Potential bioavailability of representative pyrogenic organic matter compounds in comparison to natural dissolved organic matter pools

Emily B. Graham[+*][1,2], Hyun-Seob Song[+][3], Samantha Grieger[1,4], Vanessa A. Garayburu-Caruso[1,5], James C. Stegen[1], Kevin D. Bladon[6], and Allison Myers-Pigg [1,4]

[+]equal contributors

[*]*Correspondence to*: Emily B. Graham (emily.graham@pnnl.gov)

[1] Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA, USA

[2] School of Biological Sciences, Washington State University, Richland, WA USA

[3] Department of Biological Systems Engineering, Department of Food Science and Technology, Nebraska Food for Health Center, University of Nebraska, Lincoln, NE, USA

[4] Marine and Coastal Research Laboratory, Pacific Northwest National Laboratory, Richland, WA, USA

[5] School of the Environment, Washington State University, Richland, WA USA

[6] Department of Forest Engineering, Resources, and Management, Oregon State University, Corvallis, OR, USA

20   **GRAPHICAL ABSTRACT**
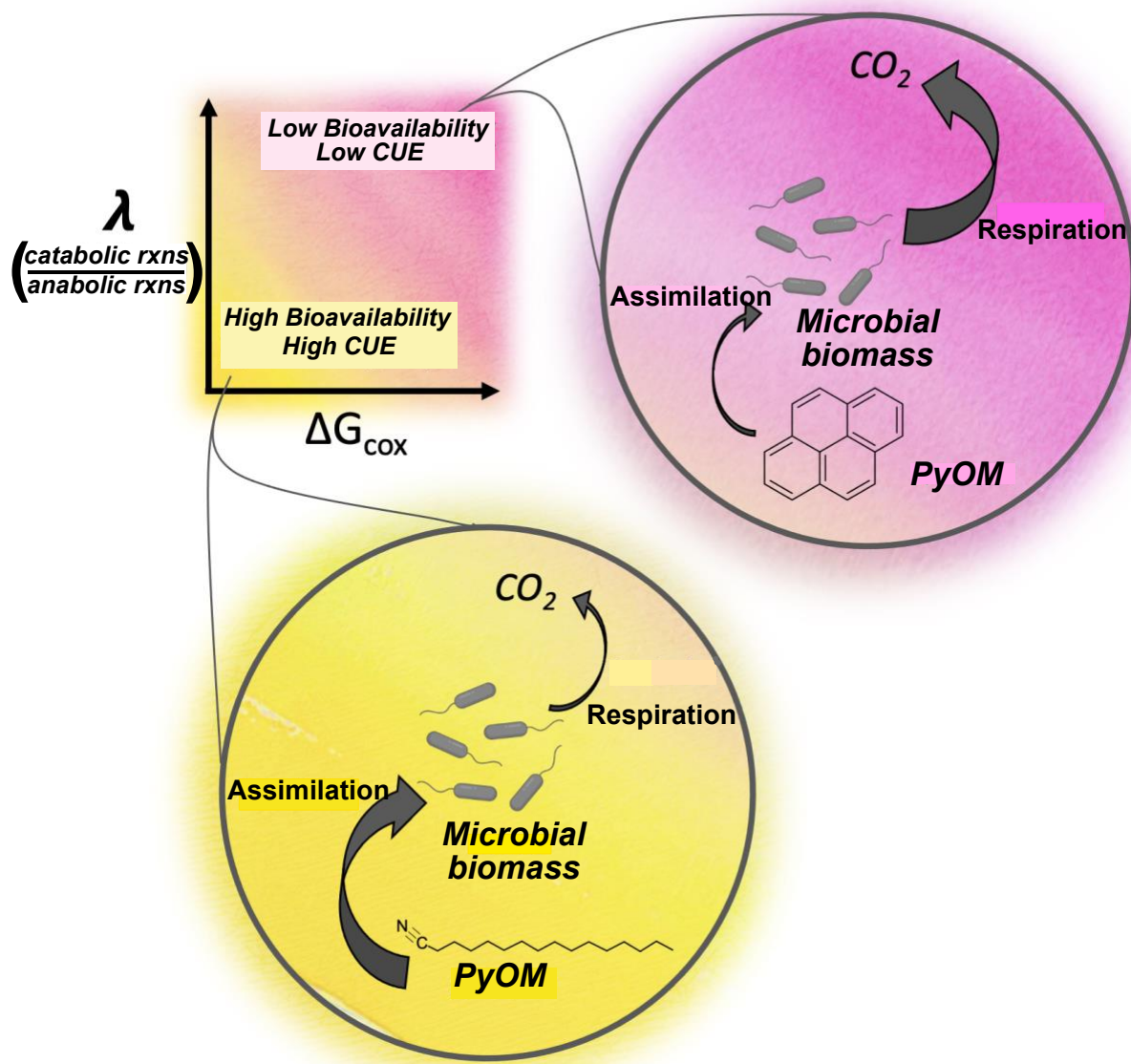
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23   **Abstract.** Pyrogenic organic matter (PyOM) from wildfires impacts river corridors globally and

24   is widely regarded as resistant to biological degradation. Though recent work suggests PyOM

25   may be more bioavailable than historically perceived, estimating bioavailability across its

26   chemical spectrum remains elusive. To address this knowledge gap, we assessed potential

27   bioavailability of representative PyOM compounds relative to ubiquitous dissolved organic

28   matter (DOM) with a substrate-explicit model. The range of potential bioavailability of PyOM

29   was greater than natural DOM; however, the predicted thermodynamics, metabolic rates, and

30   carbon use efficiencies overlapped significantly between all OM pools. Compound type (e.g.,

31   natural vs. PyOM) had approximately 6-fold less impact on predicted respiration rates than

32   simulated carbon and oxygen limitations. Within PyOM, the metabolism of specific chemistries

33   differed strongly between unlimited and oxygen limited conditions—anhydrosugars, phenols,

34   and polycyclic aromatic hydrocarbons degradations were more favorable under oxygen

35   limitation than other molecules. Notably, amino sugar-, protein-, and lignin-like PyOM had

36   lower carbon use efficiencies relative to natural DOM of the same classes, indicating potential

37   impacts in process-based model representations. Overall, our work illustrates how similar PyOM

38   bioavailability may be to that of natural DOM in the river corridor, furthering our understanding

39   of how PyOM may influence riverine biogeochemical cycling.

40   **1 Introduction**

41   Wildfires have burned an average of 1.8-million ha year^{-1} in the United States alone over

42   the past 80 years, dramatically impacting terrestrial and aquatic ecosystems (Bladon et al., 2014;

43   Shakesby and Doerr, 2006; Randerson et al., 2006; Verma and Jayakumar, 2012). As wildfire

44   activity continues to increase in response to climate change (Pierce et al., 2004; Bowman et al.,

45   2020; Flannigan et al., 2009), its impact on river corridor biogeochemistry is receiving

46   significant attention (Wagner et al., 2018; Abney et al., 2019).

47   Pyrogenic organic matter (PyOM) generated by wildfires influences river corridor

48   biogeochemistry due to the importance of organic matter as a carbon (C) and energy source.

49   Though there is substantial uncertainty in the quantification of PyOM, in part due to its chemical

50   diversity, estimates suggest that 116–385 Tg of condensed PyOM is generated per year. This

51   amounts to 300 to 500 Tg C stored in sediments, soils, and waters (Jaffé et al., 2013; Dittmar et

52   al., 2012; Hockaday et al., 2007; Santin et al., 2016) and ~10% of dissolved organic C pools in

53   surface waters (Jaffé et al., 2013). Given that organic matter drives aquatic biogeochemical

54   cycles, the loading of PyOM into river corridors has the potential to produce substantial impacts

55   on ecosystem functions and downstream drinking water treatability (Emelko et al., 2011; Hohner

56   et al., 2017).

57   While some estimates place aquatic residence times at thousands of years (Meyer and

58   Wells, 1997; Elliott and Parker, 2001; Bigio et al., 2010; Kuzyakov et al., 2014), recent work has

59   shown that PyOM may be more bioavailable (i.e., biolabile or able to be accessed and degraded

60   by microorganisms) than previously thought (Myers-Pigg et al., 2015; Norwood et al., 2013;

61   Zimmerman and Ouyang, 2019). This inference is also supported by research on biochars,

62   highlighting the diverse reactivities of combustion byproducts (Sohi et al., 2010; Mia et al.,

63   2017). PyOM bioavailability may, therefore, play an unrecognized role in global biogeochemical

64   cycles and climate feedbacks. Yet, we are just beginning to understand its potential

65   bioavailability (Zimmerman and Mitra, 2017; Wozniak et al., 2020; Masiello, 2004).

66   We used a substrate-explicit model to assess the potential bioavailability of PyOM in

67   comparison to dissolved organic matter (DOM) from global surface waters and sediments. We

68   derived 16,971 representative PyOM compounds from the primary literature describing plant

69   charcoals, biochar, smoke, burnt soil, and their leachates (Table S1). Natural DOM pool

70   composition was derived from a recent high-resolution survey of river corridor sediments and

71   surface waters (Garayburu-Caruso et al., 2020a, Figure S1). The substrate-explicit model used

72   molecular formulae to predict energetic content, metabolic efficiency, and rates of aerobic

73   metabolism for organic substrates. It has proven useful in linking DOM composition to aerobic

74   metabolism in natural environments (Song et al., 2020; Graham et al., 2017; Garayburu-Caruso

75   et al., 2020b). It provides a systematic way to formulate reaction kinetics and is agnostic of many

76   factors that have complicated a universal understanding of OM bioavailability; including

77   molecular structure, chemical inhibition, mineral-associations and physical protection, terminal

78   electron acceptors, microbial community composition and accessibility, and abiotic reactions

79   (reviewed in Arndt et al. (2013)). We hypothesize that PyOM compounds have a greater

80   similarity in their potential bioavailability to natural DOM pools than expected based on

81   historical literature.

82

83   **2 Results and discussion**

84   Our model-based approach enabled us to directly compare known combustion products to

85   thousands of ubiquitous DOM compounds, which would have been unfeasible to directly assess

86   in a laboratory setting. Key outputs from the model include lambda (λ, the number of catabolic

87   reactions that must occur to provide the energy required for the synthesis of one mole of biomass

88   carbon), Gibbs free energy of C oxidation (ΔG_{Cox}), under standard conditions with a modification

89   to pH 7, carbon use efficiency (CUE), and the rate of aerobic metabolism (as oxygen consumed

90   per mol-C biomass produced) under three scenarios: (a) no limitations, (b) C-limitation and (c)

91   oxygen (O_2) limitation. Though the relative magnitude of these metrics will vary based on the

92   specific stoichiometry of a molecule, highly bioavailable compounds are indicated by low λ and

93   ΔG_{Cox} coinciding with high CUE and metabolic rates. Low λ values denote efficient energetics

94   of catabolism in producing biomass through anabolism. Low ΔG_{Cox} denotes high thermodynamic

95   favorability in an electron donor half reaction associated with organic matter, and high CUE

96   reflects more C assimilated into biomass per unit C respired.

97

98   *2.1 Potential Bioavailability of Pyrogenic Organic Matter*

99   Though previous work has shown that sediment and surface water DOM is altered by

100   wildfires (Cawley et al., 2018; Jaffé et al., 2013; Wagner et al., 2018), our results suggest that the

101   chemically distinct pools of PyOM transported to aquatic systems may have similar potential

102   bioavailability to DOM. We found that the ranges of ΔG_{Cox}, λ, and CUE overlapped substantially

103   between PyOM and DOM in sediments and surface waters (Figure 1 and 2a-b, Figure S2).

104   Predicted CUE of PyOM classes was also comparable to literature values reported by others

105   (Saifuddin et al., 2019; Domeignoz-Horta et al., 2020; Pold et al., 2020). While λ, ΔG_{Cox} and

106   CUE were significantly different when comparing all three groups (ANOVA, $P < 0.001$, F =

107   16.12, 604.9, and 461.2 respectively), λ did not vary significantly between sediment DOM and

108   PyOM (Tukey HSD, $P = 0.21$) and was more similar between sediment DOM and PyOM than

109   between the surface water and sediment DOM pools (mean difference, surface water-sediment

110   DOM = 0.0025, sediment DOM-PyOM = 0.0019) . The range of all PyOM metrics was greater

111   than that of surface water or sediment DOM, reflecting the known heterogenous nature of PyOM

112   chemistries. Though the λ of many PyOM compounds was higher than the maximum observed

113   in either DOM pool, minimum values of λ were lowest for PyOM (surface water DOM = 0.026,

114   sediment DOM = 0.015, PyOM = 0.010), indicating that a portion of wildfire-derived molecules

115   may be more bioavailable than extant DOM pools. It is also interesting that surface water and

116   sediment DOM had greater dissimilarity in ΔG_{Cox} and CUE than any comparison involving

117   PyOM. For example, the mean differences in ΔG_{Cox} and CUE between surface water and

118   sediment DOM were 7.34 kJ/mol-C and 0.058. The differences between PyOM and surface

119   water were 4.28 kJ/mol-C for ΔG_{Cox} and 0.024 for CUE. Overall, statistical differences were not

120   surprising given an extremely large sample size (sediment $n = 398$, surface water $n = 811$, PyOM

121    $n = 16,971$), and the low effect sizes denote that differences in bioavailability between PyOM

122   and DOM were minimal despite statistical separation.

123   These results signal a strong overlap between the potential bioavailabilites of PyOM and

124   DOM pools; however, within PyOM compounds, there was variability in ΔG_{Cox}, λ, and CUE

125   consistent with heterogeneous chemistries (Figure 1 and 2). This is not surprising, given the

126   diversity of PyOM molecules generated by wildfires of different burn severities and source

127   materials (Wagner et al., 2015; Wagner et al., 2018; Neary et al., 2005), some of which are

128   chemically similar to natural DOM.

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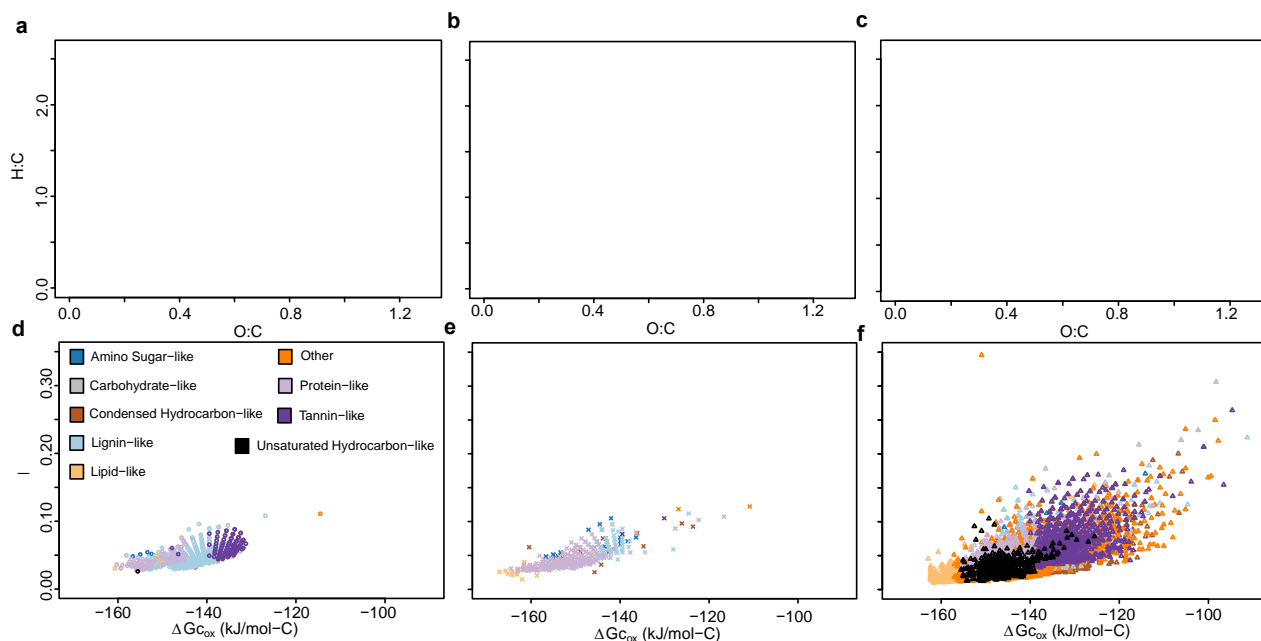
Figure 1. Comparison of natural DOM and PyOM. (**a-c**) Van Krevelen diagrams of molecules that were present in at least 95% of (**a**) surface water or (**b**) sediment samples versus (**c**) representative PyOM. (**d-f**) Distribution of energy content (ΔG_{Cox}) vs. metabolic efficiency (λ) in (**d**) surface water and (**e**) sediment DOM vs (**f**) representative PyOM. Higher values on the x-axis (ΔG_{Cox}) represent less energetically favorable molecules, while higher values on the y-axis (λ) represent higher rates of catabolism relative to anabolism. Colors for all panels indicate inferred chemical classes.
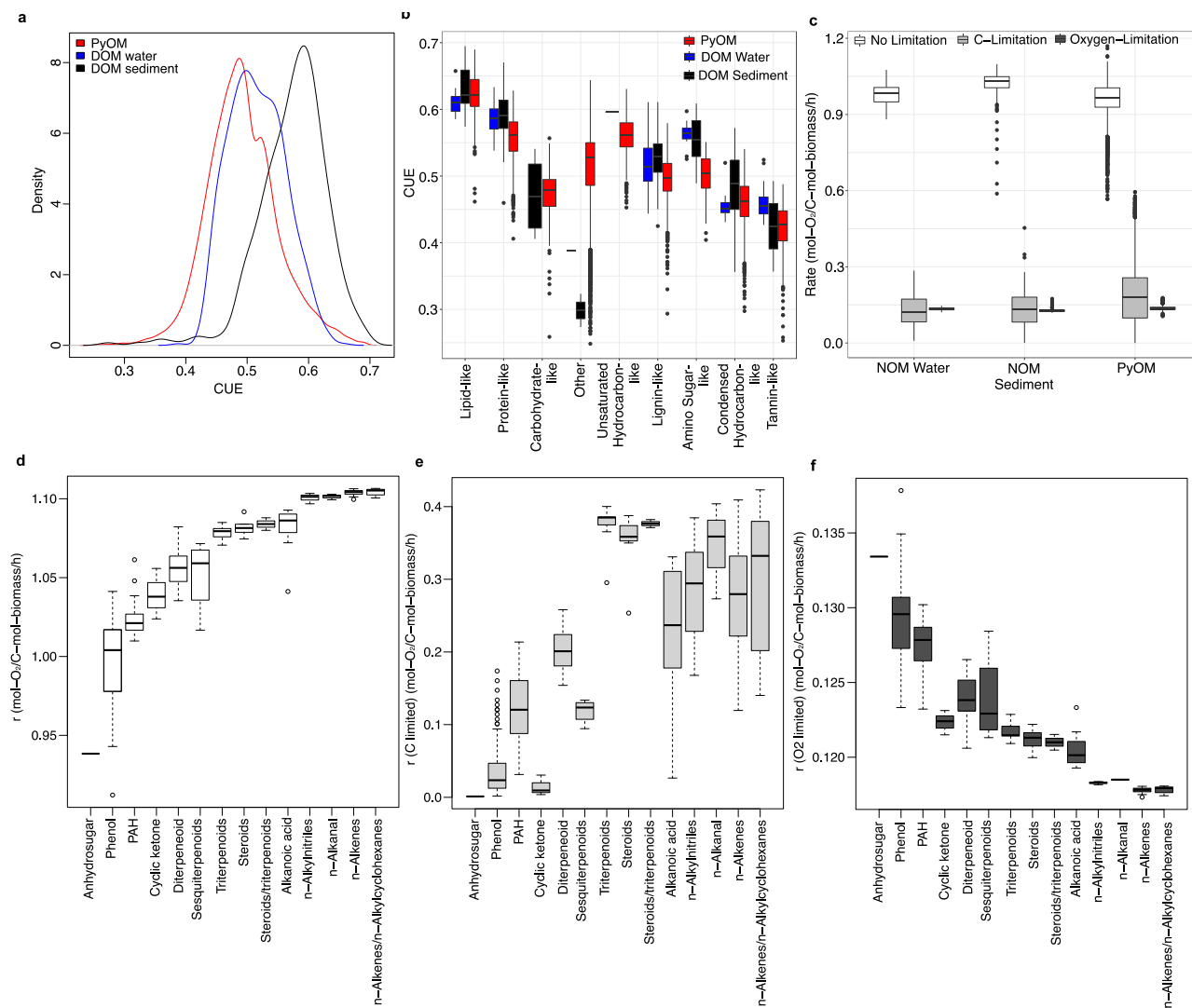
Figure 2. Predicted CUE and metabolism of DOM and PyOM. (**a**) shows the probability density function (PDF) of CUE in surface water DOM (blue), sediment (black) DOM, and PyOM (red). The PDF reflects the relative likelihood that value of a random sample drawn from a particular group would equal the value on the x-axis. (**b**) depicts CUE of surface water DOM, sediment DOM, and PyOM by inferred chemical class. (**c**) depicts the predicted metabolism of surface water DOM, sediment DOM, and PyOM under no elemental limitations (no fill), C-limitation (light grey fill), and oxygen-limitation (dark grey fill). (**d-f**) represent metabolic rates for PyOM molecules with known chemical classes under (**d**) no elemental limitations, (**e**) C-limitation, and (**f**) oxygen-limitation. For boxplots, median values are denoted by a bar, hinges correspond to the first and third quartiles, and whiskers extend to the largest/smallest value no further than 1.5 times the inter-quartile range from the hinge. Data beyond the end of the whiskers are plotted individually.

153   Interestingly, the large overlaps in predicted CUE across PyOM and DOM pools suggests

154   that PyOM decomposition in rivers could emit proportionally similar amounts of CO_2 to natural

155   DOM (Figure 2a-b). CUE is used in many microbially-explicit decomposition models to

156   constrain organic matter bioavailability (reviewed in Graham and Hofmockel, 2022). Therefore,

157   predicted CUE offers a path for assimilating PyOM in microbially-explicit models. Such an

158   approach could be used to directly evaluate the impact of PyOM on global C cycles, and help

159   lead to better incorporation of PyOM impacts in models (Santin et al., 2020). We specifically

160   highlight lower CUE of amino sugar-like, protein-like, and lignin-like PyOM in comparison to

161   corresponding classes of natural DOM, pointing to potential shifts needed in the representation

162   of these classes in process-based models in ecosystems impacted by wildfires.

163

164   *2.2 Inferred Metabolism of Pyrogenic Organic Matter.*

165   Predicted rates of PyOM metabolism were also similar to DOM pools (Figure 2c),

166   reinforcing comparable bioavailability. Elemental limitations had a ~6-fold larger impact on

167   predicted metabolic rates than changes in compound type (e.g., surface water DOM, sediment

168   DOM, and PyOM). Metabolic rates averaged across all compound types were 0.965, 0.179, and

169   0.136 mol O/mol C biomass produced for unlimited, C-limited, and oxygen-limited conditions

170   respectively. In contrast when metabolic rates were averaged across elemental limitations within

171   each compound group, we found that surface water DOM, sediment DOM, and PyOM had

172   nearly identical mean predicted rates of metabolism (ANOVA P = 0.14 F = 1.922, all pairwise

173   comparisons P > 0.10). While metabolic rates within a given limitation scenario were statistically

174   different across compound types (ANOVA, all P < 0.001, F = 197.4, 145.9, and 265.9), effect

175   sizes were low in comparison to changes across elemental limitations. Still, PyOM displayed

176   statistically elevated rates of metabolism under C- and oxygen-limitation versus both surface

177   water and sediment DOM (Tukey HSD, $P < 0.001$). Given that riverbed sediments can reach

178   anoxia within millimeters of the sediment-water interface, elevated rates of PyOM metabolism

179   under oxygen limitation may be ecologically relevant at ecosystem scales.

180   When considering the impact of elemental limitations on PyOM metabolism, predicted

181   rates were strongly inhibited under low C and oxygen conditions. Predicted PyOM metabolism

182   was over 5 times lower when C or oxygen was scarce. Low decomposition rates under C and

183   oxygen limitation could be one reason for the observed persistence of PyOM in depositional

184   features that tend to be anoxic. Still, it is worthwhile to note that metabolism of all PyOM classes

185   under low C or oxygen was predicted to be substantially slower than without elemental

186   limitations, indicating PyOM compounds may both actively cycle in well-oxygenated surface

187   waters with fresh C inputs and persist over long periods of time in O_2-limited sediments.

188   When examining PyOM with known chemistries (e.g., instead of inferred chemical

189   classes from FTICR-MS), anhydrosugars, phenols, and polycyclic aromatic hydrocarbons

190   (PAHs) were less negatively impacted by oxygen limitation than any other group (Figure 2 d-f).

191   Previous work has demonstrated that microorganisms are capable of decomposing chemically

192   complex organic molecules, such as long-chained and/or aromatic hydrocarbons under low

193   oxygen availability (Bushnell and Haas, 1941; Pozdnyakova, 2012; Rabus et al., 2016; Coates et

194   al., 1997). Similar microbial metabolic pathways may also be capable of degrading pyrogenically

195   modified phenols, and PAHs in natural settings and could be investigated with future laboratory

196   work.

197   Notably, our work also supports the notion that black nitrogen could be more bioavailable

198   than other PyOM pools. While we only examined one class of PyOM molecules containing

199 nitrogen in detail (n-alkylnitriles), it had among the highest predicted CUE and metabolic rates.

200 We posit this may be due, in part, to its chemical structure that includes pyrrole-type moieties,

201 which are relatively biodegradable (Knicker, 2010; De La Rosa and Knicker, 2011).

202

203 *2.3 Correspondence to Empirical Investigations.*

204   While the substrate-explicit modelling approach used here has been validated in natural

205 settings, we acknowledge that it does not account for all chemical and physical attributes

206 influencing PyOM bioavailability. This includes DOM structure and size, abiotic reactions, and

207 chemical complexation with minerals and particulates. Below we note aspects of the model

208 predictions that are inconsistent with experimental evidence and highlight the role of laboratory

209 studies in evaluating PyOM bioavailability.

210   For instance, n−alkenes and related compounds tended to have high modelled

211 bioavailability despite being relatively stable in the environment (Wiesenberg et al., 2004;

212 Smittenberg et al., 2004). These compounds are characterized by carbon-carbon double bonds,

213 which are not considered by the model and may decrease bioavailability. However, n-alkanes

214 generated through combustion tend to have reduced chain length in comparison to their unburned

215 counterparts (Knicker et al., 2013), and thus may be relatively bioavailable compared to

216 unburned n-alkanes. Additionally, we note that previous work has shown fast degradation of

217 combustion-derived lipids in soils (Knicker et al., 2013); as well as high n-alkene metabolism in

218 anaerobic sediments and high lipid reactivities at the sediment-water interface (Grossi et al.,

219 2008; Wilkes et al., 2016; Yongdong et al., 2015; Mbadinga et al., 2011; Canuel and Martens,

220 1996). While work on aerobic n-alkene metabolism is limited, the comparative bioavailability of

221 n-alkenes and known degradation pathways suggests that sediment microbiomes may metabolize

222 them as part of natural biogeochemical cycles.

223   Another notable discrepancy is the low potential bioavailability of anhydrosugars when

224 compared to other PyOM compounds. Experimentally, anhydrosugars are highly bioavailable in

225 oxic conditions, with a half-life of less than seven days (Norwood et al., 2013). The model may

226 therefore not adequately account for some enzyme-catalyzed reactions such as levoglucosan

227 kinase or levoglucosan dehydrogenase that may be common enzymes in aquatic microorganisms

228 (Bacik and Jarboe, 2016; Suciu et al., 2019).

229   Because of these nuances, the analysis presented here is best used as bounding estimates

230 for experimental validation and as a holistic comparison to DOM bioavailability. Still, the span

231 of compounds investigated here, and their comparison to DOM pools, provides a breadth of

232 investigation that is unfeasible without model-based approaches.

233

234 **3 Conclusions**

235   Our work supports the hypothesis that PyOM may have similar overall bioavailability as

236 compared to natural sources of DOM and provides a foundation for targeted experiments

237 investigating specific components of the PyOM continuum. Globally intensifying wildfires are

238 increasing the production of PyOM with potential implications for water supplies, which are

239 critical for domestic, industrial, agricultural, and ecological needs. Yet, many fundamental

240 questions such as "how much" PyOM exists in ecosystems, "how fast" it cycles, and "how old"

241 it is remain largely unknown (Abiven and Santin, 2019). Our work provides the first

242 comprehensive computational assessment of the potential bioavailabilities of various PyOM

243 chemistries in comparison to natural DOM pools. The comparable potential bioavailability to

244   DOM revealed that PyOM may be actively transformed in aquatic ecosystems and may be an

245   increasing source of C emissions to the atmosphere as the prevalence of wildfires increases.

246

247   **Code and Data Availability**

248   Code is available at: https://github.com/hyunseobsong/lambda. Data describing DOM pool

249   chemistry are published as a data package (Goldman et al., 2020) (available at:

250   doi:10.15485/1729719) and are discussed in more detail by Garayburu-Caruso et al. (2020a).

251

252   **Author Contributions**

253   EBG conceived of the manuscript and was responsible for writing the manuscript and generating

254   all figures. HSS performed all modelling. SG determined PyOM compounds for modelling based

255   on extensive literature review, with guidance from AMP. VGC and JCS contributed data and

256   insight on DOM pool chemistry. All authors contributed to revisions.

257

258   **Competing Interests**

259   The authors declare that they have no conflict of interest.

260

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268 the River Corridor SFA at PNNL and facilitated by the U.S. Department of Energy

269 Environmental Molecular Science Laboratory User Facility.

270

271 **APPENDIX. Materials and methods**

272   An extended version of our methods is available in the Supporting Information.

273   To assess the potential bioavailability of PyOM, we searched primary literature for

274 representative compounds of the PyOM continuum. Specifically, we targeted characteristic

275 organic compounds from controlled burns of various fuel types representing a range of moisture,

276 temperature, and oxygen conditions (Table S1). The chosen compounds focused on biomass

277 burning alteration products derived from plant charcoals, biochars, smoke, burnt soil and their

278 leachates, which are often used to characterize PyOM in the environment. This included

279 compounds such as theoretical black carbon-like compounds (defined here as condensed

280 aromatic core structures polysubstituted with O-containing functionalities (Wagner et al., 2017),

281 anhydrosugars, and polycyclic aromatic hydrocarbons (PAHs). The list also included compounds

282 created and/or transformed from biomass burning, such as those derived from biopolymers like

283 lignin (e.g., methoxyphenols), waxes (e.g., n-alkenes from thermal dehydration of n-alkanols),

284 and resins (e.g., thermally oxidized diterpenoids) (Oros and Simoneit, 2001b, a). We also include

285 molecular formula assigned via FTICR-MS spectra collected on biochar leachates to increase

286 data type comparability with common methods used in natural organic matter studies. The

287 selected set of compounds spans the chemical continuum of PyOM but is not intended to be

288 exhaustive. In total, our literature search for PyOM chemistries yielded 16,971 unique molecular

289   formulae. When a formula was found in more than one study or material type, this was noted in

290   Table S1, however only unique formulae were considered for the model.

291   After generating a set of representative compounds, we used a substrate-explicit

292   modelling framework developed by Song et al. (2020) to characterize the potential

293   bioavailability of each compound and predict its rate of decomposition. The model uses

294   molecular formulae to predict energetic content, metabolic efficiency, and rates of aerobic

295   metabolism, while it does not account for structural components of organic molecules (e.g.,

296   double bonds, folding patterns, cross-linkages). This enables flexibility in application to high-

297   throughput mass spectrometry techniques that yield chemical formulae but not structural

298   information (e.g., FTICR-MS) for comparison to environmental DOM. Despite its limitations,

299   the substrate-explicit model used here has proven useful in linking DOM composition to aerobic

300   metabolism in natural environments (Song et al., 2020; Graham et al., 2017; Garayburu-Caruso

301   et al., 2020b), and its structure is consistent with Harvey et al. (2016) who argued for the

302   importance of thermodynamic estimates of PyOM bioavailability that underlie this model. It was

303   chosen to allow for comparison of PyOM to the most comprehensive assessment of global

304   aquatic DOM pools to date (Garayburu-Caruso et al., 2020a).

305   Briefly, the substrate-explicit model uses the elemental stoichiometry of organic

306   molecules, based on molecular formulae, to predict the number of catabolic reactions that must

307   occur to provide the energy required for the synthesis of one mole of biomass carbon. This

308   quantity is described by the parameter lambda (λ) in which lower λ values denote more efficient

309   energetics of catabolism in producing biomass through anabolism. The model also predicts the

310   Gibbs free energy of C oxidation (ΔG_{Cox}), under standard conditions with a modification to pH 7

311   adjusted from LaRowe and Van Cappellen (2011) by Song et al. (2020), as well as C use

312   efficiency (CUE) as defined by Saifuddin et al. (2019). Lower ΔG_{Cox} denotes higher

313   thermodynamic favorability in an electron donor half reaction associated with organic matter,

314   and higher CUE reflects more C assimilated into biomass per unit C respired. We also predicted

315   the rate of aerobic metabolism (as oxygen consumed per mol-C biomass produced) under three

316   scenarios commonly observed in aquatic ecosystems: (a) C-limitation, (b) oxygen (O_2)

317   limitation, and (c) both C and O_2-limitation. For more details of the substrate-explicit modelling

318   approach used, please see Song et al. (2020). Each metric (λ, ΔG_{Cox}, CUE, metabolic rates)

319   denotes a different aspect of potential bioavailability. Though the relative magnitude of the

320   metrics in comparison to each other will vary based on the specific stoichiometry of a molecule,

321   highly bioavailable compounds are indicated by low λ and ΔG_{Cox} coinciding with high CUE and

322   metabolic rates.

323   Three sets of organic molecules were used as model inputs: measured global dissolved

324   (1) surface water and (2) sediment DOM pools, extracted in H_2O and analyzed by FTICR-MS as

325   per Garayburu-Caruso et al. (2020a); and (3) literature-derived PyOM compounds as described

326   above. Inputs to the model from the PyOM compounds were unique molecular formulae,

327   grouped in subsequent analysis by their corresponding compound classes (Table S1). If one

328   molecular formula was represented by several PyOM compounds (e.g., $C_{10}H_{16}O_2$, which

329   corresponds to the sesquiterpenoid cis-Thujan-10-oic acid and 3-, 4- substituted methylcatechol

330   phenols), we assigned multiple compound classes to that molecular formula. Surface water and

331   sediment DOM pools were filtered to compounds occurring in 95% of samples to yield a dataset

332   of globally ubiquitous DOM (surface water $n = 811$ molecules, sediment $n = 398$ molecules).

333   The purpose of this filtering was to focus on widespread natural organic matter chemistries by

334   removing compounds that were specific to subsets of rivers. Formulae assignment and inferred

335   chemical classes via van Krevelen diagrams in DOM pools are described by Garayburu-Caruso

336   et al. (2020a). As a whole, 16,332 compounds were found only in PyOM, 197 were found only in

337   surface water DOM, and 167 were found only in sediment DOM. We compared modelling

338   outputs from representative PyOM to outputs of ubiquitous DOM pools to infer relative

339   bioavailability using ANOVA and Tukey HSD statistical tests with R software v 4.1.0 (R Core

340   Team, 2021). Visualizations were produced using base R and 'ggplot2' (Wickham, 2011). All

341   model outputs are available in Tables S2–S4.

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