2$ with the top plot showing KMD for oxygen series and the bottom COO series. Newly formed formulas are shown in red while conserved are black.

3.3 Reaction with $\text{O}_2^{\bullet-}$

Similar to the $^1\text{O}_2$ experiments, TMAH thermochemolysis was used to investigate the oxidative degradation effect $\text{O}_2^{\bullet-}$ exposure had on the lignin phenol biomarker signature. The control extract lignin exhibited a low (Ad/Al)v ratio consistent with a minimally degraded lignin sample (Fig. 6, cation base extract). The $\text{O}_2^{\bullet-}$ exposed lignin showed no significant difference in (Ad/Al)v compared to the controls (Fig. 6). Solid potassium $\text{O}_2^{\bullet-}$ showed a much larger (Ad/Al)v ratio compared to + $\text{O}_2^{\bullet-}$ treatment and the control lignin extract (Fig. 6)

Figure 6. Ratio of vanillic acid to vanillin (Ad/Al)v, in initial base extract, solid potassium $\text{O}_2^{\bullet-}$, and 0.15M $\text{O}_2^{\bullet-}$ exposed lignin DOM samples. TMAH thermochemolysis was performed in triplicate for all samples with error bars indicating standard deviation of measurements.

Of the 1927 total formulas assigned, 1595 of the formulas in the + $\text{O}_2^{\bullet-}$ treatment contained only C, H, and O atoms (~83%). A number of new CHO containing formulas, 525, were produced from $\text{O}_2^{\bullet-}$ exposure, with 247 falling into the area located directly above the region typically associated with lignin type compounds. These new formulas

have an H/C value greater than 1.5, suggesting that they possess more aliphatic characteristics than the un-reacted control extract of lignin. The generation of new aliphatic formulas is also evident in the 41% decrease in the AI_{mod} value for CHO formulas (AI_{mod} 0.18), indicating a trend toward more saturated formulas in $O_2^{\cdot-}$ treated lignin. To investigate further differences between the new formulas vs. those present in the controls, Kendrick mass defect plots were analyzed for COOH, O_2 , and H_2 series for the $O_2^{\cdot-}$ experiments (Fig. 7).

The KMD analysis for COOH (Fig. 7) shows that, when new formulas are present in series with conserved formulas, new formulas differing in mass by COOH typically plot at higher nominal masses. This result suggests addition of COOH groups by $O_2^{\cdot-}$ to an existing compound, which would increase the mass relative to formulas present initially, rather than removal of COOH from some formulas present initially which would decrease the mass relative to initial formulas. KMD plots for oxygen show a similar trend, with new points in the series falling at higher masses indicating the addition of oxygen following exposure to $O_2^{\cdot-}$, rather than loss from formulas already present. The H_2 series (Fig. 7); however, shows that newly produced formulas result from both the addition and removal of hydrogen, with new formulas plotting at the upper and lower mass ranges of the KMD series. Despite the addition of oxygen to existing formulas, the whole lignin extract following $O_2^{\cdot-}$ treatment shows an 18% decrease in average molecular mass for the CHO formulas.

Figure 7. Kendrick mass defect plots for lignin following reaction with $O_2^{\bullet-}$ with the top plot showing KMD for O_2 series, the middle COO series, and the bottom H_2 series. Conserved formulas (black) are displayed alongside new formulas (red) and new aliphatic formulas having $H/C > 1.5$ (blue).

3.4 ROS reactivity with lignin

The relative reactivity of each ROS toward lignin derived DOM may be inferred from the FTICR-MS data by comparing formulas from each experiment that were completely removed by the treatment selective for each ROS: 1O_2 , $O_2^{\bullet-}$ (this study) and $\cdot OH$ (Waggoner et al., 2015). Formulas displayed in the van Krevelen diagram represent

the CHO containing formulas from each ROS treatment that were present in the initial samples and completely removed following exposure to the ROS tested (Fig. 8). There were strong differences in the formulas removed from lignin between ROS treatments, e.g., between $^1\text{O}_2$, $\text{O}_2^{\cdot-}$ and $\cdot\text{OH}$ (Fig. 8). Singlet oxygen exposure resulted in removal of 442 formulas. These formulas lost had an average mass of 513 m/z and O/C value of 0.48. Exposure to $\text{O}_2^{\cdot-}$ removed 631 formulas characterized by an average mass of 460 m/z and O/C value of 0.29. Reaction of organic matter with $\cdot\text{OH}$ resulted in loss of 1042 formulas characterized by an average mass of 585 m/z and O/C ratio of 0.54. These results show that $\cdot\text{OH}$ removed larger, more oxidized formulas compared to $^1\text{O}_2$ and $\text{O}_2^{\cdot-}$, which tended to remove smaller and less oxidized (more saturated) formulas (Fig. 8).

Figure 8: Van Krevelen plot of CHO containing formulas lost from Dismal Swamp lignin extract after $^1\text{O}_2$ (red), $\text{O}_2^{\cdot-}$ (blue), and $\cdot\text{OH}$ (green) exposure. Lost formulas common to both $^1\text{O}_2$ and $\text{O}_2^{\cdot-}$ are shown in black.

4. Discussion

4.1 Lignin extract from Dismal Swamp is representative of terrestrially-derived dissolved organic matter

415 The abundance of formulas common to the lignin extract and to DOM from
416 Dismal demonstrate that both are enriched in organic matter associated with lignin and
417 lignin breakdown products. This is consistent with the source of DOM in the Dismal
418 Swamp, Virginia. In this system, DOM is derived mainly from the decaying vegetation
419 in an ombrotrophic peat (Hartman et al., 2015). Rain water permeates through the peat,
420 comprised mostly of woody plant remains, and extracts organic matter with
421 characteristics similar to the organic matter in the peat. Because most of this decaying
422 vegetation in the peat is wood, there is a substantial component of the DOM in Dismal
423 Swamp derived from lignin (Sleighter and Hatcher, 2008). The lignin extract therefore is
424 representative of organic matter prior to dissolution of decaying woody debris and export
425 to sunlit surface waters where DOM may be altered by ROS.

426 *4.2 Alteration of lignin extract by $^1\text{O}_2$*

427 Upon exposure to $^1\text{O}_2$ generated by Rose Bengal, the base extracted lignin
428 changed in composition in a manner consistent with oxidative degradation as measured
429 by both FTICR-MS and GCxGC-TOF-MS following TMAH thermochemolysis
430 compared to the dark and light controls (Fig. 3). One of the largest changes in
431 composition was a change in the phenol biomarkers vanillic acid and vanillin, commonly
432 designated as G6 and G4, respectively (McKinney et al., 1995). The much greater
433 (Ad/Al)_v ratio in the replicate $^1\text{O}_2$ treated lignin (Fig. 2) compared to dark and light
434 controls was consistent with photo-oxidized lignin (Opsahl and Benner, 1998). Previous
435 work demonstrated that with increasing organic matter oxidation the amount of the more
436 oxidized acidic lignin phenol components (vanillic and syringic acids) increases relative
437 to the corresponding less oxidized aldehydes (vanillin and syringaldehyde) (Hedges and

Ertel, 1982; Opsahl and Benner, 1998). Consistent with the results of the TMAH analysis, many new formulas were identified via FTICR-MS in the +Rose Bengal treatment compared to the dark and light controls suggesting oxidation of lignin phenolic units.

The new formulas produced in the +Rose Bengal treatment identified by FTICR-MS are predominantly grouping in the tannin-like region and in the upper H/C portion of the lignin region (Fig. 4). The tannin-like formulas observed following exposure to $^1\text{O}_2$ in the +Rose Bengal treatment were not abundant in the initial lignin, suggesting that these formulas are most likely oxidized lignin compounds. Consistently, previous work demonstrated that $^1\text{O}_2$ -oxidation of terrestrially-derived DOM resulted in production of formulas plotting in the same high H/C region of the lignin-like compounds as the new formulas identified here (Cory et al. (2010). While the new formulas in this study plotting in the lignin-like region are in good agreement with products of $^1\text{O}_2$ -oxidation of terrestrially-derived DOM identified by Cory et al. (2010), no tannin-like compounds were observed at higher O/C values in their study due to quenching of these formulas by the presence of high concentrations of Rose Bengal used to selectively react DOM with $^1\text{O}_2$.

Additionally, the KMD analyses consistently showed new formulas produced indicative of additions of oxygen and carboxyl groups (Fig. 5). Because addition of oxygen atoms alone would not add to COOH series, the generation of a greater number of COOH groups suggests possible opening of the aromatic ring associated with lignin (Fig. 9). This potential ring-opening scheme shows the depolymerization and demethylation of lignin followed by cleavage of the aromatic ring, resulting in carboxylic acid containing

compounds as suggested in previous work involving $\cdot\text{OH}$ (Waggoner et al., 2015). Loss of these carboxyl groups and subsequent condensation could also be responsible for the new formulas observed in the aromatic portion of the tannin-like region that were not observed in the starting lignin extract (Waggoner et al., 2015).

Figure 9: Reactions of lignin induced by radical-initiated enzymatic processes in soils. The main reactions are those described by Crawford and Crawford (1980).

It is possible that oxygenated peaks may have been present to some degree in the lignin prior to the $^1\text{O}_2$ exposure (i.e., prior to treatment with Rose Bengal). Differences in oxygenated peaks between dark or light control and $^1\text{O}_2$ exposed lignin could be due to lower ionization efficiencies or quenching by compounds in the control (unreacted) lignin compared to $^1\text{O}_2$ exposed lignin. However, increased oxygen functionality should yield compounds with greater ionization efficiencies, due to charge site availability. Therefore the presence of new formulas in the $^1\text{O}_2$ -exposed lignin is consistent with formulas

477 produced by reaction with $^1\text{O}_2$. Experimental replicates further support the presence of
478 these new formulas, as well as the reproducibility of the analysis, with lignin extracts
479 exhibiting 90 percent formula similarity between replicates (Sleighter et al., 2012).

480 The TMAH and FTICR results suggest that the alteration of the lignin extract was
481 primarily due to reaction with $^1\text{O}_2$ as opposed to reaction of the lignin extract with other
482 ROS. While the lignin extract likely produces ROS upon exposure to light (Appiani and
483 McNeill, 2015; Grandbois et al., 2008; Latch and McNeill, 2006), light controls
484 (containing no Rose Bengal) showed no changes in composition compared to dark
485 controls. This result suggests no detectable alteration of the lignin extract from ROS
486 generated by the lignin or from direct photo-degradation of the lignin extract. Little
487 alteration was expected to result from the photochemical reactions initiated by the lignin
488 extract because (1) absorbance by Rose Bengal was greater than the lignin extract in the
489 region of maximum output of the light source used (400-700 nm), and (2) ROS
490 production from the lignin extract was expected to be less than the production of $^1\text{O}_2$ by
491 Rose Bengal, given that yields of photochemical production of ROS from terrestrially-
492 derived DOM are highest in the UV (Mopper and Kieber, 2000) where output from the
493 light source was relatively low. Consistent with the expectation that alteration of lignin
494 extract should be highest in the +Rose Bengal treatment compared to the dark or light
495 control, there was no significant differences in the (Ad/Al)v ratios between dark and light
496 controls. Because no ROS would be expected to be produced by lignin in the dark
497 control, this result indicates no detectable alteration of the lignin extract during the 48-
498 hour light exposure from production of ROS by the lignin extract in the light control (Fig.
499 3).

4.3 Alteration of lignin extract by $O_2^{\cdot-}$

Exposure of the lignin extract to $O_2^{\cdot-}$ altered the composition of the lignin in different ways compared to 1O_2 exposure. The (Ad/Al)_v ratio following $O_2^{\cdot-}$ exposure was less than half that of the 1O_2 –exposed lignin (Figures 2, 5), suggesting that $O_2^{\cdot-}$ exposure resulted in less oxidative degradation as measured by TMAH thermochemolysis. The (Ad/Al)_v values obtained from the direct addition of solid potassium superoxide to the lignin extract were much higher than the $O_2^{\cdot-}$ treatment in DMSO, suggesting oxidative degradation similar to exposure to 1O_2 . This observation is consistent with evidence in the literature that 1O_2 is likely generated when potassium superoxide is added directly to aqueous solution (Khan, 1981) and demonstrates the value of the $O_2^{\cdot-}$ in DMSO treatment as an approach to isolate the effects of $O_2^{\cdot-}$ and 1O_2 .

As was found for the 1O_2 –exposed lignin, $O_2^{\cdot-}$ exposed lignin shows new formulas plotting in the higher O/C ratio region of the van Krevelen plot typically associated with tannin-like compounds (Fig. 4). While the (Ad/Al)_v ratio obtained for the 1O_2 exposed lignin suggests addition of oxygen to the lignin structure consistent with these higher O/C formulas, the much lower (Ad/Al)_v ratio in the $O_2^{\cdot-}$ exposed lignin suggests that oxidative degradation may not be the only mechanism of alteration of lignin by $O_2^{\cdot-}$. In addition to oxidation, $O_2^{\cdot-}$ mediated reactions with lignin may lead to loss of the methoxyl group from lignin via demethylation reactions to form catechol-like structures (Crawford, 1981). Catechols and their derivatives can undergo ring opening and carboxylation upon reaction with $O_2^{\cdot-}$, (Lee-Ruff et al., 1976), consistent with the production of high O/C formulas in the $O_2^{\cdot-}$ treatment.

A prominent alteration of lignin after $O_2^{\bullet-}$ exposure was that roughly 50% of the new formulas plot at a higher H/C ratio ($H/C > 1.40$) in an area directly above the region associated with lignin. These new formulas have a substantially decreased DBE (5 down from 10) when compared to the conserved formulas (i.e., those common to treated and control lignin), and a modified aromaticity index (AI_{mod}) (Koch and Dittmar, 2006, 2016) value of 0.02. This result suggests substantially more aliphatic compounds containing oxygen functional groups in the $O_2^{\bullet-}$ exposed lignin compared to the control (Fig. 4). These findings are in good agreement with previous work suggesting that photo-degradation of terrestrially-derived DOM transforms unsaturated aromatics to more saturated compounds (Gonsior et al., 2009; Tremblay et al., 2007). Production of O-containing aliphatics after exposure to $O_2^{\bullet-}$ is consistent with a small increase in the acid/aldehyde ratio compared to the controls (Fig. 6) because newly produced aliphatic formulas may no longer contain the methoxy phenol required for the $(Ad/Al)_v$ assessment of lignin degradation.

The KMD analysis indicates a high degree of oxygenation for $O_2^{\bullet-}$ exposed lignin compared to the control. For example, KMD analysis of oxygen shows numerous homologous series comprised of many formulas, with new formulas generally plotting at higher nominal masses within these series. This finding indicates that incorporation of oxygen into lignin formulas from $O_2^{\bullet-}$ exposure has little detectable effect on the $(Ad/Al)_v$ of the lignin. This result may be explained by the fact that many of the formulas contributing to the long oxygen series are those of the newly formed aliphatic formulas, and would therefore not influence the lignin phenol based $(Ad/Al)_v$ measurement. KMD analyses also provided evidence for the addition of carboxyl groups to lignin from $O_2^{\bullet-}$

exposure. Formation of carboxyl groups is consistent with reactions of $O_2^{\cdot-}$ with catechol-like compounds resulting in ring opening discussed above (Lee-Ruff et al., 1976). Closer examination of the KMD results also show that a large number of the new formulas in the carboxyl plot belong to series not observed in the initial lignin extract, suggesting that the structures of these formulas are not a simple transformation of pre-existing formulas by addition or removal of carboxyl or oxygen groups, but rather undergo multiple alteration steps.

The KMD for H_2 series demonstrates another possible alteration of lignin by $O_2^{\cdot-}$, where the addition of H_2 to conserved formulas results in new, more aliphatic, formulas with a lower DBE (Fig. 7). The plot shows that in some cases loss of H_2 from a conserved formula can also generate new non-aliphatic formulas (Fig. 7), suggesting that $O_2^{\cdot-}$ exposure may result in both hydrogenation as well as proton abstraction from lignin. Superoxide has been proposed to serve as a proton-coupled electron shuttle in biological systems, typically involving trace metals (Afanas'ev, 1991; Miller et al., 2003); however, it may be possible that a similar mechanism is occurring in lignin extracts (Lee-Ruff et al., 1976; Sawyer and Valentine, 1981). If $O_2^{\cdot-}$ were acting as a proton shuttle capable of reacting with dienes, little production of condensed aromatic formulas would be expected. Previous work observed production of condensed aromatics from $\cdot OH$ reaction with lignin derived DOM (Waggoner et al., 2015) and suggested that these formulas form through a Diels-Alder type cyclization mechanism (Waggoner et al., 2015). This mechanism could be quenched if the dienes required were protonated by $O_2^{\cdot-}$. The subsequently formed cation could then react with $O_2^{\cdot-}$ anion producing more aliphatic formulas observed with higher O/C ratios (Fig. 4).

4.4 Assessment of organic matter oxidation by ROS in natural waters

The findings from this and previous studies suggest that $^1\text{O}_2$, $\text{O}_2^{\cdot-}$, and $\cdot\text{OH}$ interact with different classes of terrestrially-derived organic matter (Fig. 8). Superoxide appears to react with, and eliminates formulas having the lowest O/C ratios and lowest average mass (460 m/z), followed by $^1\text{O}_2$ (513 m/z) and $\cdot\text{OH}$ (585 m/z). The mass trend could suggest that sterical considerations play some part in the reactivity; however, closer inspection of the formulas likely selectively transformed by each ROS indicate that differences in the number of oxygen atoms in the formulas account for most of the selectivity, with carbon contents remaining relatively constant (26-28 carbons) for the formulas removed. Previous work also suggests that the reactivity of ROS with DOM may depend on oxygen content (Cory et al., 2010). These findings may suggest that compounds containing fewer oxygen atoms are most activated toward reaction with $\text{O}_2^{\cdot-}$, allowing for the addition of O_2 , carboxylation, and ring opening. The formulas removed by $^1\text{O}_2$ are characterized as having a greater number of oxygen atoms, and higher DBE relative to those removed by $\text{O}_2^{\cdot-}$, suggesting that $^1\text{O}_2$ is reacting with the more oxygen substituted components present in the lignin extract. A greater reactivity of $^1\text{O}_2$ toward oxygenated aromatics is also supported by the increased (Ad/Al)_v ratio observed following TMAH when compared to $\text{O}_2^{\cdot-}$ (Figures 2, 6). Lastly, data obtained from previous work (Waggoner et al., 2015) indicates that $\cdot\text{OH}$ is reacting with and removing formulas with the greatest overall mass, greatest number of oxygen atoms, and roughly equivalent DBE to those removed by $^1\text{O}_2$. An increase in oxygen functionality, particularly hydroxyl group substitution on aromatic systems, has been demonstrated to increase $\cdot\text{OH}$ scavenging in certain types of compounds (Cao et al., 1997; Wang et al.,

1997). We suggest that compounds present within the lignin extract also show an increase in radical scavenging ability as the amount of oxygen functionality increases. These findings are in good agreement with previous work showing the antioxidant properties of humic acids (Aeschbacher et al., 2012), as well as soil permafrost DOM (Ward and Cory, 2016). It is also possible that the increased oxygen functionality from exposure to $^1\text{O}_2$ and $\text{O}_2^{\cdot-}$ may in turn enable further reaction with $\cdot\text{OH}$ and the generation of condensed aromatics and CRAM-like molecules.

4.5 Comparison to alteration of DOM in natural waters

Many studies have concluded that a substantial fraction of the large quantity of terrestrial DOM annually exported to the oceans (0.4×10^{15} g of C) must be altered during transport from rivers to coastal and open marine waters (Andrews et al., 2000; Cory et al., 2014). As DOM is transported from rivers to coastal waters, DOM composition shifts from higher molecular weight, more aromatic compounds, into lower molecular weight aliphatic compounds (Opsahl and Benner, 1998; Tremblay et al., 2007). Findings from this study on the effects of ROS on lignin are generally consistent with observed modification of terrestrially-derived DOM composition along the river to marine transect. For example, net changes in lignin composition from reactions with all ROS are a decrease in molecular weight ($\text{O}_2^{\cdot-}$ and $\cdot\text{OH}$) and a shift to more aliphatic formulas (all ROS).

Further evidence for the role of ROS in transforming terrestrially-derived DOM during export from land to water is that many formulas produced by reaction of lignin with each ROS have been detected in riverine DOM draining the Dismal Swamp (Sleighter et al., 2010). For example, 78 % of the formulas produced by reaction of

lignin with $\text{O}_2^{\cdot-}$ were also identified in riverine DOM. For $^1\text{O}_2$ and $\cdot\text{OH}$, 45 % and 20 % of formulas generated by reaction of lignin with these ROS, respectively, matched formulas in riverine DOM. However, it is important to note that formula match only identifies potential products of ROS in riverine DOM given that there likely exist multiple pathways that produce the same formulas. In addition, there may be large structural differences between these formulas, as it is possible to have many isomers for a given formula (Sleighter and Hatcher, 2011).

These findings may also have implications for use of proxies to detect contributions from lignin in marine DOM. For example, ca.18% of the total CHO formulas in coastal or offshore marine DOM match those produced by exposing lignin to either $\text{O}_2^{\cdot-}$ or $^1\text{O}_2$. These matching formulas in the coastal or offshore marine DOM, predominantly aliphatic in nature, are not typically attributed to terrestrially-derived organic matter because they differ in composition from formulas associated with lignin (Figure 10). Our results suggest that some biomarker proxies for lignin may underestimate contributions from inputs of terrestrially-derived DOM that has been altered by ROS.

While results of this study provide information on potential transformations of terrestrially-derived DOM by ROS, it is not possible to relate these findings to the relative importance of each ROS in transforming DOM in natural waters. For example, photochemical transformations of DOM by each ROS depend on the apparent quantum yield for production of each ROS (which can vary by at least an order of magnitude for most ROS (Andrews et al., 2000; Page et al., 2014; Peterson et al., 2012; Powers and Miller, 2014; Vaughan and Blough, 1998)). In addition, at present it is difficult to

quantitatively evaluate the relative importance of each ROS studied here on DOM composition due to potential heterogeneous concentrations of ROS within DOM (Latch and McNeill, 2006), as well as presence of quenchers such as salts or carbonates that may influence reactions of ROS with DOM along the riverine to marine transect (Burns et al., 2010).

Table 1: Percentage of new formulas from each ROS experiment found at various sampling locations along a transect spanning from the Elizabeth River to off the coast of VA. Data used for comparison based on Sleighter et. al. (2010).

Transect Location	% New Formulas Matching		
	$O_2^{\cdot-}$	1O_2	$\cdot OH$
Dismal Swamp (DS)	48.6	34.4	11.9
Great Bridge (GB)	46.3	21.8	9.8
Town Point (TP)	60.6	26.5	9.4
Chesapeake Bay (CBB)	33.0	17.7	5.5
Offshore Coastal (OSC)	14.7	9.9	1.1
No Match	21.9	54.9	79.7

Figure 10: Superoxide newly produced CHO formulas (Top left) with matching formulas from transect study (Lower left), along with new CHO formulas from singlet oxygen (Top right) and corresponding matches to transect data (Lower right). Transect data is displayed from inland at the Dismal Swamp (DS) to Virginia off shore coastal (OSC).

5. Conclusions

The work described here demonstrates potential molecular level alteration of lignin by the primary ROS encountered upon export from terrigenous systems. Each ROS studied here, or in previous work, is produced both in the light and in the dark by DOM in natural waters (Appiani and McNeill, 2015; Cory et al., 2010; Diaz et al., 2013; Garg et al., 2011; Page et al., 2013; Page et al., 2014). Findings from this study suggest that each ROS may selectively transform lignin, and indicate that differences in the number of oxygen atoms in the formulas account for most of the selectivity between ROS with lignin. Substantial matches in formulas between ROS-transformed lignin with

riverine and marine DOM are consistent with prior work concluding that ROS are important in altering terrestrially-derived DOM composition during riverine export (Andrews et al., 2000; Cory et al., 2009; Cory et al., 2010; Cory et al., 2014; Page et al., 2014; Peterson et al., 2012; Ward and Cory, 2016), resulting in DOM that is of lower molecular weight and more aliphatic compared to lignin. Overall, our study provides insight into the modification of DOM by the dominant ROS, and how the input of terrestrial DOM to coastal waters may be masked by such modification.

Acknowledgments

This work was funded by the Old Dominion University Frank Batten Endowment. We would like to thank the COSMIC (College of Science Major Instrument Cluster) facility at Old Dominion University for use of the ESI-FTICR-MS, Dr. Rachel Sleighter for providing transect data, as well as Dr. Wassim Obeid for writing the Matlab script used to process the data. RMC was supported by NSF CAREER award 1351745.

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