

RUNNING TITLE: OC oxidation processes across vegetation

Carbon inputs from riparian vegetation limit oxidation of physically-bound organic carbon via biochemical and thermodynamic processes

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### **Key points.**

- Riparian vegetation protects bound-OC stocks
- Biochemical and metabolic OC oxidation processes vary with vegetation
- Common thermodynamic principles underlie OC oxidation regardless of vegetation

1 **Abstract.**

2           In light of increasing terrestrial carbon (C) transport across aquatic boundaries, the  
3 mechanisms governing organic carbon (OC) oxidation along terrestrial-aquatic interfaces are  
4 crucial to future climate predictions. Here, we investigate biochemistry, metabolic pathways, and  
5 thermodynamics corresponding to OC oxidation in the Columbia River corridor using ultra-high  
6 resolution C characterization. We leverage natural vegetative differences to encompass variation  
7 in terrestrial C inputs. Our results suggest that decreases in terrestrial C deposition associated  
8 with diminished riparian vegetation induce oxidation of physically-bound OC. We also find that  
9 contrasting metabolic pathways oxidize OC in the presence and absence of vegetation and—in  
10 direct conflict with the ‘priming’ concept—that inputs of water-soluble and thermodynamically  
11 favorable terrestrial OC protects bound-OC from oxidation. In both environments, the most  
12 thermodynamically favorable compounds appear to be preferentially oxidized regardless of  
13 which OC pool microbiomes metabolize. In turn, we suggest that the extent of riparian  
14 vegetation causes sediment microbiomes to locally adapt to oxidize a particular pool of OC, but  
15 that common thermodynamic principles govern the oxidation of each pool (e.g., water-soluble or  
16 physically-bound). Finally, we propose a mechanistic conceptualization of OC oxidation along  
17 terrestrial-aquatic interfaces that can be used to model heterogeneous patterns of OC loss under  
18 changing land cover distributions.

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## 22 1. Introduction

23 Soils and nearshore sediments comprise a C reservoir that is 3.2 times larger than the  
24 atmospheric C pool [Burd *et al.*, 2016], yet Earth System Models (ESMs) struggle to integrate  
25 mechanisms of OC oxidation into predictions of atmospheric carbon dioxide concentrations  
26 [Todd-Brown *et al.*, 2013; Wieder *et al.*, 2013; Wieder *et al.*, 2015]. In particular, OC oxidation  
27 in nearshore habitats constitutes a significant uncertainty in atmospheric C flux [Aalto *et al.*,  
28 2003; Battin *et al.*, 2009] and knowledge on C cycling along these transitional ecosystems is  
29 necessary to accurately predict global C cycling [Burd *et al.*, 2016]. Terrestrial C inputs into  
30 aquatic systems have nearly doubled since pre-industrial times; an estimated 2.9 Pg C now  
31 crosses terrestrial-aquatic interfaces annually (vs. 0.9 Pg C yr<sup>-1</sup> stored within forested  
32 ecosystems) [Battin *et al.*, 2008; Regnier *et al.*, 2013]. The magnitude of this flux has garnered  
33 significant recent attention [Battin *et al.*, 2008; Battin *et al.*, 2009; Regnier *et al.*, 2013], yet the  
34 biochemical, metabolic, and thermodynamic mechanisms governing OC oxidation along aquatic  
35 interfaces remain a crucial uncertainty in climate predictions. New molecular techniques are  
36 providing insight into OC dynamics [Mason *et al.*, 2016; Malak M Tfaily *et al.*, 2015; M.M.  
37 Tfaily *et al.*, 2017], but we still lack an understanding of why some OC remains stabilized for  
38 millennia whereas other OC is rapidly oxidized [Schmidt *et al.*, 2011].

39 The ability of microorganisms to oxidize complex OC is an important constraint on C  
40 cycling, as OC is a mixture of compounds with different propensities for biotic oxidation [J  
41 Hedges and Oades, 1997; J I Hedges *et al.*, 2000]. Within terrestrial research, OC oxidation is  
42 often framed within the concept of ‘priming’, whereby microbial oxidation of chemically-  
43 complex, less bioavailable OC is fueled by the addition of more bioavailable and  
44 thermodynamically favorable OC compounds [Kuzyakov, 2010]. However, the applicability of

45 priming in aquatic environments is unclear [*Bengtsson et al.*, 2014; *Bianchi*, 2011; *Guenet et al.*,  
46 2010]. Aquatic systems, and in particular nearshore environments, frequently experience mixing  
47 of terrestrial and aquatic C sources with distinct chemical character, providing a theoretical basis  
48 for priming expectations [*Bengtsson et al.*, 2014; *Guenet et al.*, 2010]. Consistent with priming,  
49 *Guenet et al.* [2010] have proposed that this mixing generates “hotspots” or “hot moments” of  
50 biological activity facilitated by complementary C resources. Alternatively, OC stabilization in  
51 sediments is tightly linked to organomineral interactions, which provide physical protection from  
52 extracellular enzyme activity [*J I Hedges and Keil*, 1995; *Hunter et al.*, 2016; *Rothman and*  
53 *Forney*, 2007], and the strength of these interactions may override any influence of priming.  
54 Early investigations of priming effects in aquatic systems have been inconclusive, with evidence  
55 both for [*Dorado-García et al.*, 2016] and against [*Bengtsson et al.*, 2014; *Catalán et al.*, 2015]  
56 priming mechanisms.

57         Several new perspectives have attempted to move beyond frameworks, such as priming,  
58 that depend on strict chemical definitions to predict OC oxidation [*Burd et al.*, 2016; *Cotrufo et*  
59 *al.*, 2013; *Lehmann and Kleber*, 2015]. Recent work proposes that the probability of OC  
60 oxidation is related to a spectrum of chemical properties and that even very complex OC can be  
61 oxidized when more thermodynamically favorable OC is depleted or isolated from  
62 microorganisms. For example, *Lehmann and Kleber* [2015] have proposed a ‘soil continuum  
63 hypothesis’ whereby OC is a gradient of continually decomposing compounds that are variably  
64 accessible for biotic oxidation, with no notion of chemically labile versus recalcitrant  
65 compounds. Similarly, *Burd et al.* [2016] have suggested that OC oxidation is a ‘logistical  
66 problem’ involving the ability of microorganisms to access and metabolize compounds. Both

67 concepts capture the emerging belief that chemically-complex, less thermodynamically favorable  
68 OC can be oxidized when more favorable compounds are inaccessible.

69 Here, we address a critical knowledge gap in predicting the global C balance [*Aalto et al.*,  
70 2003; *Battin et al.*, 2009; *Burd et al.*, 2016; *Regnier et al.*, 2013]—mechanisms governing OC  
71 oxidation along terrestrial-aquatic interfaces. Specifically, we investigate the biochemistry,  
72 microbial metabolism, and thermodynamics of OC oxidation in nearshore water-soluble and  
73 physically-bound (i.e., mineral and microbial) OC pools along a freshwater terrestrial-aquatic  
74 interface. We leverage natural variation in riparian vegetation along the Columbia River in  
75 Eastern Washington State, the largest river in the U.S. west of the Continental Divide [*Ebel et*  
76 *al.*, 1989; *Moser et al.*, 2003], to examine these mechanisms in the context of spatial variation in  
77 terrestrial C deposition. Consistent with the priming paradigm, we hypothesize that (a) C  
78 deposition associated with riparian vegetation increases total aerobic metabolism and enhances  
79 oxidation of bound-OC stocks, while (b) areas without riparian vegetation foster lower rates of  
80 aerobic metabolism with minimal oxidation of bound-OC.

81

## 82 **2. Materials and Methods**

### 83 *2.1. Site Description*

84 This study was conducted along the Columbia River shoreline within the Hanford 300A  
85 (approximately 46° 22' 15.80"N, 119° 16' 31.52"W) in eastern Washington State [*Graham et al.*,  
86 2016a; 2017; *Slater et al.*, 2010; *Zachara et al.*, 2013]. The Columbia River experiences  
87 shoreline geographic variation in vegetation patterns, substrate geochemistry, and microbiome  
88 composition [*Arntzen et al.*, 2006; *Lin et al.*, 2012; *Peterson and Connelly*, 2004; *Slater et al.*,  
89 2010; *James C Stegen et al.*, 2016; *James C. Stegen et al.*, 2012; *Zachara et al.*, 2013].

90 Accordingly, the Hanford Reach of the Columbia River embodies an ideal natural system in  
91 which to examine heterogeneity of terrestrial OC inputs and subsequent OC oxidation  
92 mechanisms.

93 Liquid N<sub>2</sub>-frozen sediment profiles (0-60 cm) were collected at two transects within  
94 shoreline stretches with or without riparian vegetation (hereafter, V and NV for ‘vegetated’ and  
95 ‘not vegetated’, Table 1) perpendicular to the Columbia River in March 2015, separated by a  
96 distance of ~170m. V was characterized by a moderately sloping scour zone, small boulders; and  
97 vegetation consisted of woody perennials *Morus rubra* (Red Mulberry) and *Ulmus rubra*  
98 (Slippery Elm), with a closed canopy. Upper bank samples were collected within the root zone.  
99 In contrast, NV was characterized by a gradually sloping scour zone and cobbled armor layer.  
100 We collected profiles at three locations in each transect with 5m spacing within a spatial domain  
101 of ~175 x 10m. In each transect, the lower bank profile was located at ~0.5m (vertical distance)  
102 below the water line and the upper bank profile was located ~0.5m (vertical distance) above the  
103 water line approximately 10m horizontal distance, with the third profile situated at the midpoint.  
104 Each profile was sectioned into 10-cm intervals from 0-60cm, and OC composition (see below  
105 for Methods) did not differ across upper (0-10cm) to lower (50-60cm) sections in each profile.  
106 To provide sufficient sample size (n >15 at each transect) for cross-site comparisons, each 10-cm  
107 section was used as a replicate sample.

108

## 109 2.2. Sample Collection

110 Liquid N<sub>2</sub>-frozen sediment profiles were collected as outlined in *Moser et al.* [2003]  
111 using a method developed by *Lotspeich and Reed* [1980] and modified by *Rood and Church*  
112 [1994]. A pointed stainless steel tube (152 cm length, 3.3 cm outside diameter, 2.4 cm inside

113 diameter) was driven into the river bed to a depth of ~60cm. N<sub>2</sub>(l) was poured down the tube for  
114 ~15 minutes, until a sufficient quantity of material had frozen to the outside of the rod. The rod  
115 and attached material was removed from the river bed with a chain hoist suspended beneath a  
116 tripod. Profiles were placed over an aluminum foil lined cooler containing dry ice. Frozen  
117 material was removed by with a mallet. The material was then wrapped in the foil and  
118 transported on dry ice to storage at -80°C. In the lab, profiles were sectioned into 10cm depth  
119 intervals from 0-60 cm (n = 6 per profile, except for NV3 which was sectioned only from 30-  
120 60cm; total n = 33)

121

### 122 2.3. Physicochemistry

123 Details concerning physicochemical assays are provided in the Supporting Information.  
124 Briefly, we determined the particle distribution of sediments by separating size fractions via  
125 sieving; total nitrogen, sulfur, and carbon content were determined using an Elementar vario EL  
126 cube (Elementar Co.Germany); NH<sub>4</sub><sup>+</sup> was extracted with KCl and measured with Hach Kit  
127 2604545 (Hach, Loveland, Co); iron content was measured with a ferrozine assay; and all other  
128 ion concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS)  
129 on HCl extractions. Aerobic metabolism was determined with a resazurin reduction assay,  
130 modified from *Haggerty et al.* [2009]

131

### 132 2.4. FT-ICR-MS solvent extraction and data acquisition

133 We leverage state of science chemical extraction protocols combined with Electrospray  
134 ionization (ESI) and Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry  
135 (MS) to infer differences in OC character among our samples. Previously, *Tfaily et al.* [2015;

136 2017] have demonstrated the optimization of OC characterization from soils and sediments by  
137 sequential extraction with polar and non-polar solvents tailored to the sample set of interest.  
138 Tfaily's extraction procedures have been coupled to ESI FT-ICR-MS to distinguish OC pools  
139 among ecosystems and soil types [Malak M Tfaily et al., 2015; M.M. Tfaily et al., 2017] as well  
140 as to provide information on the metabolism of distinct OC pools among samples within a single  
141 environment [Bailey et al., 2017]. Other common OC characterization methods such as nuclear  
142 magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FT-IR), and  
143 gas chromatography MS only analyze a limited number of compound classes [Kögel-Knabner,  
144 2002; Kögel-Knabner, 2000]. In contrast, ESI FT-ICR-MS introduces intact organic molecules  
145 into the MS without fragmentation and allows for the detection of a wide range of chemical  
146 compounds [Malak M Tfaily et al., 2015; M.M. Tfaily et al., 2017]. The use of 12 Tesla (T) FT-  
147 ICR-MS offers high mass resolving power (>1M) and mass measurement accuracy (<1 ppm),  
148 and while nascent in its application within complex environmental systems, it has emerged as a  
149 prevailing method for determining OC chemistry of natural organic matter [Kim et al., 2003;  
150 Koch et al., 2005; Malak M Tfaily et al., 2011; Tremblay et al., 2007]. Moreover, Tfaily et al.  
151 [2015; 2017] have demonstrated that sequential extraction with targeted solvents can  
152 preferentially select OC pools with differing chemical character (e.g., lipid-like vs. carbohydrate-  
153 like).

154 Here, we used three solvents with different polarities —water (H<sub>2</sub>O), methanol (CH<sub>3</sub>OH,  
155 hereafter “MeOH”) and chloroform (CHCl<sub>3</sub>)—to sequentially extract a large diversity of organic  
156 compounds from samples, according to Tfaily et al. [2015; 2017]. Water extractions were  
157 performed first, followed by MeOH and then CHCl<sub>3</sub>. Previous work has shown that each solvent  
158 is selective towards specific types of compounds [Malak M Tfaily et al., 2015]. Water is a polar

159 solvent with a selection bias for carbohydrates with high O/C ratios, amino-sugars and other  
160 labile polar compounds [*Malak M Tfaily et al.*, 2015]; and, as nearshore environments frequently  
161 experience wetting, water extractions represent an estimation of readily accessible OC  
162 compounds in these environments. Conversely, CHCl<sub>3</sub> is selective for non-polar lipids associated  
163 with mineral interactions and cellular membranes (i.e., physically-bound OC) [*Malak M Tfaily et*  
164 *al.*, 2015]. Because MeOH has a polarity in between that of water and CHCl<sub>3</sub>, it extracts both  
165 water-soluble and bound-OC pools (i.e., a mix of compounds that water and CHCl<sub>3</sub> extract), and  
166 *Tfaily et al.* [2015] have demonstrated compositional overlap between water-soluble and MeOH  
167 extracted OC pools. In this study, we are interested in the differences in OC composition  
168 between pure water-soluble and bound-OC pools, and we will focus our discussion on H<sub>2</sub>O- and  
169 CHCl<sub>3</sub>-extractions only. We use H<sub>2</sub>O- and CHCl<sub>3</sub>-extracted OC as proxies for readily  
170 bioavailable (i.e., weakly bound) vs. less bioavailable (i.e., mineral- and microbial-bound) pools,  
171 respectively.

172         Extracts were prepared by adding 1 ml of solvent to 100 mg bulk sediment and shaking in  
173 2 mL capped glass vials for two hours on an Eppendorf Thermomixer. Samples were removed  
174 from the shaker and left to stand before spinning down and pulling off the supernatant to stop the  
175 extraction. The residual sediment was dried with nitrogen gas to remove any remaining solvent,  
176 and then the next solvent was added. The CHCl<sub>3</sub> and H<sub>2</sub>O extracts were diluted in MeOH to  
177 improve ESI efficiency. *Tfaily et al.* [2015] estimated the OC extraction efficiency to be ~15%.  
178 *Tfaily et al.* [2015] have previously demonstrated extraction efficiencies as low as 2% to be  
179 representative of OC pool composition. We further note that numerous studies have established  
180 FT-ICR-MS as a robust method for distinguishing compositional differences among OC pools

181 [Herzprung *et al.*, 2017; Kellerman *et al.*, 2015; Rossel *et al.*, 2016; Ward and Cory, 2015;  
182 Zhang *et al.*, 2016].

183 Ultra-high resolution mass spectrometry of the three different extracts from each sample  
184 was carried out using a 12 Tesla Bruker Solarix FT-ICR-MS located at the Environmental  
185 Molecular Sciences Laboratory (EMSL) in Richland, WA, USA. As per Tfaily *et al.* [2017], we  
186 performed weekly calibration using a tuning solution containing C<sub>2</sub>F<sub>3</sub>O<sub>2</sub>, C<sub>6</sub>HF<sub>9</sub>N<sub>3</sub>O,  
187 C<sub>12</sub>HF<sub>21</sub>N<sub>3</sub>O, C<sub>20</sub>H<sub>18</sub>F<sub>27</sub>N<sub>3</sub>O<sub>8</sub>P<sub>3</sub>, and C<sub>26</sub>H<sub>18</sub>F<sub>39</sub>N<sub>3</sub>O<sub>8</sub>P<sub>3</sub> with *m/z* ranging from 112 to 1333  
188 (Agilent Technologies, Santa Clara, CA USA), and instrument settings were optimized using  
189 Suwannee River Fulvic Acid (IHSS). The instrument was flushed between samples using a  
190 mixture of water and methanol. Blanks were analyzed at the beginning and the end of the day to  
191 monitor for background contaminants.

192 The extracts were injected directly into the mass spectrometer and the ion accumulation  
193 time was optimized for all samples to account for differences in OC concentration. The ion  
194 accumulation time ranged between 0.5 and 1s. A standard Bruker electrospray ionization (ESI)  
195 source was used to generate negatively charged molecular ions. Samples were introduced to the  
196 ESI source equipped with a fused silica tube (30 µm i.d.) through an Agilent 1200 series pump  
197 (Agilent Technologies) at a flow rate of 3.0 µL min<sup>-1</sup>. Experimental conditions were as follows:  
198 needle voltage, +4.4 kV; Q1 set to 50 *m/z*; and the heated resistively coated glass capillary  
199 operated at 180 °C.

200

### 201 2.5. FT-ICR-MS data processing

202 One hundred forty-four individual scans were averaged for each sample and internally  
203 calibrated using an organic matter homologous series separated by 14 Da (–CH<sub>2</sub> groups). The

204 mass measurement accuracy was less than 1 ppm for singly charged ions across a broad  $m/z$   
205 range (100-1200  $m/z$ ). The mass resolution was  $\sim 350K$  at 339  $m/z$ . Data Analysis software  
206 (BrukerDaltonik version 4.2) was used to convert raw spectra to a list of  $m/z$  values applying  
207 FTMS peak picker module with a signal-to-noise ratio (S/N) threshold set to 7 and absolute  
208 intensity threshold to the default value of 100.

209 Putative chemical formulae were then assigned using in-house built software following  
210 the Compound Identification Algorithm (CIA), proposed by *Kujawinski and Behn* [2006],  
211 modified by *Minor et al.* [2012], and previously described in *Tfaily et al.* [2017]. Chemical  
212 formulae were assigned based on the following criteria: S/N >7, and mass measurement error <1  
213 ppm, taking into consideration the presence of C, H, O, N, S and P and excluding other elements.  
214 To ensure consistent formula assignment, we aligned all sample peak lists for the entire dataset  
215 to each other in order to facilitate consistent peak assignments and eliminate possible mass shifts  
216 that would impact formula assignment. We implemented the following rules to further ensure  
217 consistent formula assignment: (1) we consistently picked the formula with the lowest error and  
218 with the lowest number of heteroatoms and (2) the assignment of one phosphorus atom requires  
219 the presence of at least four oxygen atoms.

220

## 221 2.6. Identification of putative biochemical transformations using FT-ICR-MS

222 To identify potential biochemical transformation pathways, we followed the procedure  
223 detailed by *Breitling et al.* [2006] and employed by *Bailey et al.* [2017]. In essence, the mass  
224 difference between  $m/z$  peaks extracted from each spectrum with S/N>7 were compared to  
225 commonly observed mass differences associated with biochemical transformations. All possible  
226 pairwise mass differences were calculated within each extraction type, and differences (within

227 1ppm) were matched to a list of 92 common biochemical transformations (e.g., gain or loss of  
228 amino groups or sugars). For example, a mass difference of 99.07 corresponds to a gain or loss  
229 of the amino acid valine, while a difference of 179.06 corresponds to the transfer of a glucose  
230 molecule. Pairs of peaks with a mass difference within 1 ppm of our transformation list were  
231 considered to be related by the corresponding compound. This approach is feasible with FT-ICR-  
232 MS data because the set of peaks in each sample are related by measureable and clearly defined  
233 mass differences corresponding to gains and losses of compounds. It has been previously used by  
234 *Bailey et al.* [2017] to demonstrate differences in biochemical transformations among soils  
235 incubated with different microbial inoculate and among pore size classes in complex soil  
236 matrices.

237

### 238 *2.7. Identification of putative microbial metabolic pathways using FT-IR-MS*

239 Additionally, a set of putative microbial metabolic pathways in each sample can be  
240 identified by locating chemical formulae assigned to m/z's within metabolic pathways defined in  
241 the Kyoto Encyclopedia of Genes and Genomes (KEGG, Release, 80.0, <http://www.kegg.jp>)  
242 [*Kanehisa and Goto*, 2000]. For example, a peak with a mass of 400.3356 was assigned formula  
243 C<sub>20</sub>H<sub>16</sub>O<sub>9</sub> and mapped to KEGG pathway 'map00254' (Aflatoxin biosynthesis). While only a  
244 subset of compounds detected by FT-ICR-MS are defined within the KEGG database (i.e., peaks  
245 must be assigned a chemical formula and that chemical formula must be present in a KEGG  
246 pathway), we found 415 unique peaks that were assigned putative molecular formulae *and* that  
247 corresponded to compounds present in KEGG pathways. Additionally, we defined assignments  
248 at the pathway level (i.e., by "map" number) instead of using enzyme level classification (i.e.,

249 EC number) in order to aggregate compounds found within the same pathways. This was done to  
250 facilitate functional interpretation.

251 Although we acknowledge our results do not represent a comprehensive analysis of all  
252 microbial metabolic pathways present in a sample, we assume that KEGG pathways containing  
253 more peaks detected by FT-ICR-MS within a sample are more likely to be active than those with  
254 fewer mapped peaks. We further reduced possible random matches by assessing correlations  
255 with aerobic metabolisms as described in the ‘Statistical Methods’ section below, and we  
256 compare results across samples to yield insight into microbial pathways in each sample beyond  
257 that which can be garnered from biochemical transformations. The results are, however,  
258 conceptually congruent with those derived from the biochemical transformation analyses  
259 described in the preceding sub-section. The KEGG pathway and transformation analyses are  
260 independent of each other, yet provided consistent insights and thus together they provide greater  
261 confidence in our interpretations.

262

## 263 *2.8. Statistical Methods*

264 All statistical analyses were conducted using R software (<https://www.r-project.org/>). FT-  
265 ICR m/z intensities were converted into presence/absence data prior to analysis because  
266 differences in m/z intensity are influenced by ionization efficiency as well as relative abundance  
267 [Kujawinski and Behn, 2006; Minor et al., 2012; Malak M Tfaily et al., 2015; M.M. Tfaily et al.,  
268 2017].

269 To examine differences in OC composition between transects, we used the ‘vegan’  
270 package to construct a Sorenson dissimilarity matrix for all m/z’s identified (i.e., we included  
271 peaks with or without assigned formula) within each OC pool (water-soluble or physically-

272 bound). Differences between vegetation states (i.e., V vs. NV) were tested with PERMANOVA  
273 (999 permutations, ‘vegan’) and visualized using Non-metric Multidimensional Scaling (NMDS,  
274 ‘vegan’). One sample (NV, profile 1, depth 30-40cm) was removed due to peak interference  
275 during FT-ICR-MS, and three samples (NV, profile 2, depths 00-10cm, 10-20cm, 20-30cm) were  
276 excluded because were unable to collect sufficient sample mass for all analyses.

277 To reveal transformations associated with aerobic metabolism and to study differences in  
278 those transformations across vegetation states, we determined the number of times a given  
279 transformation occurred within each OC pool in each sample. Specifically, for each of the 92  
280 compounds in our set of biochemical transformations, we counted the number of times in each  
281 sample that transformation was observed to yield an estimate of the prevalence or ‘abundance’ of  
282 each transformation in each sample. We correlated these abundance estimates to rates of  
283 metabolism using Pearson’s product-moment correlation coefficient. Positive relationships were  
284 inferred as biochemical transformations possibly associated with biotic OC oxidation. To  
285 determine variation in biochemical transformations across vegetation states, we calculated Bray-  
286 Curtis dissimilarity from the abundance of biochemical transformations that positively correlated  
287 with aerobic metabolism at either vegetation state, visualized them with non-metric  
288 Multidimensional Scaling visualization (NMDS, ‘vegan’), and statistically evaluated them with  
289 PERMANOVA (999 permutations, ‘vegan’). We refer to H<sub>2</sub>O- and CHCl<sub>3</sub>-soluble OC pools at  
290 V and NV, respectively, as V-W (‘vegetated water’), V-B (‘vegetated bound’), NV-W (‘not  
291 vegetated water’), and NV-B (‘not vegetated bound’) for the remainder of the manuscript.

292 Similar to our analyses of biochemical transformations, we found the number of m/z’s  
293 that mapped to a given KEGG pathway. We make the assumption that pathways with more m/z’s  
294 mapped to them have a higher probability of actively contributing to biogeochemical function.

295 To identify which pathways were most likely to contribute to aerobic metabolism, we correlated  
296 the number of m/z's mapped to a given KEGG pathway within each sample to aerobic  
297 metabolism. Those pathways with positive correlations were interpreted as contributing to OC  
298 oxidation, and the following analysis was conducted only with positively correlated KEGG  
299 pathways. The number peaks mapping to each KEGG pathway in a sample was normalized by  
300 the total number of peaks mapping to any positively correlated KEGG pathway in the sample to  
301 yield data as a relative abundance. Pathways were clustered based on their relative abundance in  
302 each vegetation state and pool type. Clusters were determined using the 'hclust' algorithm in R  
303 with the 'complete linkage' clustering method and visualized using the 'pheatmap' package.

304 Finally, we examined associations between aerobic metabolism and OC thermodynamics  
305 by calculating the Gibbs Free Energy of OC oxidation under standard conditions ( $\Delta G^{\circ}_{\text{Cox}}$ ) from  
306 the Nominal Oxidation State of Carbon (NOSC) as per *La Rowe and Van Cappellen* [2011].  
307 NOSC was calculated from the number of electrons transferred in OC oxidation half reactions  
308 and is defined by the equation:

$$309 \quad (1) \text{NOSC} = -((-Z + 4a + b - 3c - 2d + 5e - 2f)/a) + 4$$

310 , where a, b, c, d, e, and f are, respectively, the numbers of C, H, N, O, P, S atoms in a given  
311 organic molecule and Z is net charge of the organic molecule (assumed to be 1). In turn,  $\Delta G^{\circ}_{\text{Cox}}$   
312 was estimated from NOSC following *La Rowe and Van Cappellen* [2011]:

$$313 \quad (2) \Delta G^{\circ}_{\text{Cox}} = 60.3 - 28.5(\text{NOSC})$$

314 Values of  $\Delta G^{\circ}_{\text{Cox}}$  are generally positive, indicating that OC oxidation must be coupled to the  
315 reduction of a terminal electron acceptor. While  $\Delta G^{\circ}_{\text{Cox}}$  varies according to the availability of  
316 terminal electron acceptors, our system is primarily oxic, allowing us to infer oxygen as the  
317 primary electron acceptor in most reactions and make direct comparisons across samples.

318 Additionally, though the exact calculation of  $\Delta G^{\circ}_{\text{Cox}}$  necessitates an accurate quantification of all  
319 species involved in every chemical reaction in a sample, the use of NOSC as a practical basis for  
320 determining  $\Delta G^{\circ}_{\text{Cox}}$  has been validated [Arndt *et al.*, 2013; LaRowe and Van Cappellen, 2011].

321 Here, we assessed relationships between aerobic metabolism and  $\Delta G^{\circ}_{\text{Cox}}$  of OC  
322 compounds identified in each OC pool (determined by FT-ICR-MS analysis) using linear  
323 regressions in each vegetation state, in which aerobic metabolism (determined by resazurin  
324 assay) was the independent variable and average  $\Delta G^{\circ}_{\text{Cox}}$  of all m/z's with assigned formula was  
325 the dependent variable.

326

### 327 **3. Results and Discussion**

#### 328 *3.1. Shifts in physicochemical, metabolic, and OC character between vegetation states*

329 Differences in vegetation states corresponded to differences in physicochemistry, aerobic  
330 metabolism, and OC pool composition. V was characterized by mature trees near the water line  
331 and was nutrient-rich relative to NV (Figure S1-3). V displayed comparatively high  
332 concentrations of total C and rates of aerobic metabolism (Figure S1-3). In contrast, NV  
333 consisted of vegetation-free, cobble-ridden shoreline with sandier soils, low total C, and low  
334 aerobic metabolism (Figure S1-3).

335 Compositional difference in OC pools indicated a possibility for distinct OC oxidation  
336 processes between the vegetation states (Figure 1), as preferential oxidation of certain OC  
337 compounds in each state would be expected to generate an observable difference in OC pool  
338 composition. Further, total organic OC content explained only 38% of aerobic metabolic rates  
339 ( $R^2 = 0.38$ ,  $P < 0.0001$ , Figure S4), leaving open the possibility that OC compositional

340 differences may be related to differences in aerobic metabolism at each vegetation state. The  
341 following sections explore this possibility.

342

### 343 *3.2. Associations between C transformations and aerobic metabolism*

344         Given compositional differences in OC between vegetation states and known impacts of  
345 C chemistry on metabolic functioning in other systems [*Castle et al.*, 2016; *Graham et al.*,  
346 2016b], we hypothesized that biochemical transformations related to rates of aerobic metabolism  
347 would be unique to each vegetation state.

348         Consistent with this hypothesis, transformation analysis indicated that the biochemical  
349 processes associated with OC oxidation were significantly different between the vegetation  
350 states. Specifically, OC transformations that increased in abundance with increases in aerobic  
351 metabolism were significantly different at each vegetation state (PERMANOVA, H<sub>2</sub>O P = 0.022  
352 and CHCl<sub>3</sub> P = 0.002, Figure 3 a-b, Table 2). In comparing differences in transformations  
353 occurring within the water-soluble OC pool, we observed higher abundances of amino- and  
354 sugar-associated transformations for V-W relative to NV-W. Twenty-six of these  
355 transformations were identified as contributing to aerobic metabolism in V-W, while none were  
356 identified in NV-W. These V-W transformations were primarily associated with simple C  
357 molecules (e.g., glucose, alanine, and lysine, Table 2). Conversely, within the bound-OC pool,  
358 38 transformations were identified as contributing to aerobic metabolism in NV-B, compared to  
359 only 11 in V-B. In both cases, these transformations consisted of a greater proportion of complex  
360 C molecules (e.g., pyridoxal phosphate, palmitic acid, and glyoxylate, Table 2) than in water-  
361 soluble pools.

362           The larger number of transformations associated with aerobic metabolism in V-W vs. V-  
363 B, and the larger number in NV-B vs. NV-W, suggests that aerobic metabolism in vegetated and  
364 unvegetated areas depend on water-soluble and bound-OC pools, respectively. We note some  
365 oxidation of the bound-OC pool under vegetated conditions, but only 11 correlations were  
366 observed between V-B transformations and aerobic metabolism suggesting a relatively minor  
367 role, especially considering that there were 38 significant correlations for NV-B. These  
368 differences suggests that an increased supply of bioavailable compounds in vegetated areas leads  
369 to bound-OC being less involved in aerobic metabolism, relative to unvegetated areas where  
370 bound-OC appears to be heavily involved in aerobic metabolism. The concept of priming  
371 [Kuznyakov, 2010] would predict the opposite pattern—a greater supply of bioavailable OC  
372 should increase the contributions of less bioavailable OC (here, bound-OC) to aerobic  
373 metabolism. Our results run counter to a priming mechanism and indicate that the supply of  
374 bioavailable compounds—associated with riparian vegetation—diminishes the contribution of  
375 bound-OC to aerobic metabolism and, in turn, protects bound-OC pools. Mineral-stabilized OC  
376 therefore has greater potential to remain sequestered along river corridors with spatially and  
377 temporally consistent inputs of bioavailable OC, potentially derived from riparian vegetation.  
378 The fate of OC that moves across the terrestrial-aquatic continuum may therefore be impacted by  
379 land use change [Foley *et al.*, 2005] in ways not currently represented in ESMs.

380

### 381 *3.3. Associations between microbial metabolic pathways and aerobic metabolism*

382           Because we observed stark differences in the identity of OC transformations that  
383 correlated with aerobic metabolism across vegetation states, we hypothesized that the microbial  
384 metabolic pathways associated with OC transformations were also dependent on vegetation state.

385 Indeed, pathways associated with OC oxidation were distinct at V vs. NV, supporting our  
386 hypothesis that there were differences in the metabolic processing of OC in the presence or  
387 absence of riparian vegetation. Specifically, while the metabolism of plant-derived compounds  
388 appeared to be a major driver of aerobic respiration at both vegetation states, metabolism at V  
389 mostly involved readily bioavailable plant derivatives in the water-soluble OC pool, and  
390 metabolism at NV was associated with plant derivatives in the bound-OC pool (Figure 4).

391 In V-W, two primary pathways were involved in metabolism of plant compounds, each  
392 contained within its own hierarchical cluster (map01110: Biosynthesis of secondary metabolites;  
393 map00941: Flavonoid biosynthesis). A concomitant cluster of plant-associated metabolisms with  
394 lower abundance in V-W (Cluster 4) was also positively correlated to aerobic metabolism  
395 (Figure 4). Secondary metabolites (map01110) are largely comprised of plant-derived  
396 compounds such as flavonoids [Agati *et al.*, 2012], terpenoids [Tholl, 2015], and nitrogen-  
397 containing alkaloids [Willaman and Schubert, 1961], while flavonoids [Agati *et al.*, 2012] are  
398 one of those most abundant plant-derived compounds. Associations with aflatoxin [Trail *et al.*,  
399 1995], flavone/flavonol [Agati *et al.*, 2012], and phenylpropanoids [Hahlbrock and Scheel, 1989]  
400 (Cluster 4) bolster this association between plant-associated metabolic pathways and aerobic  
401 metabolism in V-W.

402 Although correlations between plant-associated KEGG pathways and aerobic metabolism  
403 could indicate the persistence of plant secondary metabolites rather than microbial metabolism,  
404 our results indicate a central role for vegetation in water-soluble OC oxidation in either case. For  
405 example, if KEGG associations were attributable to plant metabolism instead of microbial  
406 metabolism, correlations between plant-associated pathways and aerobic metabolism in V-W  
407 would indicate an indirect relationship between plant growth and microbial oxidation of OC,

408 whereby plant byproducts support microbial communities in oxidizing other portions of the OC  
409 pool.

410 In contrast to V-W, NV-W did not display associations between plant-associated  
411 metabolic pathways and OC oxidation. All correlations indicated broad metabolic processes  
412 including membrane transport and carbohydrate metabolism that may indicate utilization of other  
413 resources (Cluster 3, Figure 4).

414 Instead, we observed relationships between plant-associated metabolisms and OC  
415 oxidation within NV-B. For example, correlations were strongest in Cluster 1, which contained  
416 pathways of cutin, suberine, and wax biosynthesis [*King et al.*, 2007; *Raffaele et al.*, 2009;  
417 *Shepherd and Wynne Griffiths*, 2006], alpha-linolenic acid metabolism [*Crawford et al.*, 2000;  
418 *Creelman and Mulpuri*, 2002], and biosynthesis of secondary metabolites [*Agati et al.*, 2012;  
419 *Tholl*, 2015; *Willaman and Schubert*, 1961] (Figure 4). Each of these pathways denotes the  
420 synthesis or metabolism of a plant-associated lipid compound. Because no specific metabolisms  
421 were correlated to OC oxidation in the water-soluble pool, we hypothesize that these lipid-based  
422 metabolisms comprise the primary KEGG-identifiable pathways associated with OC oxidation in  
423 areas without riparian vegetation. We also observed one cluster of pathways that correlated with  
424 metabolism at V-B (Cluster 7) and contained plant-associated metabolic pathways such as  
425 linoleic acid metabolism [*Crawford et al.*, 2000; *Creelman and Mulpuri*, 2002] and  
426 brassinosteroid biosynthesis [*Bishop*, 2007], indicating some oxidation of lipid plant material in  
427 the bound-OC pool under vegetated conditions. We therefore propose that plant-derived lipid  
428 compounds serve as a secondary substrate for OC oxidation in shorelines with riparian  
429 vegetation, given that most correlations at V were detected in the water-soluble pool.

430

431 3.4. *Thermodynamics of carbon oxidation*

432 Finally, we hypothesized that microbes would preferentially oxidize more  
433 thermodynamically favorable compounds at both sites, consistent with common thermodynamic  
434 constraints on biogeochemical cycles [Burgin *et al.*, 2011; Hedin *et al.*, 1998; Helton *et al.*,  
435 2015]. Because we observed evidence for preferential OC oxidation of the water-soluble OC  
436 pool at V and of the bound-OC pool at NV, we further hypothesized that thermodynamic-based  
437 preference of OC oxidation would be observable only in the preferred substrate pool within each  
438 vegetation state. Consistent with this hypothesis, aerobic metabolism was positively correlated to  
439 average  $\Delta G^{\circ}_{\text{Cox}}$  in V-W ( $R^2 = 0.22$ ,  $P = 0.03$ , Fig 5a) and NV-B ( $R^2 = 0.54$ ,  $P = 0.001$  Fig 5b),  
440 but these variables were not correlated in V-B or NV-W. In both cases, aerobic metabolism  
441 corresponded to a depletion of more thermodynamically favorable OC (i.e., OC became less  
442 favorable as aerobic metabolism increased), resulting in progressively less favorable  
443 thermodynamic conditions.

444 The priming conceptual framework would predict that terrestrial inputs associated with  
445 riparian vegetation should condition microbial communities to oxidize less thermodynamically  
446 favorable C, such as that found in the bound-OC pool. In such a scenario, inputs of  
447 thermodynamically favorable carbon should—by minimizing community-level energy  
448 constraints—allow for the rise of microbial physiologies that can oxidize less favorable C  
449 [Kuzuyakov, 2010]. In this case, a significant relationship between thermodynamic favorability  
450 and aerobic metabolism in the V-W pool should lead to a similar relationship within the V-B  
451 pool. Our results reveal a strong relationship within the V-W pool, but not in the V-B pool,  
452 thereby rejecting an influence of priming. Instead, our results suggest that bound-OC pools are  
453 protected by thermodynamically favorable compounds that serve as preferred substrate.

454 In contrast to our expectation that water-soluble OC associated with riparian vegetation  
455 would increase oxidation of bound-OC pools, we observed evidence consistent with inhibition of  
456 bound-OC oxidation by thermodynamically favorable water-soluble compounds. Priming has  
457 been actively debated in aquatic research [*Bengtsson et al.*, 2014; *Bianchi*, 2011; *Guenet et al.*,  
458 2010], and a number of other studies have been unable to detect a priming effect in sediment and  
459 aqueous habitats [*Bengtsson et al.*, 2014; *Catalán et al.*, 2015].

460 The mechanisms resulting in priming are not well understood, but the phenomenon has  
461 been associated with nutrient and energy limitations in soil environments [*Kuzyakov*, 2010]. For  
462 instance, under nutrient limitation microorganisms may oxidize chemically-complex OC to  
463 garner resources (e.g., nitrogen mining), while shared resources that facilitate OC oxidation (e.g.,  
464 extracellular enzymes) are more likely to facilitate ecological cheating under energy limiting  
465 conditions [*Blagodatskaya and Kuzyakov*, 2008; *Catalán et al.*, 2015; *Guenet et al.*, 2010;  
466 *Kuzyakov*, 2010]. Our system is oligotrophic, containing a fraction of the total C content  
467 observed in other systems (Figure S1) such that C limitation rather than nutrient limitation might  
468 drive OC oxidation dynamics. In such a case, readily bioavailable C inputs would be rapidly  
469 oxidized but microbial communities may be well-adapted to rely on alternative energy sources  
470 (e.g.,  $\text{NH}_4^+$ , Fe) that may be more available than bound-OC pools.

471

### 472 3.5. Conceptual model for OC oxidation at terrestrial-aquatic interfaces

473 Based on our work, we propose a conceptual model of OC oxidation along terrestrial-  
474 aquatic interfaces in which the oxidation of bound-OC is limited by terrestrial inputs from  
475 riparian vegetation (Fig 6. a-b). Riparian vegetation sustains inputs of water-soluble compounds  
476 to nearshore OC pools, resulting in a larger thermodynamically favorable, water-soluble OC pool

477 (Figure 6b). This leads to higher overall C content in nearshore sediments and elevated rates of  
478 aerobic respiration relative to areas with less riparian vegetation. However, our data suggest that  
479 in the presence of riparian vegetation microbial carbon oxidation primarily uses the water-  
480 soluble OC pool with minimal oxidation of bound-OC due to physical and/or thermodynamic  
481 protection of this pool. For instance,  $\Delta G^{\circ}_{\text{Cox}}$  was lower in water-soluble OC pools than in bound-  
482 OC, and a large presence of this thermodynamically favorable pool may provide adequate  
483 substrate to sustain metabolic functioning, limiting the need to metabolize less  
484 thermodynamically favorable OC. Additionally, organomineral interactions can protect bound-  
485 OC from extracellular enzyme activity [Hunter *et al.*, 2016], inhibiting the bioavailability of OC  
486 by sequestering it within larger aggregates.

487 In contrast, non-vegetated riparian zones provide little input into water-soluble OC pools  
488 (Fig 6a), and rates of metabolism and C pool sizes are lower in these environments. Carbon  
489 oxidation in these non-vegetated zones occurs primarily within the bound-OC pool, albeit more  
490 slowly and as product of different biochemical and metabolic pathways than in vegetated  
491 environments (e.g., complex C transformations and lipid-based metabolism of plant derivatives).  
492 We posit that water-soluble pools in non-vegetated sediments are sufficiently small that investing  
493 in enzymes needed to metabolize this OC pool results in a net energy loss. Instead, microbes in  
494 unvegetated areas must invest in cellular machinery to access bound-OC, and our results  
495 imply that the cellular machinery needed to access bound-OC is distinct from the machinery  
496 needed to access water-soluble OC.

497 Interestingly, aerobic metabolism within both types of sediments is related to a depletion  
498 of thermodynamically favorable compounds; however, this dynamic is associated with water-  
499 soluble OC pools in vegetated zones vs. bound-OC pools in non-vegetated zones. That is,

500 microorganisms in both environments are constrained to the metabolism of their primary  
501 substrate pool but preferentially oxidize more thermodynamically favorable compounds within  
502 that pool. This dynamic suggests that microorganisms are conditioned to metabolize a subset of  
503 compounds within sediment OC, possibly defined by thermodynamic or physical protection  
504 mechanisms, but operate under common thermodynamic constraints once adapted to oxidize a  
505 certain OC pool.

506

### 507 *3.6. Broader Implications*

508 Our results indicate that terrestrial C inputs associated with riparian vegetation protect  
509 bound-OC from oxidation, possibly aiding long-term storage of mineral-bound pools along river  
510 corridors, and our work is particularly relevant to global patterns of CO<sub>2</sub> emissions in light of  
511 changes in land cover and increases in C fluxes across the terrestrial-aquatic interface. The  
512 magnitude, distribution, and chemical quality of terrestrial C fluxes into aquatic environments  
513 are perturbed by shifts in land cover (e.g., due to agriculture, urbanization, and climate-driven  
514 vegetation change) [Fang *et al.*, 2005; Knapp *et al.*, 2008]. These fluxes have been examined  
515 primarily for their own propensity to be oxidized along land-to-sea continuums [Battin *et al.*,  
516 2008; Battin *et al.*, 2009; Regnier *et al.*, 2013], but we also suggest a role for these fluxes in  
517 stabilizing mineral-bound carbon within nearshore environments. For example, vegetation  
518 removal, impervious surfaces, and drainage systems coincident with urbanization alter terrestrial  
519 C runoff patterns, both changing their magnitude and creating preferential deposition flowpaths  
520 [Fraleley *et al.*, 2009; Imberger *et al.*, 2011; Smith and Kaushal, 2015]. Agricultural drainage  
521 systems also lead to preferential flowpaths as well as spatiotemporal variation in the quantity and  
522 quality of terrestrial-aquatic fluxes [Graeber *et al.*, 2012; Larson *et al.*, 2014], an effect that

523 strongly influences C cycling given that 40% of the earth's land is cultivated [*Foley et al.*, 2005;  
524 *Graeber et al.*, 2012]. We propose that changes in the distribution of these fluxes through space  
525 and time may impact OC oxidation both in the C transported along these flow paths and within  
526 sediments that are differentially exposed to terrestrial OC.

527 Furthermore, vegetation distributions in natural ecosystems are predicted to shift in  
528 response to altered precipitation regimes. Associated changes in plant phenology, morphology,  
529 and establishment will impact the quantity, quality, and distribution of terrestrial material  
530 entering aquatic systems [*Knapp et al.*, 2008], and we currently have an incomplete  
531 understanding of how these patterns will vary across ecosystems and precipitation patterns [*Fang*  
532 *et al.*, 2005; *Knapp et al.*, 2008]. A mechanistic framework for C oxidation that captures impacts  
533 of heterogeneity in vegetation in river corridors will therefore aid in predicting how terrestrial-  
534 aquatic interfaces respond to ongoing perturbations. Here, we demonstrate a potential for  
535 increases in the intensity of terrestrial C fluxes to lead to larger mineral-bound C pools by  
536 physically and thermodynamically protecting these pools; and conversely, a potential for  
537 oxidation of mineral-bound C pools in areas with diminished terrestrial C inputs.

538 Earth System Models depend on mathematical representations of C cycling, and the  
539 continued development of these models is tightly coupled to conceptual advances drawn from  
540 field-based observations [*Burd et al.*, 2016; *Six et al.*, 2002]. Despite recent progress, these  
541 models are still missing key regulatory processes [*Todd-Brown et al.*, 2013; *Wieder et al.*, 2013;  
542 *Wieder et al.*, 2015]. To address this knowledge gap, we propose a new conceptual framework of  
543 OC dynamics based on analysis of *in situ* observational data that explicitly considers a central  
544 challenge in model improvement—biochemical, metabolic, and thermodynamic mechanisms  
545 governing OC oxidation along terrestrial-aquatic interfaces. Our results directly contrast those

546 expected within a ‘priming’ framework, and we advance that water-soluble thermodynamically  
547 favorable OC associated with riparian vegetation protects thermodynamically less favorable  
548 bound-OC from oxidation. We also demonstrate differences in biochemical and metabolic  
549 pathways associated with metabolism of water-soluble and bound-OC pools in the presence or  
550 absence of riparian vegetation, furthering a processed-based understanding of terrestrial-aquatic  
551 interfaces.

552 Our conceptualization of OC oxidation may also be applicable beyond terrestrial-aquatic  
553 interfaces, as many ecosystems experience spatiotemporal variability in the quantity of  
554 thermodynamically favorable water-soluble OC. For instance, vegetation senescence generates  
555 pulses of bioavailable C into most temperate and tropical ecosystems. Our research provides an  
556 opportunity to enhance the mechanistic underpinning of OC oxidation process representations  
557 within ESMs—an imperative under heterogeneous landscapes and unknown future land cover  
558 distributions—and proposes interactions between OC thermodynamics and mineral-inhibition of  
559 OC oxidation as a key future research need.

560

#### 561 **Author Contributions.**

562 EBG was responsible for conceptual development and data analysis and was the primary writer  
563 with guidance from JCS and MT. ARC, AEG, CTR, ECR, DWK, and JCS were responsible for  
564 experimental design and data collection. MT was responsible for all FT-ICR processing. All  
565 authors contributed to manuscript revisions.

566

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575

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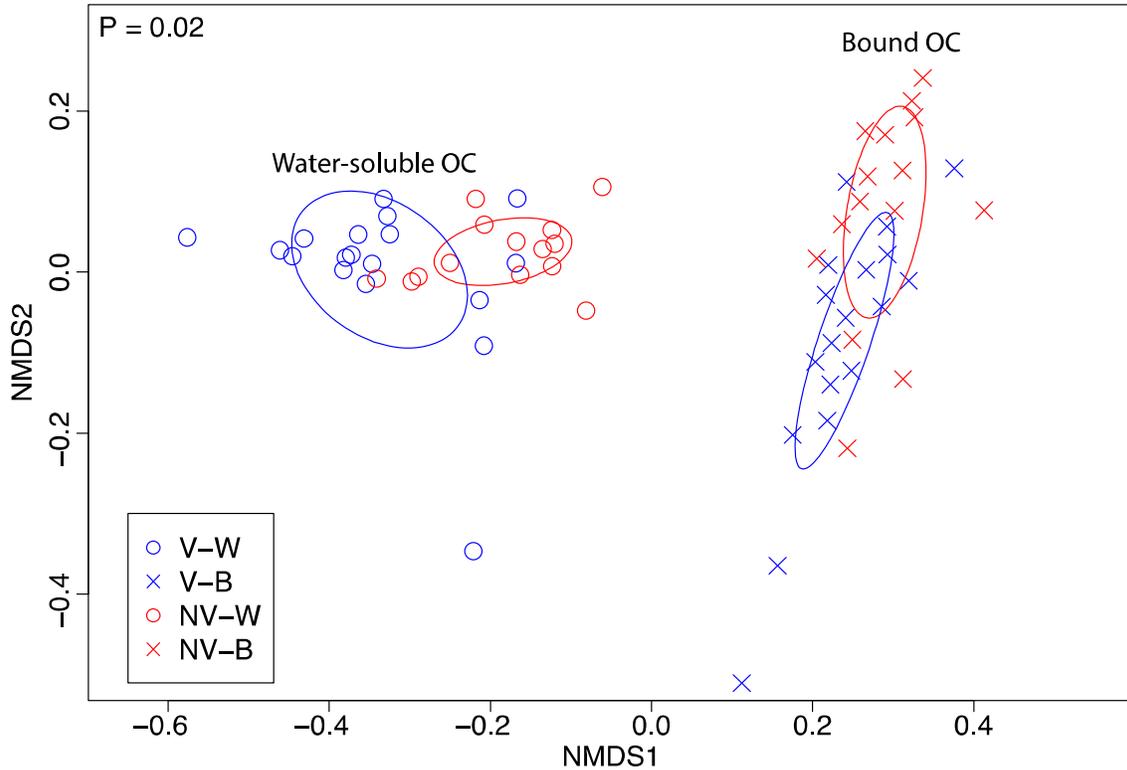
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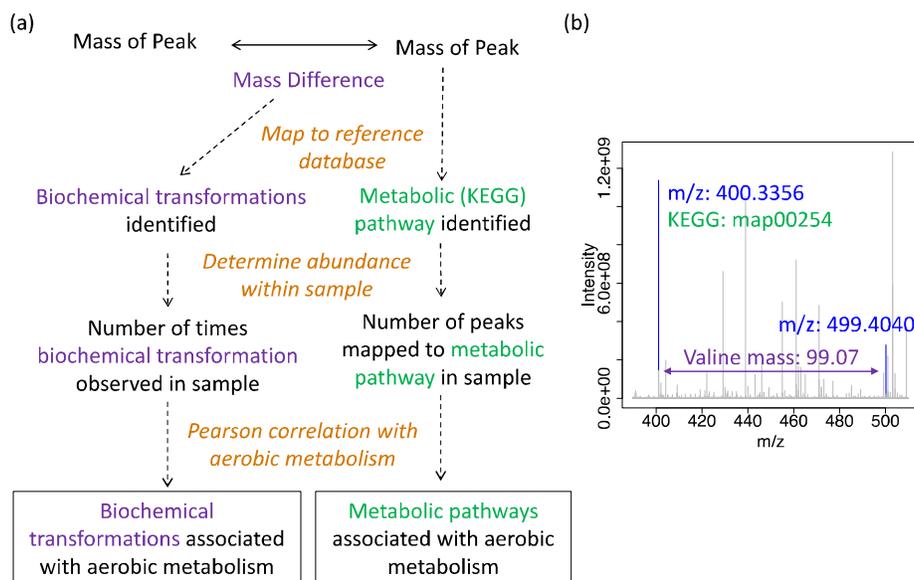
811 **Figure Captions and Table Legends.**

Figure 1



812

813 **Figure 1. NMDS visualization of dissimilarity in OC pool composition.** Water-soluble and  
814 bound-OC pools are represented by open circles and x's, respectively. Samples associated with  
815 riparian vegetation are blue, and those in areas without vegetation are red. The P-value reflects  
816 differences among all groups, as assessed by PERMANOVA. Ellipses represent the standard  
817 deviation of the average axis scores for each group, generated using the 'ordiellipse' function in  
818 the 'vegan' package. Within each extraction, the composition of OC pools was significantly  
819 different across vegetation states (both  $P = 0.001$ ).



820

821 **Figure 2. Methodology for inferring biochemical transformations and metabolic pathways.**

822 Panel (a) depicts our workflow for analyzing biochemical transformations and metabolic

823 pathways. Biochemical OC transformations (purple) were identified by mapping mass

824 differences in pairwise m/z peak comparisons to a set of 92 known masses transferred in

825 common biochemical transformations (e.g., glucose, amines). Metabolic pathways (green) were

826 identified by mapping all chemical formula assigned to m/z peaks to the KEGG database. Within

827 each sample, the abundance of each biochemical transformation and the number of peaks

828 mapping to each metabolic pathway were then correlated to aerobic metabolism to garner

829 insights into OC oxidation processes. Panel (b) displays an example portion of our FT-ICR-MS

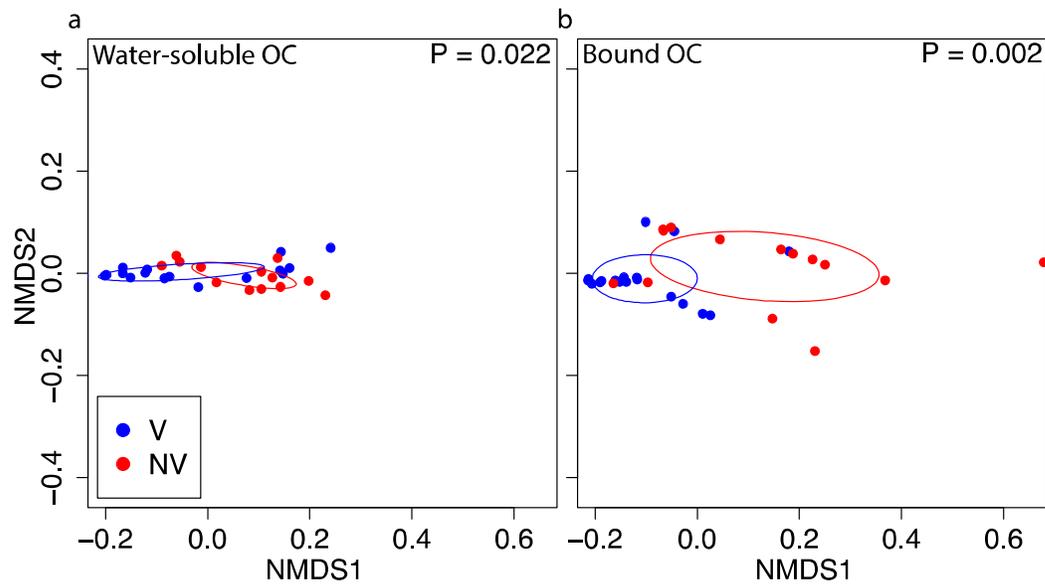
830 spectra overlain with peak assignments (blue), a biochemical transformation (mass difference

831 between peaks, denoted in purple), and a metabolic pathway (associated with the left-hand peak,

832 denoted in green).

833

Figure 3



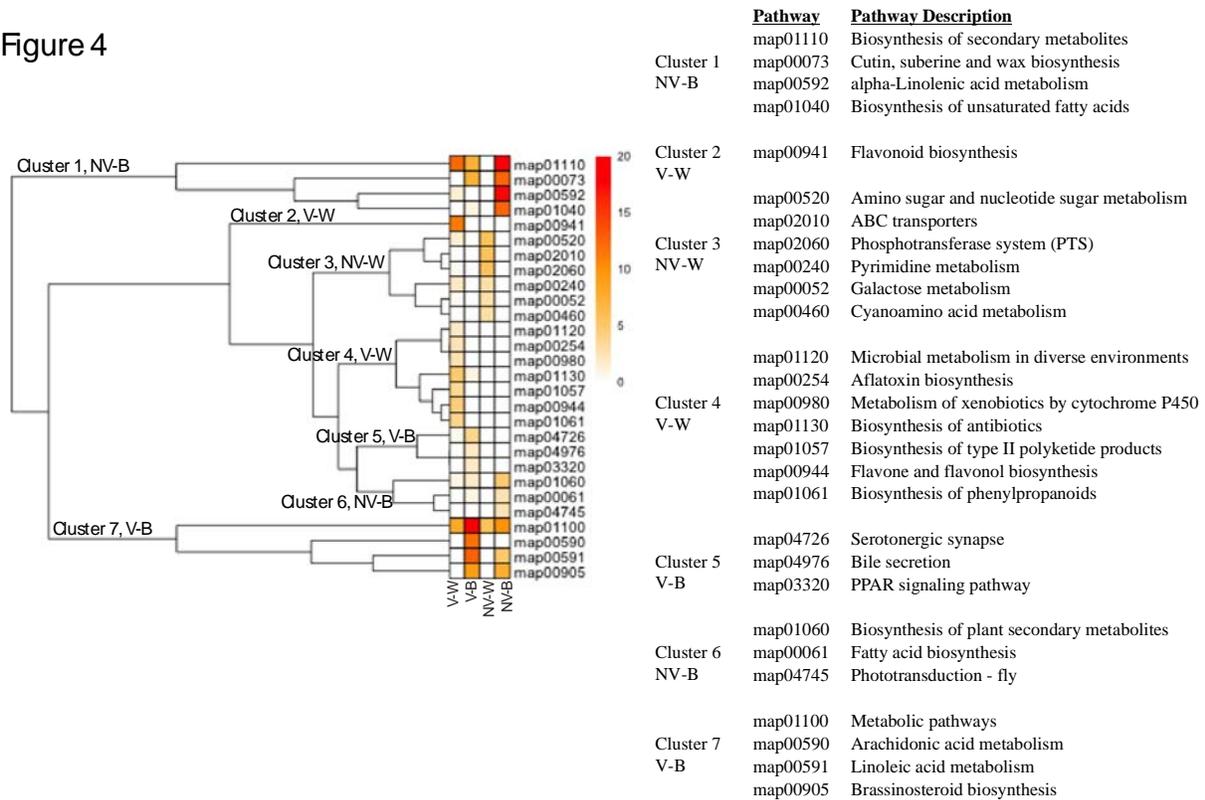
834  
835 **Figure 3. NMDS visualization of biochemical transformation partitioning among vegetation**

836 **states.** Biochemical transformations that were correlated to aerobic metabolism were  
837 significantly different among vegetation states in both the (a) water-soluble and (b) bound-OC  
838 pools. V and NV are denoted in blue and red, respectively, and significance values are derived  
839 from PERMANOVA. Ellipses represent the standard deviation of the average axis scores for  
840 each group, generated using the 'ordiellipse' function in the 'vegan' package.

841

842

Figure 4



843

844 **Figure 4. KEGG pathways associated with aerobic metabolism.** A hierarchical clustering

845 heatmap shows KEGG pathways positively associated with aerobic metabolism. Colors move

846 from white to red from a scale of 0% to 20%, showing percent relative abundance of each

847 pathway in each group. Pathways are described and divided by cluster and listed in the legend.

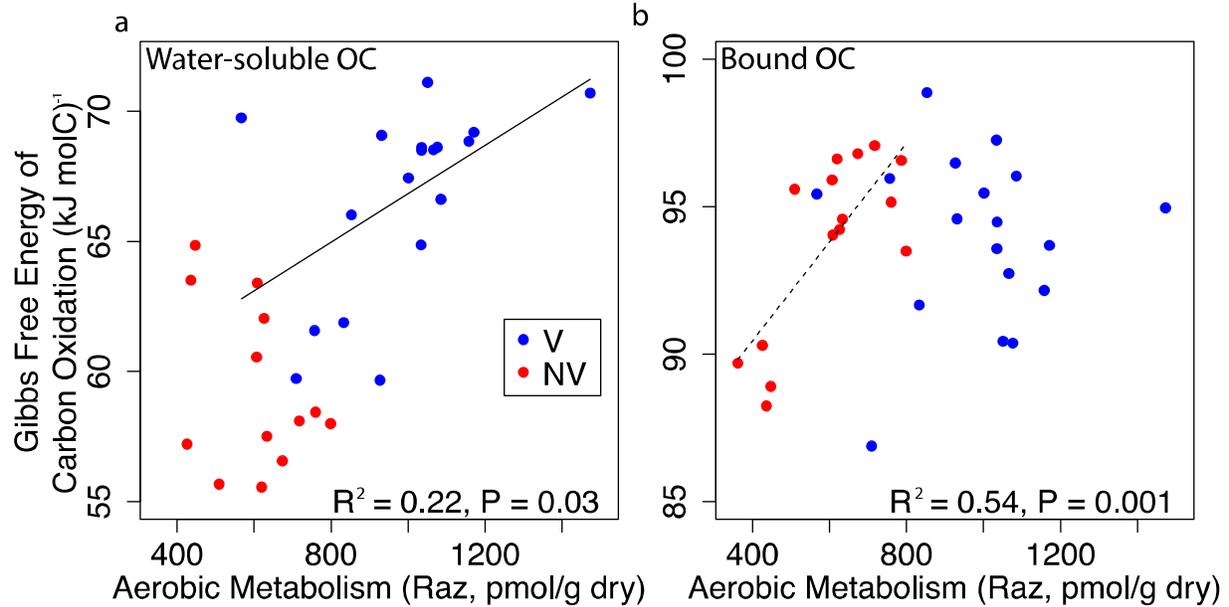
848 V-W, V-B, NV-W, and NV-B are placed on branches that yield clusters with which they are

849 predominantly associated.

850

851

Figure 5



852

853 **Figure 5. Correlations between Gibbs free energy of carbon oxidation ( $\Delta G^{\circ}_{\text{Cox}}$ ) and aerobic**

854 **metabolism.** (a) and (b) display linear regressions relating  $\Delta G^{\circ}_{\text{Cox}}$  to aerobic metabolism in

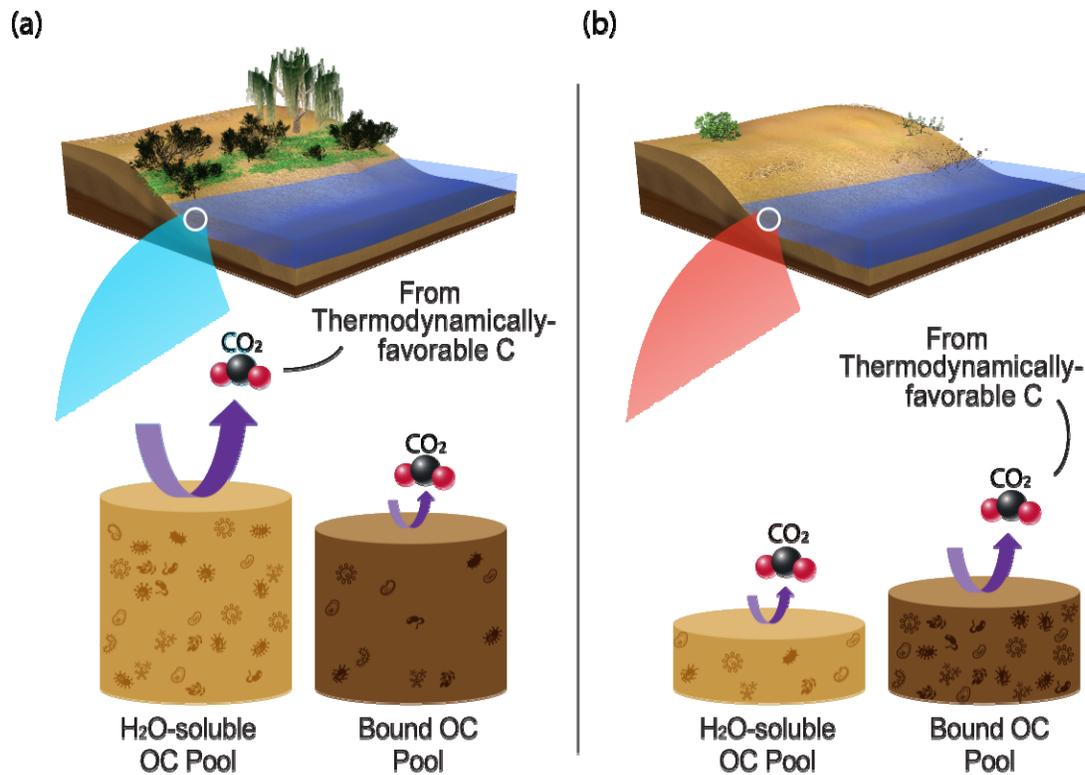
855 water-soluble and bound-OC pools, respectively. Aerobic metabolism is expressed as pmoles of

856 resazurin reduced to resorufin per gram dry weight over a 48hr incubation period (Raz, see

857 Supplemental Information). V and NV are denoted in blue and red. Solid lines show significant

858 relationship at V; dashed lines show significant relationship at NV.

859



Figure

860

861 **6. Conceptualization of relationship between riparian vegetation and OC oxidation.** We

862 propose a conceptualization of OC oxidation at terrestrial-aquatic interfaces whereby (a) more

863 riparian vegetation results in greater terrestrial C deposition and larger water-soluble and bound-

864 OC pools. However, water-soluble OC is preferentially oxidized, which protects the bound-OC

865 pool. Conversely, (b) areas depleted in riparian vegetation experience lower inputs to water-

866 soluble OC pools and show lower rates of OC oxidation. This results in smaller OC pools (water-

867 soluble and bound) and microbial adaptation for oxidation of the bound-OC pool. In both cases,

868 the most thermodynamically favorable portions of the OC pool being metabolized are

869 preferentially oxidized. Height of the cylinders denotes pool sizes, and arrow thickness denotes

870 flux magnitude.

871

872

873 **Table 1. Acronyms and abbreviations used in this paper.**

<b>Abbreviation/Acronym</b>	<b>Description</b>
V	Transect with dense riparian vegetation (i.e., 'vegetated')
NV	Transect with sparse riparian vegetation (i.e., 'not vegetated')
V-W	Transect V, water extraction (water-soluble OC)
V-B	Transect V, chloroform extraction (bound-OC)
NV-W	Transect NV, water extraction (water-soluble OC)
NV-B	Transect NV, chloroform extraction (bound-OC)
H <sub>2</sub> O	Water
CHCl <sub>3</sub>	Chloroform
C	Carbon
OC	Organic carbon
FT-ICR-MS	Fourier transform ion cyclotron resonance mass spectrometry
KEGG	Kyoto Encyclopedia of Genes and Genomes
$\Delta G^{\circ}_{\text{Cox}}$	Gibbs free energy of C oxidation

875 **Table 2. Biochemical transformations correlated with aerobic metabolism in each OC pool**  
 876 **and vegetation state.**

	Pearson's $r$
<b>V-W</b>	
biotinyl_(-H)_C10H15N2O3S	0.74
uridine_5_diphosphate_(-H2O)_C9H12N2O11P2	0.67
cytosine_(-H)_C4H4N3O	0.65
uridine_5_monophosphate_(-H2O)_C9H11N2O8P	0.65
guanine_(-H)_C5H4N5O	0.61
guanosine_(-H2O)_C10H11N5O4	0.59
adenine_(-H)_C5H4N5	0.59
glutathione_(-H2O)_C10H15N3O5S	0.57
uracil_(-H)_C4H3N2O2	0.56
glucose_C6H12O6	0.53
C6H10O6	0.53
Aspartic_Acid_C4H5NO3	0.52
Glucuronic_Acid_(-H2O)	0.52
Lysine_C6H12N2O	0.51
D-Ribose_(-H2O)_(-ribosylation)	0.50
secondary_amine	0.50
Alanine_C3H5NO	0.50
C6H10O5	0.49
monosaccharide_(-H2O)	0.49
Threonine_C4H7NO2	0.49
Glutamic_Acid_C5H7NO3	0.48
pentose_C5H8O4	0.47
acetotacetate_(-H2O)_C4H4O2	0.47
Glutamine_C5H8N2O2	0.47
pyridoxal_phosphate_(-H2O)_C8H8NO5P	0.47
<b>V-B</b>	
isoprene_addition_(-H)_C5H7	0.61
phosphate	0.56
primary_amine	0.55
Glucuronic_Acid_(-H2O)	0.53
glyoxylate_(-H2O)_C2O2	0.53
malonyl_group_(-H2O)_C3H2O3	0.52
D-Ribose_(-H2O)_(-ribosylation)	0.49
pyrophosphate	0.49
acetotacetate_(-H2O)_C4H4O2	0.49
hydrogenation_dehydrogenation_H2	0.47

877

<b>NV-W</b>	
NONE	NA
<b>NV-B</b>	
Adenosine_5_monophosphate_(-H2O)_C10H12N5O6P	0.92
adenylate_(-H2O)_C10H12N5O6P	0.92
pyridoxal_phosphate_(-H2O)_C8H8NO5P	0.73
acetylation_(-H2O)_C2H2O	0.70
ketol_group_(-H2O)	0.70
Isoleucine_C6H11NO	0.69
Leucine_C6H11NO	0.69
ethyl_addition_(-H2O)_C2H4	0.69
Threonine_C4H7NO2	0.69
Valine_C5H9NO	0.68
Carboxylation_CO2	0.68
Glycine_C2H3NO	0.67
Formic_Acid_(-H2O)_CO	0.67
Serine_C3H5NO2	0.67
hydroxylation_(-H)_O	0.67
palmitoylation_(-H2O)_C16H30O	0.67
pentose_C5H8O4	0.66
secondary_amine	0.66
condensation/dehydration_H2O	0.66
C2H2_C2H2	0.66
erythrose_(-H2O)	0.66
CH4_O	0.65
methanol_(-H2O)	0.65
glyoxylate_(-H2O)_C2O2	0.65
NH_CH2	0.64
Alanine_C3H5NO	0.63
acetotacetate_(-H2O)_C4H4O2	0.63
Proline_C5H7NO	0.62
hydrogenation_dehydrogenation_H2	0.61
Histidine_C6H7N3O	0.60
malonyl_group_(-H2O)_C3H2O3	0.59
Cysteine_C3H5NOS	0.58
glcnac_C8H13N1O5	0.57
Methionine_C5H9NOS	0.57
Arginine_C6H12N4O	0.56
Aspartic_Acid_C4H5NO3	0.56

D-Ribose(→H<sub>2</sub>O)ribosylation)

0.55