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

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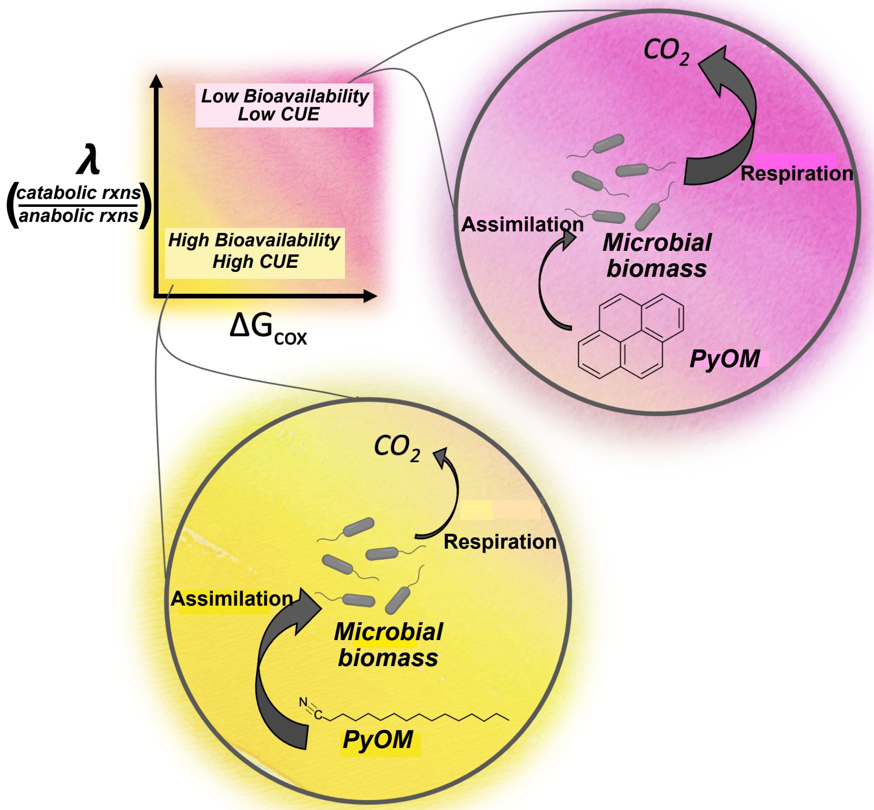
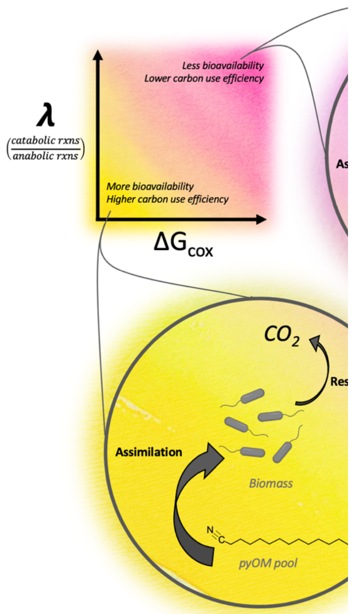
GRAPHICAL ABSTRACT





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ABSTRACT

Pyrogenic organic matter (PyOM) from wildfires impacts river corridors globally and is widely regarded as resistant to biological degradation. Though recent work suggests PyOM may be more bioavailable than historically perceived, estimating bioavailability across its chemical spectrum remains elusive. To address this knowledge gap, we assessed the potential bioavailability of representative PyOM compounds in relation to ubiquitous dissolved organic matter (DOM) with a substrate-explicit model. The range of potential bioavailability of PyOM was greater than natural DOM; however, the predicted thermodynamics, metabolic rates, and carbon use efficiencies overlapped significantly between all OM pools. Compound type (e.g., natural vs. PyOM) had approximately 6-fold less impact on predicted respiration rates than simulated carbon and oxygen limitations. Within PyOM, the metabolism of specific chemistries differed strongly between unlimited and oxygen limited conditions—anhydrosugars, phenols, and polycyclic aromatic hydrocarbons degradations were more favorable under oxygen limitation than other molecules. Notably, amino sugar-, protein-, and lignin-like PyOM had lower carbon use efficiencies relative to natural DOM of the same classes, indicating potential impacts in process-based model representations. Overall, our work illustrates how similar PyOM bioavailability may be to that of natural DOM in the river corridor, furthering our understanding of how PyOM may influence riverine biogeochemical cycling.

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Deleted: Overall, we found similar potential bioavailability between PyOM and sediment and surface water DOM. Predicted thermodynamics and carbon use efficiencies of PyOM and DOM were statistically indistinguishable. Within PyOM, phenols and black carbon (BC, defined by Wagner et al. (2017)) had lower metabolic efficiency than other PyOM and DOM compounds, and oxygen limitation had less impact on BC metabolism than on other PyOM classes.

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1 Introduction

Wildfires have burned an average of 1.8-million ha year^{-1} in the United States alone over the past 80 years, dramatically impacting terrestrial and aquatic ecosystems (Bladon et al., 2014; Shakesby and Doerr, 2006; Randerson et al., 2006; Verma and Jayakumar, 2012). As wildfire activity continues to increase in response to climate change (Pierce et al., 2004; Bowman et al., 2020; Flannigan et al., 2009), its impact on river corridor biogeochemistry is receiving significant attention (Wagner et al., 2018; Abney et al., 2019).

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Pyrogenic organic matter (PyOM) generated by wildfires influences river corridor biogeochemistry due to the importance of organic matter as a carbon (C) and energy source. Though there is substantial uncertainty in the quantification of PyOM, in part due to its chemical diversity, estimates suggest that 116–385 Tg C of condensed PyOM is generated per year. This amounts to 300 to 500 Tg C stored in sediments, soils, and waters (Jaffé et al., 2013; Dittmar et al., 2012; Hockaday et al., 2007; Santín et al., 2016) and ~10% of dissolved organic C pools in surface waters (Jaffé et al., 2013). Given that organic matter drives aquatic biogeochemical cycles, the loading of PyOM into river corridors has the potential to produce substantial impacts on ecosystem functions and downstream drinking water treatability (Emelko et al., 2011; Hohner et al., 2017).

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While some estimates place aquatic residence times at thousands of years (Meyer and Wells, 1997; Elliott and Parker, 2001; Bigio et al., 2010; Kuzyakov et al., 2014), recent work has shown that PyOM may be more bioavailable (i.e., biolabile or able to be accessed and degraded by microorganisms) than previously thought (Myers-Pigg et al., 2015; Norwood et al., 2013; Zimmerman and Ouyang, 2019). This inference is also supported by research on biochars, highlighting the diverse reactivities of combustion by-products (Sohi et al., 2010; Mia et al.,

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2017). PyOM bioavailability may, therefore, play an unrecognized role in global biogeochemical cycles and climate feedbacks. Yet, we are just beginning to understand its potential bioavailability (Zimmerman and Mitra, 2017).

D We used a substrate-explicit model to assess the potential bioavailability of PyOM in comparison to dissolved organic matter (DOM) from global surface waters and sediments. We derived 16,971 representative PyOM compounds from the primary literature describing plant charcoals, biochar, smoke, burnt soil, and their leachates (Table S1). Natural DOM pool composition was derived from a recent high-resolution survey of river corridor sediments and surface waters (Garayburu-Caruso et al., 2020a, Figure S1). The substrate-explicit model used molecular formulae to predict energetic content, metabolic efficiency, and rates of aerobic metabolism for organic substrates. It has proven useful in linking DOM composition to aerobic metabolism in natural environments (Song et al., 2020; Graham et al., 2017; Garayburu-Caruso et al., 2020b). It provides a systematic way to formulate reaction kinetics and is agnostic of many factors that have complicated a universal understanding of OM bioavailability; including molecular structure, chemical inhibition, mineral-associations and physical protection, terminal electron acceptors, microbial community composition and accessibility, and abiotic reactions (reviewed in Arndt et al. (2013)). We hypothesize that PyOM compounds have a greater similarity in their potential bioavailability to natural DOM pools than expected based on historical literature.

2 Results and discussion

Our model-based approach enabled us to directly compare known combustion products to thousands of ubiquitous DOM compounds, which would have been unfeasible to directly assess

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in a laboratory setting. Key outputs from the model include lambda (λ, the number of catabolic reactions that must occur to provide the energy required for the synthesis of one mole of biomass carbon), Gibbs free energy of C oxidation (ΔG_{Cox}), under standard conditions with a modification to pH 7, carbon use efficiency (CUE), and the rate of aerobic metabolism (as oxygen consumed per mol-C biomass produced) under three scenarios: (a) no limitations, (b) C-limitation and (c) oxygen (O_2) limitation. Though the relative magnitude of these metrics will vary based on the specific stoichiometry of a molecule, highly bioavailable compounds are indicated by low λ and ΔG_{Cox} coinciding with high CUE and metabolic rates. Low λ values denote efficient energetics of catabolism in producing biomass through anabolism. Low ΔG_{Cox} denotes high thermodynamic favorability in an electron donor half reaction associated with organic matter, and high CUE reflects more C assimilated into biomass per unit C respired.

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2.1 Potential Bioavailability of Pyrogenic Organic Matter

Though previous work has shown that sediment and surface water DOM is altered by wildfires (Cawley et al., 2018; Jaffé et al., 2013; Wagner et al., 2018), our results suggest that the chemically distinct pools of PyOM transported to aquatic systems may have similar potential bioavailability to DOM. We found that the ranges of ΔG_{Cox}, λ, and CUE overlapped substantially between PyOM and DOM in sediments and surface waters (Figure 1 and 2a-b, Figure S2). Predicted CUE of PyOM classes was also comparable to literature values reported by others (Saifuddin et al., 2019; Domeignoz-Horta et al., 2020; Pold et al., 2020). While λ, ΔG_{Cox} and CUE were significantly different when comparing all three groups (ANOVA, $P < 0.001$, $F = 16.12$, 604.9, and 461.2 respectively), λ did not vary significantly between sediment DOM and PyOM (Tukey HSD, $P = 0.21$) and was more similar between sediment DOM and PyOM than

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between the surface water and sediment DOM pools (mean difference, surface water-sediment DOM = 0.0025, sediment DOM-PyOM = 0.0019) . The range of all PyOM metrics was greater than that of surface water or sediment DOM, reflecting the known heterogenous nature of PyOM chemistries. Though the λ of many PyOM compounds was higher than the maximum observed in either DOM pool, minimum values of λ were lowest for PyOM (surface water DOM = 0.026, sediment DOM = 0.015, PyOM = 0.010), indicating that a portion of wildfire-derived molecules may be more bioavailable than extant DOM pools. It is also interesting that surface water and sediment DOM had greater dissimilarity in ΔG_{Cox} and CUE than any comparison involving PyOM. For example, the mean differences in ΔG_{Cox} and CUE between surface water and sediment DOM were 7.34 kJ/mol-C and 0.058. The differences between PyOM and surface water were 4.28 kJ/mol-C for ΔG_{Cox} and 0.024 for CUE. Overall, statistical differences were not surprising given an extremely large sample size (sediment $n = 398$, surface water $n = 811$, PyOM $n = 16{,}971$), and the low effect sizes denote that differences in bioavailability between PyOM and DOM were minimal despite statistical separation.

These results signal a strong overlap between the potential bioavailabilites of PyOM and DOM pools; however, within PyOM compounds, there was variability in ΔG_{Cox}, λ, and CUE consistent with heterogeneous chemistries (Figure 1 and 2). This is not surprising, given the diversity of PyOM chemistries generated by wildfires of different burn severities and source materials (Wagner et al., 2015; Wagner et al., 2018; Neary et al., 2005), some of which are chemically similar to natural DOM.

Deleted: Similarly, λ did not vary across groups of organic molecules (ANOVA $p = 0.09$, and Tukey HSD p (PyOM-sediment) = 0.92, p (PyOM-water) = 0.40, p (water-sediment) = 0.10). While ΔG_{Cox} and CUE were significantly different when comparing all three groups (ANOVA, $p < 0.001$), surface water and sediment DOM had greater dissimilarity in these parameters than any comparison involving PyOM. For example, the mean difference in ΔG_{Cox} and CUE between surface water and sediment DOM was 7.34 kJ/mol-C and 0.058. The differences between PyOM and both surface water and sediment were less than 7.4 kJ/mol-C for ΔG_{Cox} and 0.058 for CUE. Further, there was no evidence that CUE was different between PyOM and sediment DOM (Tukey HSD, $p = 0.20$).

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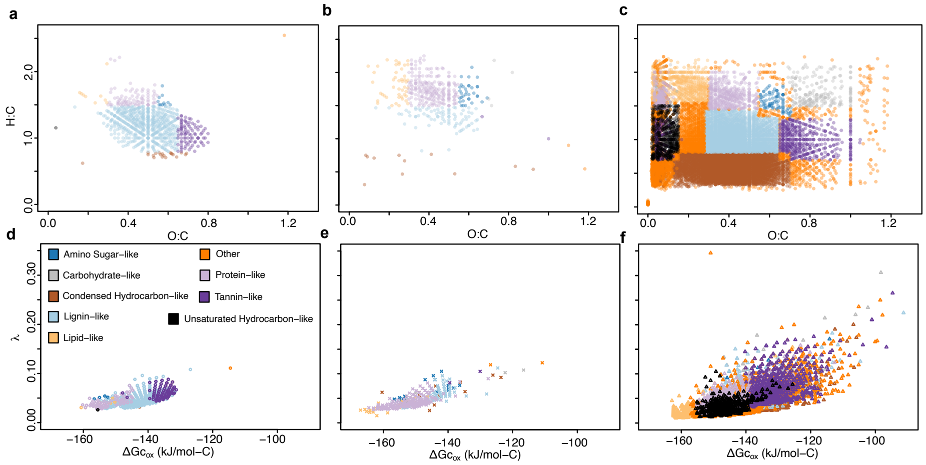

Figure 1. Comparison of natural DOM and PyOM. (**a-c**) Van Krevelen diagrams of molecules that were present in at least 95% of (**a**) surface water or (**b**) sediment samples versus (**c**) representative PyOM. (**d-f**) Distribution of energy content (ΔG_{Cox}) vs. metabolic efficiency (λ) in (**d**) 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.

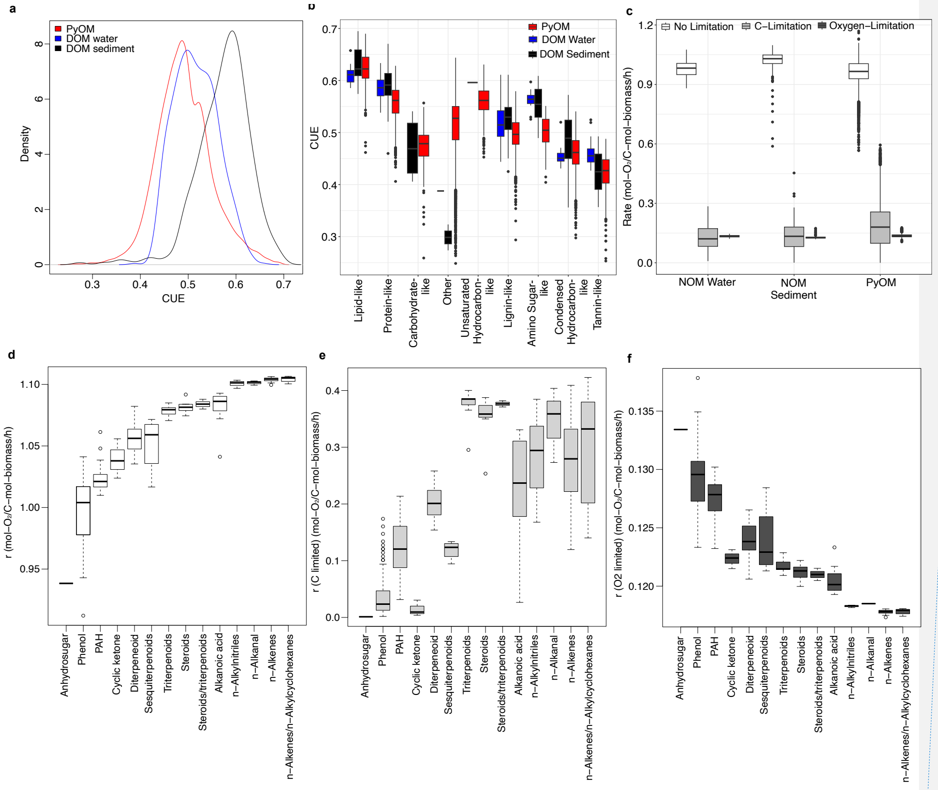

Figure 2. Predicted CUE and metabolism of DOM and PyOM. (**a**) shows the probability density function (PDF) of CUE in surface water DOM (blue), sediment (black) DOM, and PyOM (red). The PDF reflects the relative likelihood that value of a random sample drawn from a particular group would equal the value 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.

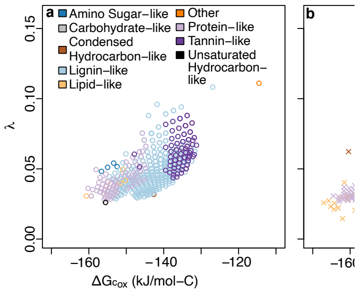

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Figure 1. Comparison of PyOM energy content (ΔG_{Cox}) and metabolic efficiency (λ) to global DOM. Ubiquitous DOM molecules detected via FTICR-MS in global (**a**) surface water and (**b**) sediment are colored by inferred chemical class. (**c**) Representative PyOM molecules are colored by known chemical properties. Because PyOM molecules were from primary literature, we could assign chemical properties at higher resolution than inferred classes from measured DOM pools. Details on inferred chemical class assignment are provided in the Supporting Information. Legends are inset in (**a**) for (**a**) and (**b**), and to the right of (**c**).

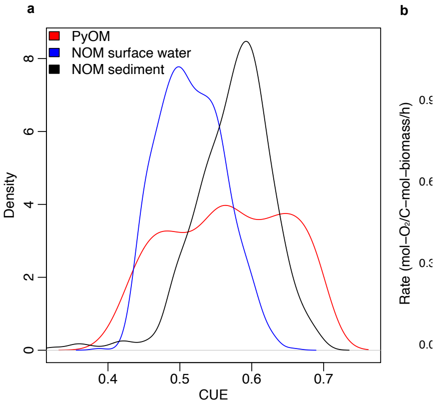

Figure 2. Predicted CUE and metabolism of DOM and PyOM. (**a**) shows the probability density function (PDF) of CUE in PyOM (red) and ubiquitous surface water (blue) and sediment (black). The PDF reflects the relative likelihood that value of a random sample drawn from a particular group would equal the value on the x-axis. (**b**) depicts the predicted metabolism of surface water DOM, sediment DOM, and PyOM. Letters in denote statistical groups. Median values are denoted by a bar, hinges correspond to the first and third quartiles (25th and 75th percentiles), and whiskers extend from the hinge to the largest/smallest value no further than 1.5 * IQR from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles), and data beyond the end of the whiskers are plotted individually.

Interestingly, the large overlaps in predicted CUE across PyOM and DOM pools suggests that PyOM decomposition in rivers could emit proportionally similar amounts of CO_2 to natural DOM (Figure 2a-b). CUE is used in many microbially-explicit decomposition models to constrain organic matter bioavailability (reviewed in Graham and Hofmockel, 2022). Therefore, predicted CUE offers a path for assimilating PyOM in microbially-explicit models. Such an approach could be used to directly evaluate the impact of PyOM on global C cycles, and help lead to better incorporation of PyOM impacts in models (Santin et al., 2020). We specifically highlight lower CUE of amino sugar-like, protein-like, and lignin-like PyOM in comparison to corresponding classes of natural DOM, pointing to potential shifts needed in the representation of these classes in process-based models in ecosystems impacted by wildfires.

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2.2 Inferred Metabolism of Pyrogenic Organic Matter.

Predicted rates of PyOM metabolism were also similar to DOM pools (Figure 2c), reinforcing comparable bioavailability. Elemental limitations had a ~6-fold larger impact on predicted metabolic rates than changes in compound type (e.g., surface water DOM, sediment DOM, and PyOM). Metabolic rates averaged across all compound types were 0.965, 0.179, and 0.136 mol O/mol C biomass produced for unlimited, C-limited, and oxygen-limited conditions respectively. In contrast when metabolic rates were averaged across elemental limitations within each compound group, we found that surface water DOM, sediment DOM, and PyOM had nearly identical mean predicted rates of metabolism (ANOVA $P = 0.14$ $F = 1.922$, all pairwise comparisons $P > 0.10$). While metabolic rates within a given limitation scenario were statistically different across compound types (ANOVA, all $P < 0.001$, $F = 197.4$, 145.9, and 265.9), effect sizes were low in comparison to changes across elemental limitations. Still, PyOM displayed

Deleted: Within PyOM, two clusters of compounds were distinctly separated from the energetic and metabolic properties of most PyOM (Figure 1c). Phenols had higher λ than the majority of PyOM and DOM compounds (all $\lambda > 0.039$, mean $\lambda = 0.084$), while BC molecules were less energetically favorable than other PyOM classes (all $\Delta G_{Cox} > -143.42$, mean $\Delta G_{Cox} = -136.40$). However, both were within the range of variability in DOM pools (Figure 1). Phenols and BC also had among the lowest CUE (means, BC = 0.47, phenols = 0.54). Phenols are traditionally associated with refractory organic matter, such as lignin and tannins, that exhibit long residence times in soils (Thevenot et al., 2010), although they have also been reported to be bioavailable in soils and waters in recent years (e.g., Thevenot et al., 2010; Ward et al., 2013). Additionally, BC in this study is defined by inferred aromaticity (i.e., the presence of condensed aromatic structures), which is also considered to have low reactivity (Kuzyakov et al., 2014; Wagner et al., 2017). Although the potential bioavailability of phenols and BC is consistent with refractory PyOM, it is within the potential bioavailability range observed in DOM, and these compounds represent only a small portion of the PyOM continuum (Wagner et al., 2018; Masiello, 2004). We note that the comparatively low predicted CUE of phenols and BC indicates that, if metabolized, their decomposition could have a greater impact on river corridor CO_2 emissions than other PyOM and DOM compounds. As a result, current understanding may substantially underestimate the size, reactivity, and hydrobiogeochemical role of PyOM (Wagner et al., 2018).¶

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statistically elevated rates of metabolism under C- and oxygen-limitation versus both surface water and sediment DOM (Tukey HSD, $P < 0.001$). Given that aquatic sediments can reach anoxia within millimeters of the sediment-water interface, elevated rates of PyOM metabolism under oxygen limitation may be ecologically relevant at ecosystem scales.

When considering the impact of elemental limitations on PyOM metabolism, predicted rates were strongly inhibited under low C and oxygen conditions. Predicted PyOM metabolism was over 5 times lower when C or oxygen was scarce. Low decomposition rates under C and oxygen limitation could be one reason for the observed persistence of PyOM in depositional features that tend to be anoxic. Still, it is worthwhile to note that metabolism of all PyOM classes under low C or oxygen was predicted to be substantially slower than without elemental limitations, indicating PyOM compounds may both actively cycle in well-oxygenated surface waters with fresh C inputs and persist over long periods of time in O_2-limited sediments.

When examining PyOM with known chemistries (e.g., instead of inferred chemical classes from FTICR-MS), anhydrosugars, phenols, and polycyclic aromatic hydrocarbons (PAHs) were less negatively impacted by oxygen limitation than any other group (Figure 2 d-f). Previous work has demonstrated that microorganisms are capable of decomposing chemically complex organic molecules, such as long-chained and/or aromatic hydrocarbons under low oxygen availability (Bushnell and Haas, 1941; Pozdnyakova, 2012; Rabus et al., 2016; Coates et al., 1997). Similar microbial metabolic pathways may also be capable of degrading pyrogenically modified phenols, and PAHs in natural settings and could be investigated with future laboratory work.

Notably, our work also supports the notion that black nitrogen could be more bioavailable than other PyOM pools. While we only examined one class of PyOM molecules containing

Deleted: Pairwise comparison of metabolic rates revealed no differences between PyOM and sediment DOM under oxygen limitation (Tukey HSD, $p = 0.23$) or without C or oxygen limitations (Tukey HSD, $p = 0.34$). However, the metabolic rates of both PyOM and sediment DOM were different than surface water DOM (Tukey HSD, all $p < 0.001$).

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Deleted: such that model predictions under oxygen limitation may translate to no meaningful difference between PyOM and DOM in natural sediments. Under C-limitation, PyOM had statistically elevated metabolism relative to both surface water and sediment DOM (Tukey HSD, all $p < 0.001$). However, we noted only small differences in rate values (means, surface water: 0.13, sediment: 0.13, PyOM: 0.17), with a similar range in sediment DOM (0.0008–0.45) and PyOM (4.75e-08–0.45). Statistical differences were not surprising given an extremely large sample size for DOM (sediment $n = 398$, surface water $n = 811$), and the low effect sizes denote that overall differences in metabolism between PyOM and DOM were minimal despite statistical separation.

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nitrogen in detail (n-alkylnitriles), it had among the highest predicted CUE and metabolic rates. We posit this may be due, in part, to its chemical structure that includes pyrrole-type moieties, which are relatively biodegradable (Knicker, 2010; De La Rosa and Knicker, 2011).

2.3 Correspondence to Empirical Investigations.

While the substrate-explicit modelling approach used here has been validated in natural settings, its underlying assumptions preclude accounting for DOM structure and size, abiotic reactions, and chemical complexation with minerals and particulates. Some aspects of model predictions are inconsistent with experimental evidence, highlighting the role of laboratory studies in evaluating PyOM bioavailability.

For instance, n−alkenes and related compounds tended to have high modelled bioavailability despite being relatively stable in the environment (Wiesenberg et al., 2004; Smittenberg et al., 2004). These compounds are characterized by carbon-carbon double bonds, which are not considered by the model and may decrease bioavailability. However, n-alkanes generated through combustion tend to have reduced chain length in comparison to their unburned counterparts (Knicker et al., 2013), and thus may be relatively bioavailable compared to un-burned n-alkanes. Additionally, we note that previous work has shown fast degradation of combustion-derived lipids in soils (Knicker et al., 2013); as well as high n-alkene metabolism in anaerobic sediments and high lipid reactivities at the sediment-water interface (Grossi et al., 2008; Wilkes et al., 2016; Yongdong et al., 2015; Mbadinga et al., 2011; Canuel and Martens, 1996). While work on aerobic n-alkene metabolism is limited, the comparative bioavailability of n-alkenes and known degradation pathways suggests that sediment microbiomes may metabolize them as part of natural biogeochemical cycles.

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Another notable discrepancy is the low potential bioavailability of anhydrosugars when compared to other PyOM compounds. Experimentally, anhydrosugars are highly bioavailable in oxic conditions, with a half-life of less than seven days (Norwood et al., 2013). The model may therefore not adequately account for some enzyme-catalyzed reactions such as levoglucosan kinase or levoglucosan dehydrogenase that may be common enzymes in aquatic microorganisms (Bacik and Jarboe, 2016; Suciu et al., 2019).

Because of these nuances, the analysis presented here is best used as bounding estimates for experimental validation and as a holistic comparison to DOM bioavailability. Still, the span of compounds investigated here, and their comparison to DOM pools, provides a breadth of investigation that is unfeasible without model-based approaches.

3 Conclusions

Our work supports the hypothesis that PyOM may have similar overall bioavailability as compared to natural sources of DOM and provides a foundation for targeted experiments investigating specific components of the PyOM continuum. Globally intensifying wildfires are increasing the production of PyOM with potential implications for source water supplies, which are critical for domestic, industrial, agricultural, and ecological needs. Yet, many fundamental questions such as “how much” PyOM exists in ecosystems, “how fast” it cycles, and “how old” it is remain largely unknown (Abiven and Santin, 2019). Our work provides the first comprehensive computational assessment of the potential bioavailabilities of various PyOM chemistries in comparison to natural DOM pools. The comparable potential bioavailability to DOM revealed that PyOM may be actively transformed within the river corridor and may be an increasing source of C emissions to the atmosphere as the prevalence of wildfires increases.

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Code and Data Availability

Code is available at: https://github.com/hyunseobsong/lambda. Data describing DOM pool chemistry are published as a data package (Goldman et al., 2020) (available at: doi:10.15485/1729719) and are discussed in more detail by Garayburu-Caruso et al. (2020a).

Author Contributions

EBG conceived of the manuscript and was responsible for writing the manuscript and generating all figures. HSS performed all modelling. SG determined PyOM compounds for modelling based on extensive literature review, with guidance from AMP. VGC and JCS contributed data and insight on DOM pool chemistry. All authors contributed to revisions.

Competing Interests

The authors declare that they have no conflict of interest.

Acknowledgements

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APPENDIX. Materials and methods

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

To assess the potential bioavailability of PyOM, we searched primary literature for representative compounds of the PyOM continuum. Specifically, we targeted characteristic organic compounds from controlled burns of various fuel types representing a range of moisture, temperature, and oxygen conditions (Table S1). The chosen compounds focused on biomass burning alteration products derived from plant charcoals, biochars, smoke, burnt soil and their leachates, which are often used to characterize PyOM in the environment. This included compounds such as theoretical black carbon-like compounds (defined here as condensed aromatic core structures polysubstituted with O-containing functionalities (Wagner et al., 2017), anhydrosugars, and polycyclic aromatic hydrocarbons (PAHs). The list also included compounds created and/or transformed from biomass burning, such as those derived from biopolymers like lignin (e.g., methoxyphenols), waxes (e.g., n-alkenes from thermal dehydration of n-alkanols), and resins (e.g., thermally oxidized diterpenoids) (Oros and Simoneit, 2001b, a). We also include molecular formula assigned via FTICR-MS spectra collected on biochar leachates to increase data type comparability with common methods used in natural organic matter studies. The selected set of compounds spans the chemical continuum of PyOM but is not intended to be exhaustive. In total, our literature search for PyOM chemistries yielded 16,971 unique molecular formulae. When a formula was found in more than one study or material type, this was noted in Table S1, however only unique formulae were considered for the model.

After generating a set of representative compounds, we used a substrate-explicit modelling framework developed by Song et al. (2020) to characterize the potential bioavailability of each compound and predict its rate of decomposition. The model uses molecular formulae to predict energetic content, metabolic efficiency, and rates of aerobic metabolism, while it does not account for structural components of organic molecules (e.g., double bonds, folding patterns, cross-linkages). This enables flexibility in application to high-throughput mass spectrometry techniques that yield chemical formulae but not structural information (e.g., FTICR-MS) for comparison to environmental DOM. Despite its limitations, the substrate-explicit model used here has proven useful in linking DOM composition to aerobic metabolism in natural environments (Song et al., 2020; Graham et al., 2017; Garayburu-Caruso et al., 2020c), and its structure is consistent with Harvey et al. (2016) who argued for the importance of thermodynamic estimates of PyOM bioavailability that underlie this model. It was chosen to allow for comparison of PyOM to the most comprehensive assessment of global aquatic DOM pools to date (Garayburu-Caruso et al., 2020a).

Briefly, the substrate-explicit model uses the elemental stoichiometry of organic molecules, based on molecular formulae, to predict the number of catabolic reactions that must occur to provide the energy required for the synthesis of one mole of biomass carbon. This quantity is described by the parameter lambda (λ) in which lower λ values denote more efficient energetics of catabolism in producing biomass through anabolism. The model also predicts the Gibbs free energy of C oxidation (ΔG_{Cox}), under standard conditions with a modification to pH 7 adjusted from LaRowe and Van Cappellen (2011) by Song et al. (2020), as well as C use efficiency (CUE) as defined by Saifuddin et al. (2019). Lower ΔG_{Cox} denotes higher thermodynamic favorability in an electron donor half reaction associated with organic matter,

Deleted: , which are often used to characterize PyOM in the environment. This included compounds such as theoretical BC compounds, anhydrosugars, and polycyclic aromatic hydrocarbons (PAHs). The list also included compounds created and/or transformed from biomass burning, such as those derived from biopolymers like lignin (e.g., methoxyphenols), waxes (e.g., n-alkenes from thermal dehydration of n-alkanols), and resins (e.g., thermally oxidized diterpenoids) (Oros and Simoneit, 2001b, a). While we recognize that recent research has applied new technologies to inferring PyOM compound presence in environmental samples (e.g., FTICR-MS), there remains high uncertainty in the confidence of formula assignment and structural information with some of these techniques. Therefore, we focused only on known, chemically identified compounds from controlled burns to represent PyOM chemistries. The selected set of compounds spans the chemical continuum of PyOM but was not intended to be exhaustive. In total, our literature search for PyOM chemistries yielded 389 compounds with 207 unique chemical formulae. ¶

and higher CUE reflects more C assimilated into biomass per unit C respired. We also predicted the rate of aerobic metabolism (as oxygen consumed per mol-C biomass produced) under three scenarios commonly observed in aquatic ecosystems: (a) C-limitation, (b) oxygen (O_2) limitation, and (c) both C and O_2-limitation. For more details of the substrate-explicit modelling approach used, please see Song et al. (2020). Each metric (λ, ΔG_{Cox}, CUE, metabolic rates) denotes a different aspect of potential bioavailability. Though the relative magnitude of the metrics in comparison to each other will vary based on the specific stoichiometry of a molecule, highly bioavailable compounds are indicated by low λ and ΔG_{Cox} coinciding with high CUE and metabolic rates.

Three sets of organic molecules were used as model inputs: measured global dissolved (1) surface water and (2) sediment DOM pools, extracted in H_2O and analyzed by FTICR-MS as per Garayburu-Caruso et al. (2020a); and (3) literature-derived PyOM compounds as described above. Inputs to the model from the PyOM compounds were unique molecular formulae, grouped in subsequent analysis by their corresponding compound classes (Table S1). If one molecular formula was represented by several PyOM compounds (e.g., $C_{10}H_{16}O_2$, which corresponds to the sesquiterpenoid cis-Thujan-10-oic acid and 3-, 4- substituted methylcatechol phenols), we assigned multiple compound classes to that molecular formula. Surface water and sediment DOM pools were filtered to compounds occurring in 95% of samples to yield a dataset of globally ubiquitous DOM (surface water $n = 811$ molecules, sediment $n = 398$ molecules).. Formulae assignment and inferred chemical classes via van Krevelen diagrams in DOM pools are described by Garayburu-Caruso et al. (2020a). As a whole, 16,332 compounds were found only in PyOM, 197 were found only in surface water DOM, and 167 were found only in sediment DOM. We compared modelling outputs from representative PyOM to outputs of ubiquitous DOM pools to infer relative bioavailability using ANOVA and Tukey HSD statistical tests with R software v 4.1.0 (R Core Team, 2021). Visualizations were produced using base R and ‘ggplot2’ (Wickham, 2011). All model outputs are available in Tables S2–S4.

Deleted: R software.

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