Inferred bioavailability of pyrogenic organic matter compared to natural organic matter from global sediments and surface waters

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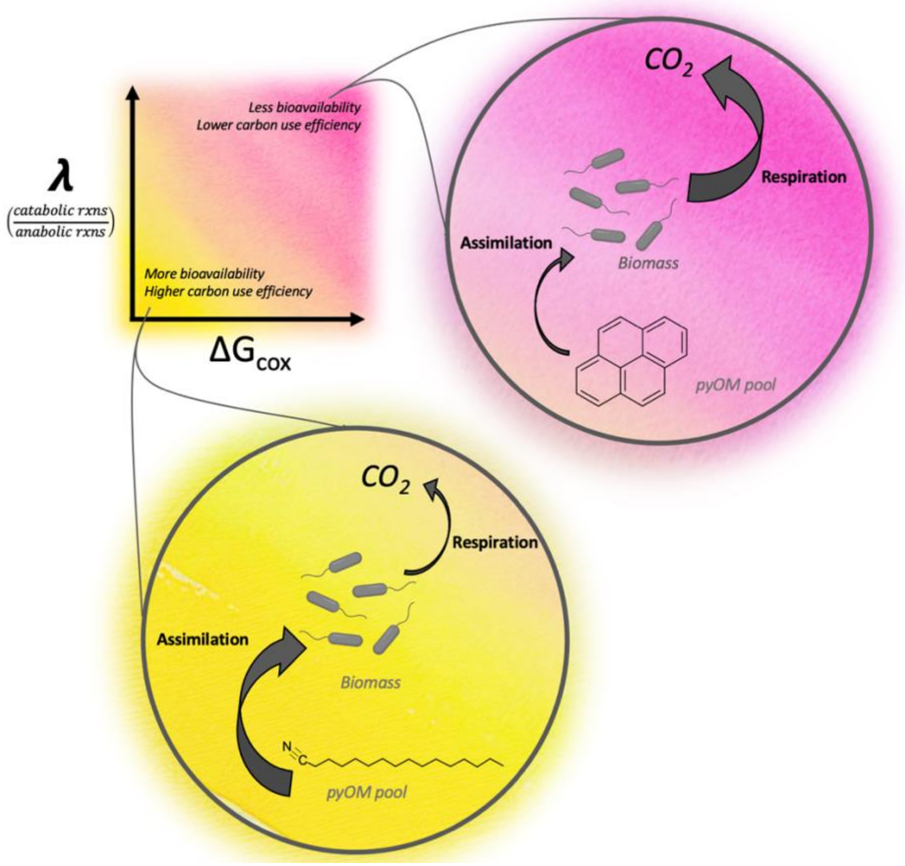
GRAPHICAL ABSTRACT



ABSTRACT

Wildfires are increasing in severity and extent, creating many negative consequences for aquatic ecosystems. Pyrogenic materials generated by wildfires are transported across terrestrial landscapes into inland waters, where ~10% of organic matter pools may be comprised of black carbon (BC), a major component of pyrogenic organic matter (PyOM). Yet, the heterogeneity of PyOM from various fuels and burn conditions complicates efforts to understand its bioavailability. We used a substrate-explicit model to predict the energy content, metabolic efficiency, and rate of aerobic decomposition of representative PyOM compounds. This enabled us to systematically evaluate a full spectrum of PyOM chemistries that is unfeasible with laboratory experiments. The model relies on the elemental stoichiometry, allowing comparison of known PyOM chemistry to formula assignments of natural organic matter (NOM) from a recent high resolution mass spectrometry assessment of global aquatic NOM. Overall, we found the range of predicted bioavailability of PyOM was similar to NOM. Phenolic and BC molecules had lower metabolic efficiency than other PyOM and NOM compounds, and BC metabolism was less negatively impacted by oxygen limitation. In total, our work supports the recent paradigm shift regarding PyOM bioavailability, highlighting its potential role in global C emissions as the prevalence of wildfires increases.

INTRODUCTION

Wildfires have burned an average of 1.8-million ha year^{-1} in the US alone over the past 80 years[1] with dramatic impacts on terrestrial and aquatic ecosystem functions.[2-5] The timing, extent, and severity of wildfire activity has increased rapidly in recent years in response to pervasive hotter and drier conditions associated with climate change,[6, 7] a trend that is expected to continue.[8] Globally, wildfires generate 116–385 Tg of black carbon (C) each year, providing a continuous source of pyrogenic organic matter (PyOM) to inland waters that totals 300 to 500 giga-metric tons of C stored in sediments, soils, and waters.[9-13] Within river corridors, black C (BC)—a major chemical constituent of PyOM—constitutes ~10% of dissolved organic C pools.[9] Given that organic matter drives biogeochemical cycles in most aquatic ecosystems, the loading of PyOM into river corridors has the potential to produce substantial negative impacts on ecosystem functions and downstream drinking water treatability.[14, 15]

Historically, PyOM has been considered to be refractory, passively transported and deposited throughout landscapes. Some estimates of PyOM residence times in aquatic landscapes extend thousands of years.[16-19] Yet, PyOM is a complex continuum of materials,[20, 21] and simple mass balances suggest depositional reservoirs, including soils and sediments, do not fully account for PyOM production via biomass burning.[22] This implies biotic and/or abiotic loss from source to sink.[23] This paradigm shift is highlighted by recent work that has shown PyOM may be more bioavailable than previously thought.[24-26] In this scenario, PyOM may constitute a significant and unconstrained contributor to global biogeochemical cycles and climate feedbacks. To address this knowledge gap, we used a predictive model of organic matter energy content, metabolic efficiency, and rate of aerobic decomposition to compare the bioavailability of PyOM to natural organic matter (NOM) in global surface waters and sediments.

MATERIALS AND METHODS

To assess the bioavailability of PyOM, we searched primary literature for representative compounds of the PyOM continuum. Specifically, we targeted characteristic organic compounds from field and laboratory 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 which are often used to characterize PyOM in the environment. This included compounds such as theoretical BC compounds (defined here as condensed aromatic core structures polysubstituted with O-containing functionalities[27]), 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).[28, 29] In total, our literature search for PyOM chemistries yielded 389 compounds with 207 unique chemical formulae.

After generating a set of representative compounds, we used a substrate-explicit modelling framework developed by Song et al.[30] to characterize the bioavailability of each compound and predict its rate of decomposition. Importantly, the model utilizes 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 enabled flexibility in application to high-throughput mass spectrometry techniques that yield chemical formulae but not structural information (e.g., Fourier Transform Ion Cyclotron Resonance Mass Spectrometry, FTICR-MS). Despite its limitations, the substrate-explicit model used here has proven useful in linking NOM composition to aerobic metabolism in natural environments[30-32], and its structure is consistent with Harvey et al.[33] 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 NOM pools to date.[34]

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 a unit carbon mole of biomass. 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[35] by Song et al.[30], as well as C use efficiency (CUE) as defined by Saifuddin et al.[36] Lower ΔG_{Cox} denotes higher thermodynamic favorability in an electron donor half reaction associated with organic matter, 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.[30] Each metric (λ, ΔG_{Cox}, CUE, metabolic rates) denotes a different aspect of 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: global dissolved (1) surface water NOM and (2) sediment NOM pools, measured by FTICR-MS as described by Garayburu-

Caruso et al.[34]; 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 NOM pools were filtered to compounds occurring in 95% of samples to yield a dataset of globally ubiquitous NOM. Formulae assignment and inferred chemical classes via Van Krevelen diagrams in NOM pools are described by Garayburu-Caruso et al.[34] We compared modelling outputs from representative PyOM to outputs of ubiquitous NOM pools to infer relative bioavailability using ANOVA and Tukey HSD statistical tests with R software. All model outputs are available in Tables S2-S4. Code is available at: https://github.com/hyunseobsong/lambda. Data describing NOM pool chemistry are published as a data package[37] (available at: doi:10.15485/1729719) and are discussed in more detail by Garayburu-Caruso et al.[34].

RESULTS AND DISCUSSION

Intensifying wildfire regimes in many parts of the world are increasing the production of PyOM with potential implications for source water supplies, which are critical for domestic, industrial, agricultural, and ecological needs. However, the influence of PyOM on river corridor biogeochemistry is inadequately understood.[1, 38-41] Here, we used a substrate-explicit model to evaluate the emerging paradigm of PyOM bioavailability and compared model outputs to comprehensive measurements of global NOM pool composition.[30-32, 34] In contrast to previous characterizations of PyOM bioavailability,[24, 25, 33, 42] the model-based approach enabled us to directly compare known combustion products to thousands of ubiquitous NOM compounds, which would have been unfeasible to directly assess in a laboratory setting.

Though previous work has shown that organic matter chemistry of sediment and surface waters can be altered by wildfires,[9, 43-45] our results suggest that the chemically distinct pools generated through combustion may have similar overall bioavailability to NOM. We found that the ranges of ΔG_{Cox}, λ, and CUE were similar between PyOM and NOM pools in sediments and surface waters (Figure 1 and 2a). Predicted CUE of PyOM classes was also comparable to literature values reported by others.[36, 46, 47] Similarly, λ did not vary across all groups of organic molecules or in post-hoc pairwise comparisons (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 NOM had greater dissimilarity in these parameters than any comparison involving PyOM. For example, the mean difference in NOM between surface water and sediment was 7.34 kJ/mol-C for ΔG_{Cox} and 0.058 for CUE. Comparatively, 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 NOM (Tukey HSD, $p = 0.20$). These results signal a strong overlap between PyOM bioavailability and NOM pools; however, within PyOM compounds, there was variability in ΔG_{Cox}, λ, and CUE consistent with a heterogeneous continuum of organic matter (Figure 1 and S1). This is not surprising, given the diversity of PyOM chemistries generated by wildfires of different burn severities and source materials,[45, 48, 49] some of which overlap with chemical classes in unaltered NOM.

Interestingly, the relative equivalence of predicted CUE across PyOM and NOM pools revealed that PyOM decomposition in rivers could emit comparable amounts of CO_2 to extant NOM pools on a per C basis (i.e., assuming equivalent pool sizes). CUE is used in many microbially-explicit decomposition models to constrain organic matter bioavailability,[50-55] and predicted CUE from PyOM pools can be easily assimilated in microbially-explicit model predictions that rely on CUE as an input parameter. Such an approach could be used to directly evaluate the impact of PyOM on global C cycles, leading to a better incorporation of PyOM impacts in model predictions.[56]

Within PyOM (Figure 1c), two clusters of compounds were distinctly separated from the energetic and metabolic properties of most PyOM. For example, phenols had greater λ values than the majority of PyOM and NOM compounds (all $\lambda > 0.039$, mean $\lambda = 0.084$), while the electron donor half reaction involving 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 NOM pools (Figure 1). Phenols and BC also had among the lowest CUE values (BC mean = 0.47 and phenols mean = 0.54). Phenols are traditionally associated with refractory organic matter, such as lignin and tannins, that exhibit long residence times in soils,[57] although they have also been reported to be bioavailable in soils and waters in recent years (e.g., [57, 58]). Additionally, BC in this study is defined by inferred aromaticity from ultrahigh resolution mass spectroscopy (i.e., the presence of condensed aromatic structures), which is also considered to have low reactivity.[19, 27] Although the bioavailability of phenols and BC is consistent with refractory PyOM, it is within the bioavailability range observed in NOM, and these compound classes represent only a small portion of chemical products from wildfires.[45] We note that the comparatively low predicted CUE of phenols and BC indicates that their decomposition could have a greater impact on river corridor CO_2 emissions than other PyOM and NOM compounds. As a result, current understanding may substantially underestimate the size, reactivity, and hydrobiogeochemical role of PyOM.[45]

Predicted rates of PyOM metabolism were also similar to NOM pools (Figure 2b), reinforcing comparable bioavailability between the two pools of organic matter. Pairwise comparison of metabolic rates revealed no evidence for differences between PyOM and sediment NOM under oxygen limitation (Tukey HSD, $p = 0.23$) and without carbon or oxygen limitations (Tukey HSD, $p = 0.34$). However, there was strong evidence that metabolic rates of both PyOM and sediment NOM were different than surface water NOM (Tukey HSD, all $p < 0.001$). Aquatic sediments can reach anoxia within millimeters of the sediment-water interface such that model predictions under oxygen limitation may translate to no meaningful difference between PyOM and NOM in natural sediments. Under C-limitation, PyOM had a statistically elevated rate of metabolism relative to both surface water and sediment NOM (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 of values in sediment NOM (0.0008–0.45) and PyOM (4.75e-08–0.45). Statistical differences were not surprising given an extremely large sample size for NOM (sediment $n = 398$, surface water $n = 811$), and the low effect sizes denote that overall differences in metabolisms between PyOM and NOM were minimal despite statistical separation.

When considering the impact of various elemental limitations on PyOM metabolism, rate predictions were strongly inhibited under low carbon and oxygen conditions. Predicted rates of PyOM metabolism were approximately six times lower when C or O_2 was scarce. Low decomposition rates such as those predicted here under oxygen and C limitation could be one reason for the observed persistence of PyOM in depositional features that tend to be anoxic with refractory C pools.[16-18] Still, it is worthwhile to note that metabolism of all PyOM classes under low O_2 or C 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[23] and persist over long periods of time in O_2-limited sediments.

Among PyOM compound classes, BC was less negatively impacted by oxygen limitation than any other group (Figure S2). 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 and/or anaerobia.[59-62] Similar microbial metabolic pathways may also be capable of degrading BC molecules 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 BC. We posit this may be due, in part, to its chemical structure that includes pyrrole-type moieties, which are relatively biodegradable.[63, 64] While we only examined one class of PyOM molecules containing nitrogen (n-alkylnitriles), it had among the highest predicted CUE and rates of aerobic metabolism.

While the substrate-explicit modelling approach used here has been validated in natural settings and enabled comparison to a rich suite of ubiquitous NOM molecules, one key limitation is its inability to account for structural characteristics of organic matter. Indeed, 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 be most favorable for metabolism, despite these compound classes having high stability in the environment and common usage as paleo-proxies in soils and sediments (e.g., [65, 66]). These compounds are characterized by carbon-carbon double bond functional groups, which may decrease bioavailability and are not considered in the model predictions. Fatty acids and n-alkanes biomass burning by-products are generally reduced in chain length in comparison to their un-burned counterparts[67], and thus may be relatively bioavailable compared to other lipids. Additionally, we note that previous work has shown fast degradation of combustion-derived lipids in soils;[67] as well as high n-alkene metabolism under anaerobic conditions (in particular by sulfate-reducing bacteria[68-71]), n-alkene metabolism in natural sediments,[71, 72] and a range in lipid reactivities at the sediment-water interface.[73] While work on n-alkene metabolism in aerobic settings is limited, the comparative bioavailability of n-alkenes and known microbial degradation pathways suggests diverse microbiomes in sediments may metabolize these compounds as part of natural biogeochemical cycles. Another notable discrepancy is the relatively low 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.[25] The model may therefore not adequately account for enzyme-catalyzed reactions, such as levoglucosan kinase or levoglucosan dehydrogenase, which cleave and phosphorylate the 1,6-intramolecular linkage,[74] and could be potentially common enzymes utilized by aquatic microorganisms.[75] Because of these nuances, the PyOM compound classes presented here are best used as bounding estimates for experimental validation, and for holistic comparison to NOM bioavailability. Still, the span of compounds investigated here, and their comparison to NOM pools, provides a breadth of PyOM investigation that is unfeasible without model-based approaches.[24, 25, 33, 42]

Our data supports the recent paradigm shift towards high PyOM bioavailability and provides a foundation for detailed laboratory experiments investigating specific components of the PyOM continuum. The importance of PyOM in hydrobiogeochemical regimes of aquatic systems is increasingly recognized, emerging from a long history of wildfire research in terrestrial ecosystems.[45] Yet, many fundamental questions, such as “how much” PyOM exists in ecosystems, “how fast” it cycles, and “how old” it is remain largely unknown, and are an active area of research.[76] Furthermore, there has been no systematic evaluation of the bioavailability of different constituents within the heterogeneous pools that comprise the PyOM continuum.[77] Our work provides the first comprehensive computational assessment of the bioavailability of various chemical classes of PyOM in comparison to NOM pools. The comparable bioavailability to NOM revealed that PyOM may be actively transformed within the river corridor and may be an increasing source of C emissions to the atmosphere in the future as the prevalence of wildfires increases.

AUTHOR INFORMATION

EBG and AMP conceived of the manuscript. EBG was responsible for writing the manuscript and drafting 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 NOM pool chemistry. All authors contributed to revisions.

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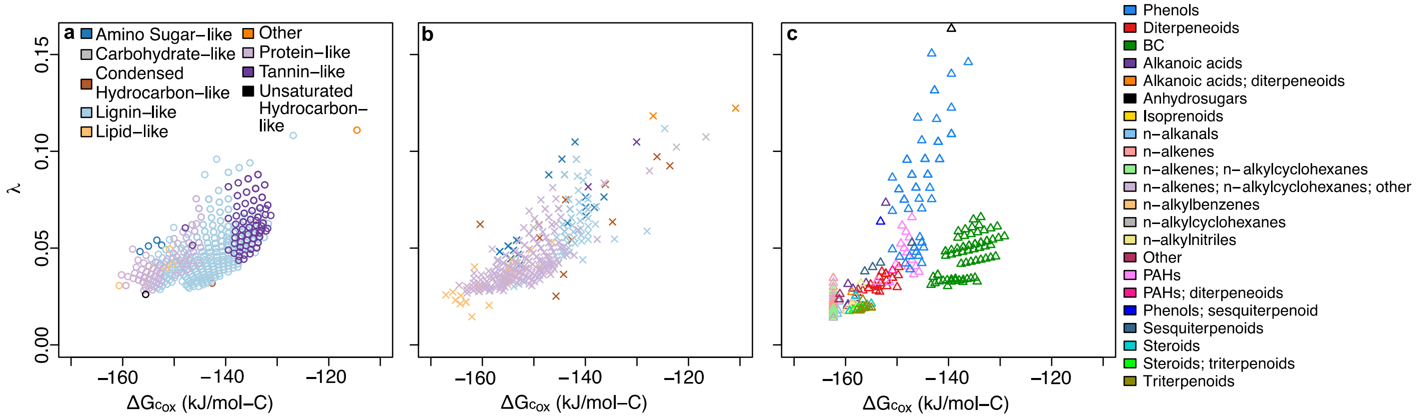

Figure 1. Comparison of PyOM energy content (ΔG_{Cox}) and metabolic efficiency (λ) to global NOM. Ubiquitous NOM molecules detected via FTICR-MS in global (**a**) surface water and (**b**) sediment are colored by inferred chemical class. (**c**) Representative PyOM molecules are colored by known chemical properties. Legends are inset in (**a**) for (**a**) and (**b**), and to the right of (**c**).

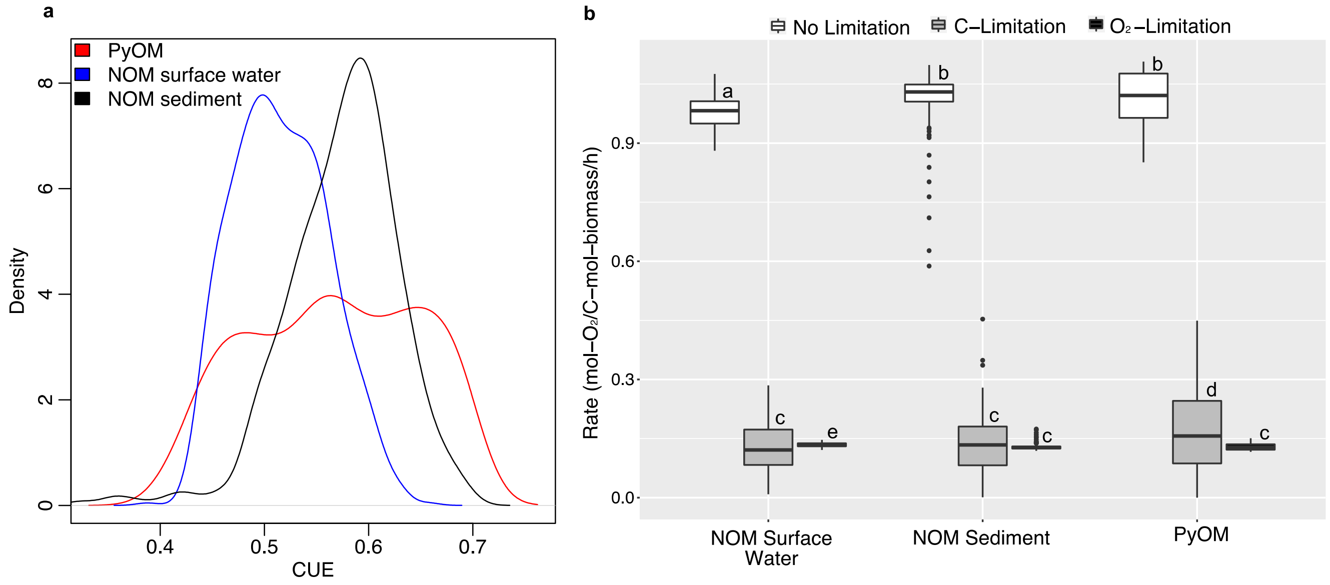

Figure 2. Carbon use efficiency (CUE) and predicted metabolism of NOM and PyOM. (**a**) shows the distribution of CUE in PyOM (red) and ubiquitous surface water (blue) and sediment (black) NOM molecules rates. (**b**) depicts the predicted metabolism of surface water NOM, sediment NOM, and PyOM. Letters in (**b**) denote statistical groups. Median values are denoted by a bar, the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles), and the upper and lower 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.

SUPPLEMENTAL FIGURES

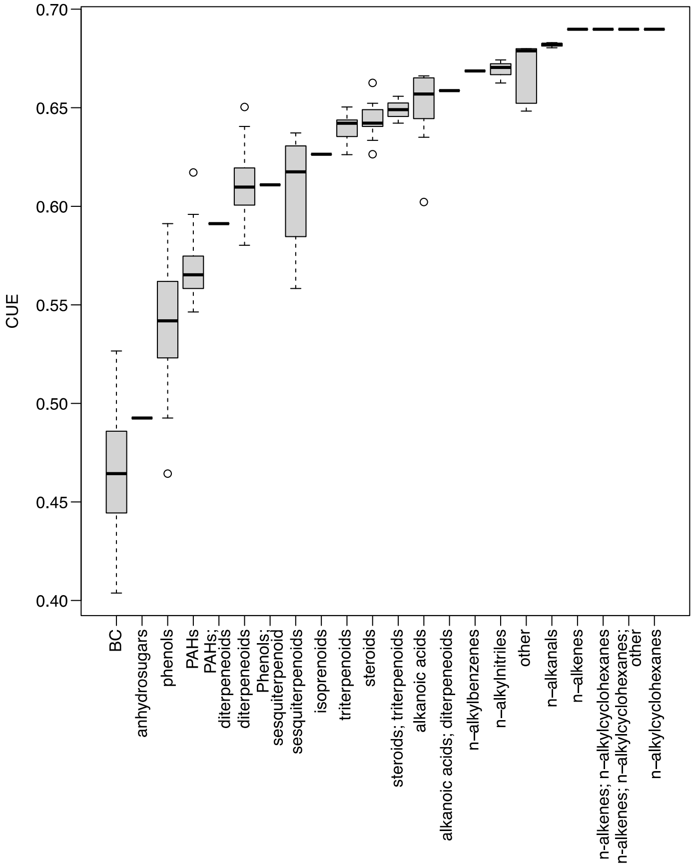

Figure S1. Carbon use efficiency (CUE) of PyOM, grouped by known chemical properties. High CUE values indicate more C incorporated into biomass vs. respired per unit C. Median values are denoted by a bar, the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles), and the upper and lower 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.

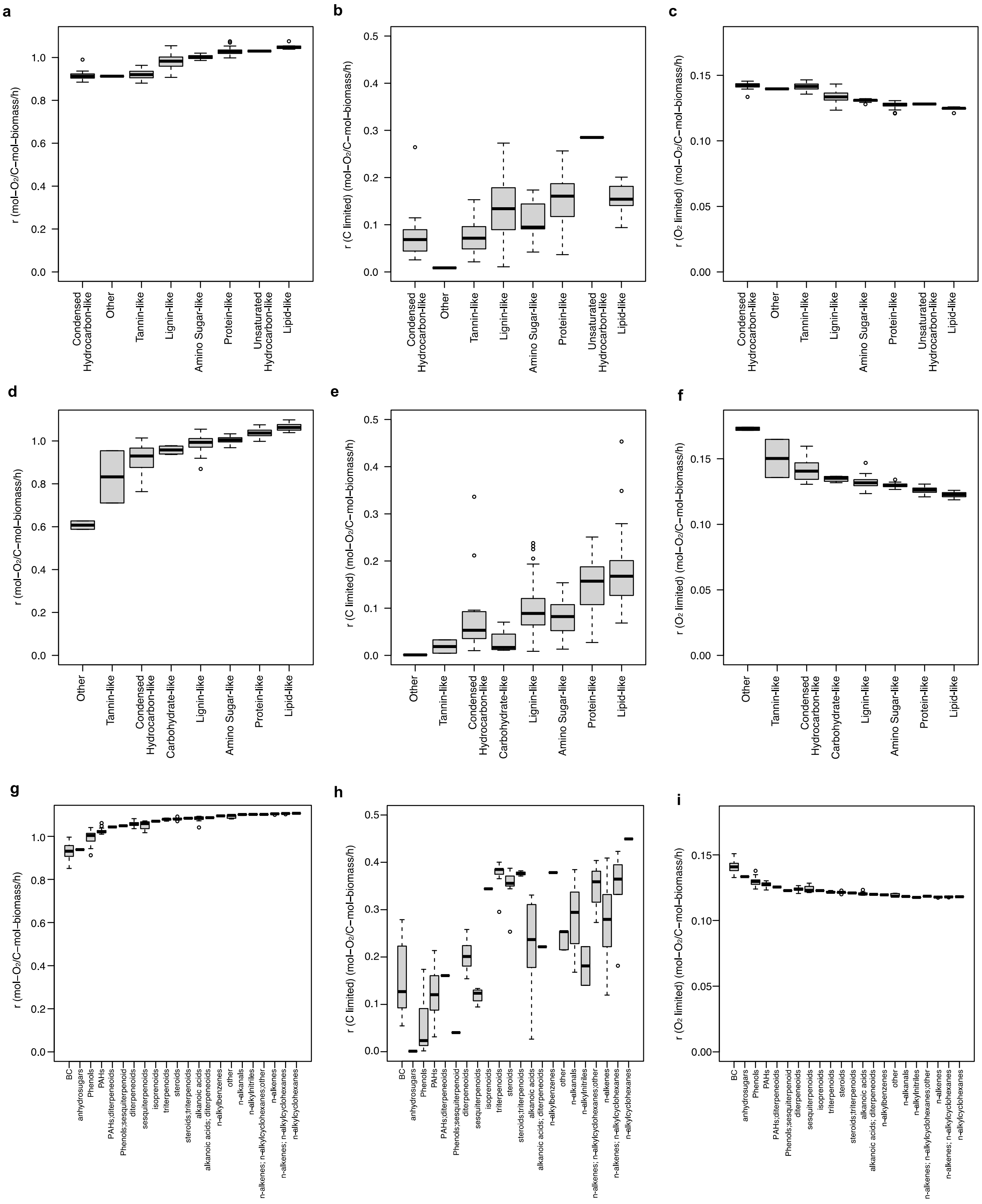

Figure S2. Predicted metabolism of ubiquitous (a–c) surface water NOM, (d–f) sediment NOM, and (g–i) PyOM molecules, grouped by inferred chemical class or known chemical properties. The first column shows predicted rates of aerobic metabolism without any elemental limitations.

C-limited and O_2-limited scenarios are show in the second and third columns, respectively. Median values are denoted by a bar, the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles), and the upper and lower 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.

Table S1. PyOM molecules and chemical properties.

Table S2. Substrate-explicit model outputs for each PyOM compound.

Table S3. Substrate-explicit model outputs for each sediment NOM compound.

Table S4. Substrate-explicit model outputs for each surface water NOM compound.

REFERENCES

1. Bladon, K. D.; Emelko, M. B.; Silins, U.; Stone, M., Wildfire and the future of water supply. In ACS Publications: 2014.

2. Shakesby, R.; Doerr, S., Wildfire as a hydrological and geomorphological agent. *Earth-Science Reviews* **2006,** *74*, (3-4), 269-307.

3. Randerson, J. T.; Liu, H.; Flanner, M. G.; Chambers, S. D.; Jin, Y.; Hess, P. G.; Pfister, G.; Mack, M.; Treseder, K.; Welp, L., The impact of boreal forest fire on climate warming. *science* **2006,** *314*, (5802), 1130-1132.

4. Verma, S.; Jayakumar, S., Impact of forest fire on physical, chemical and biological properties of soil: A review. *Proceedings of the International Academy of Ecology and Environmental Sciences* **2012,** *2*, (3), 168.

5. Smith, H. G.; Sheridan, G. J.; Lane, P. N.; Nyman, P.; Haydon, S., Wildfire effects on water quality in forest catchments: a review with implications for water supply. *Journal of Hydrology* **2011,** *396*, (1-2), 170-192.

6. Krawchuk, M. A.; Moritz, M. A., Constraints on global fire activity vary across a resource gradient. *Ecology* **2011,** *92*, (1), 121-132.

7. Pierce, J. L.; Meyer, G. A.; Jull, A. T., Fire-induced erosion and millennial-scale climate change in northern ponderosa pine forests. *Nature* **2004,** *432*, (7013), 87.

8. Flannigan, M. D.; Krawchuk, M. A.; de Groot, W. J.; Wotton, B. M.; Gowman, L. M., Implications of changing climate for global wildland fire. *International journal of wildland fire* **2009,** *18*, (5), 483-507.

9. Jaffé, R.; Ding, Y.; Niggemann, J.; Vähätalo, A. V.; Stubbins, A.; Spencer, R. G.; Campbell, J.; Dittmar, T., Global charcoal mobilization from soils via dissolution and riverine transport to the oceans. *Science* **2013,** *340*, (6130), 345-347.

10. Santin, C.; Doerr, S. H.; Kane, E. S.; Masiello, C. A.; Ohlson, M.; de la Rosa, J. M.; Preston, C. M.; Dittmar, T., Towards a global assessment of pyrogenic carbon from vegetation fires. *Global Change Biology* **2016,** *22*, (1), 76-91.

11. Dittmar, T.; De Rezende, C. E.; Manecki, M.; Niggemann, J.; Ovalle, A. R. C.; Stubbins, A.; Bernardes, M. C., Continuous flux of dissolved black carbon from a vanished tropical forest biome. *Nature Geoscience* **2012,** *5*, (9), 618-622.

12. Hockaday, W. C.; Grannas, A. M.; Kim, S.; Hatcher, P. G., The transformation and mobility of charcoal in a fire-impacted watershed. *Geochimica et Cosmochimica Acta* **2007,** *71*, (14), 3432-3445.

13. Santín, C.; Doerr, S. H.; Kane, E. S.; Masiello, C. A.; Ohlson, M.; de la Rosa, J. M.; Preston, C. M.; Dittmar, T., Towards a global assessment of pyrogenic carbon from vegetation fires. *Global Change Biology* **2016,** *22*, (1), 76-91.

14. Emelko, M. B.; Silins, U.; Bladon, K. D.; Stone, M., Implications of land disturbance on drinking water treatability in a changing climate: Demonstrating the need for “source water supply and protection” strategies. *Water research* **2011,** *45*, (2), 461-472.

15. Hohner, A. K.; Terry, L. G.; Townsend, E. B.; Summers, R. S.; Rosario-Ortiz, F. L., Water treatment process evaluation of wildfire-affected sediment leachates. *Environmental Science: Water Research & Technology* **2017,** *3*, (2), 352-365.

16. Meyer, G. A.; Wells, S. G., Fire-related sedimentation events on alluvial fans, Yellowstone National Park, USA. *Journal of Sedimentary Research* **1997,** *67*, (5), 776-791.

17. Elliott, J. G.; Parker, R., Developing a post-fire flood chronology and recurrence probability from alluvial stratigraphy in the Buffalo Creek watershed, Colorado, USA. *Hydrological Processes* **2001,** *15*, (15), 3039-3051.

18. Bigio, E.; Swetnam, T. W.; Baisan, C. H., A comparison and integration of tree-ring and alluvial records of fire history at the Missionary Ridge Fire, Durango, Colorado, USA. *The Holocene* **2010,** *20*, (7), 1047-1061.

19. Kuzyakov, Y.; Bogomolova, I.; Glaser, B., Biochar stability in soil: decomposition during eight years and transformation as assessed by compound-specific 14C analysis. *Soil Biology and Biochemistry* **2014,** *70*, 229-236.

20. Wozniak, A. S.; Goranov, A. I.; Mitra, S.; Bostick, K. W.; Zimmerman, A. R.; Schlesinger, D. R.; Myneni, S.; Hatcher, P. G., Molecular heterogeneity in pyrogenic dissolved organic matter from a thermal series of oak and grass chars. *Organic Geochemistry* **2020,** *148*, 104065.

21. Masiello, C. A., New directions in black carbon organic geochemistry. *Marine Chemistry* **2004,** *92*, (1-4), 201-213.

22. Masiello, C.; Druffel, E., Organic and black carbon 13C and 14C through the Santa Monica Basin sediment oxic-anoxic transition. *Geophysical research letters* **2003,** *30*, (4).

23. Masiello, C.; Louchouarn, P., Fire in the ocean. *Science* **2013,** *340*, (6130), 287-288.

24. Myers-Pigg, A. N.; Louchouarn, P.; Amon, R. M.; Prokushkin, A.; Pierce, K.; Rubtsov, A., Labile pyrogenic dissolved organic carbon in major Siberian Arctic rivers: Implications for wildfire-stream metabolic linkages. *Geophysical Research Letters* **2015,** *42*, (2), 377-385.

25. Norwood, M. J.; Louchouarn, P.; Kuo, L.-J.; Harvey, O. R., Characterization and biodegradation of water-soluble biomarkers and organic carbon extracted from low temperature chars. *Organic Geochemistry* **2013,** *56*, 111-119.

26. Zimmerman, A. R.; Ouyang, L., Priming of pyrogenic C (biochar) mineralization by dissolved organic matter and vice versa. *Soil Biology and Biochemistry* **2019,** *130*, 105-112.

27. Wagner, S.; Ding, Y.; Jaffé, R., A new perspective on the apparent solubility of dissolved black carbon. *Frontiers in Earth Science* **2017,** *5*, 75.

28. Oros, D. R.; Simoneit, B. R., Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 1. Temperate climate conifers. *Applied Geochemistry* **2001,** *16*, (13), 1513-1544.

29. Oros, D. R.; Simoneit, B. R., Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 2. Deciduous trees. *Applied Geochemistry* **2001,** *16*, (13), 1545-1565.

30. Song, H.-S.; Stegen, J. C.; Graham, E. B.; Lee, J.-Y.; Garayburu-Caruso, V.; Nelson, W. C.; Chen, X.; Moulton, J. D.; Scheibe, T. D., Representing Organic Matter Thermodynamics in Biogeochemical Reactions via Substrate-Explicit Modeling. *Frontiers in Microbiology* **2020**.

31. Graham, E. B.; Tfaily, M. M.; Crump, A. R.; Goldman, A. E.; Bramer, L. M.; Arntzen, E.; Romero, E.; Resch, C. T.; Kennedy, D. W.; Stegen, J. C., Carbon inputs from riparian vegetation limit oxidation of physically bound organic carbon via biochemical and thermodynamic processes. *Journal of Geophysical Research: Biogeosciences* **2017,** *122*, (12), 3188-3205.

32. Garayburu-Caruso, V. A.; Stegen, J. C.; Song, H.-S.; Renteria, L.; Wells, J.; Garcia, W.; Resch, C. T.; Goldman, A. E.; Chu, R. K.; Toyoda, J.; Graham, E. B., Carbon limitation leads to

thermodynamic regulation of aerobic metabolism. *Environmental Science & Technology Letters* **2020,** *7*, 517-524.

33. Harvey, O. R.; Myers-Pigg, A. N.; Kuo, L.-J.; Singh, B. P.; Kuehn, K. A.; Louchouarn, P., Discrimination in degradability of soil pyrogenic organic matter follows a return-on-energy-investment principle. *Environmental science & technology* **2016,** *50*, (16), 8578-8585.

34. Garayburu-Caruso, V. A.; Danczak, R. E.; Stegen, J. C.; Renteria, L.; Mccall, M.; Goldman, A. E.; Chu, R. K.; Toyoda, J.; Resch, C. T.; Torgeson, J. M., Using Community Science to Reveal the Global Chemogeography of River Metabolomes. *Metabolites* **2020,** *10*, (12), 518.

35. LaRowe, D. E.; Van Cappellen, P., Degradation of natural organic matter: a thermodynamic analysis. *Geochimica et Cosmochimica Acta* **2011,** *75*, (8), 2030-2042.

36. Saifuddin, M.; Bhatnagar, J. M.; Segrè, D.; Finzi, A. C., Microbial carbon use efficiency predicted from genome-scale metabolic models. *Nature communications* **2019,** *10*, (1), 1-10.

37. Goldman, A. E.; Chu, R. K.; Danczak, R. E.; Daly, R. A.; Fansler, S.; Garayburu-Caruso, V. A.; Graham, E. B.; McCall, M. L.; Ren, H.; Renteria, L. *WHONDRS Summer 2019 Sampling Campaign: Global River Corridor Sediment FTICR-MS, NPOC, and Aerobic Respiration*; Environmental System Science Data Infrastructure for a Virtual Ecosystem …: 2020.

38. Robinne, F.-N.; Bladon, K. D.; Silins, U.; Emelko, M. B.; Flannigan, M. D.; Parisien, M.-A.; Wang, X.; Kienzle, S. W.; Dupont, D. P., A Regional-Scale Index for Assessing the Exposure of Drinking-Water Sources to Wildfires. *Forests* **2019,** *10*, (5), 384.

39. Robinne, F.-N.; Miller, C.; Parisien, M.-A.; Emelko, M.; Bladon, K.; Silins, U.; Flannigan, M., A global index for mapping the exposure of water resources to wildfire. *Forests* **2016,** *7*, (1), 22.

40. Hallema, D. W.; Robinne, F. N.; Bladon, K. D., Reframing the challenge of global wildfire threats to water supplies. *Earth's Future* **2018,** *6*, (6), 772-776.

41. Martin, D. A., At the nexus of fire, water and society. *Philosophical Transactions of the Royal Society B: Biological Sciences* **2016,** *371*, (1696), 20150172.

42. Harvey, O. R.; Kuo, L.-J.; Zimmerman, A. R.; Louchouarn, P.; Amonette, J. E.; Herbert, B. E., An index-based approach to assessing recalcitrance and soil carbon sequestration potential of engineered black carbons (biochars). *Environmental science & technology* **2012,** *46*, (3), 1415-1421.

43. Cawley, K. M.; Hohner, A. K.; McKee, G. A.; Borch, T.; Omur-Ozbek, P.; Oropeza, J.; Rosario-Ortiz, F. L., Characterization and spatial distribution of particulate and soluble carbon and nitrogen from wildfire-impacted sediments. *Journal of soils and sediments* **2018,** *18*, (4), 1314-1326.

44. Hohner, A. K.; Rhoades, C. C.; Wilkerson, P.; Rosario-Ortiz, F. L., Wildfires Alter Forest Watersheds and Threaten Drinking Water Quality. *Accounts of chemical research* **2019**.

45. Wagner, S.; Jaffé, R.; Stubbins, A., Dissolved black carbon in aquatic ecosystems. *Limnology and Oceanography Letters* **2018,** *3*, (3), 168-185.

46. Domeignoz-Horta, L. A.; Pold, G.; Liu, X.-J. A.; Frey, S. D.; Melillo, J. M.; DeAngelis, K. M., Microbial diversity drives carbon use efficiency in a model soil. *Nature communications* **2020,** *11*, (1), 1-10.

47. Pold, G.; Domeignoz-Horta, L. A.; Morrison, E. W.; Frey, S. D.; Sistla, S. A.; DeAngelis, K. M., Carbon use efficiency and its temperature sensitivity covary in soil bacteria. *Mbio* **2020,** *11*, (1).

48. Wagner, S.; Dittmar, T.; Jaffé, R., Molecular characterization of dissolved black nitrogen via electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Organic Geochemistry* **2015,** *79*, 21-30.

49. Neary, D. G.; Ryan, K. C.; DeBano, L. F., Wildland fire in ecosystems: effects of fire on soils and water. *Gen. Tech. Rep. RMRS-GTR-42-vol. 4. Ogden, UT: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. 250 p.* **2005,** *42*.

50. Wang, G.; Jagadamma, S.; Mayes, M. A.; Schadt, C. W.; Steinweg, J. M.; Gu, L.; Post, W. M., Microbial dormancy improves development and experimental validation of ecosystem model. *The ISME journal* **2015,** *9*, (1), 226-237.

51. Abramoff, R.; Xu, X.; Hartman, M.; O'Brien, S.; Feng, W.; Davidson, E.; Finzi, A.; Moorhead, D.; Schimel, J.; Torn, M., The Millennial model: in search of measurable pools and transformations for modeling soil carbon in the new century. *Biogeochemistry* **2018,** *137*, (1-2), 51-71.

52. Moorhead, D. L.; Lashermes, G.; Sinsabaugh, R. L., A theoretical model of C-and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. *Soil Biology and Biochemistry* **2012,** *53*, 133-141.

53. Sulman, B. N.; Shevliakova, E.; Brzostek, E. R.; Kivlin, S. N.; Malyshev, S.; Menge, D. N.; Zhang, X., Diverse mycorrhizal associations enhance terrestrial C storage in a global model. *Global Biogeochemical Cycles* **2019,** *33*, (4), 501-523.

54. Wieder, W. R.; Cleveland, C. C.; Smith, W. K.; Todd-Brown, K., Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geoscience* **2015,** *8*, (6), 441-444.

55. Graham, E. B.; Hofmockel, K., A new frontier in molecular applications of ecological stoichiometry to understand global soil organic matter decomposition. **2021**.

56. Santin, C.; Doerr, S. H.; Jones, M. W.; Merino, A.; Warneke, C.; Roberts, J. M., The relevance of pyrogenic carbon for carbon budgets from fires: insights from the FIREX experiment. *Global Biogeochemical Cycles* **2020,** *34*, (9), e2020GB006647.

57. Thevenot, M.; Dignac, M.-F.; Rumpel, C., Fate of lignins in soils: a review. *Soil Biology and Biochemistry* **2010,** *42*, (8), 1200-1211.

58. Ward, N. D.; Keil, R. G.; Medeiros, P. M.; Brito, D. C.; Cunha, A. C.; Dittmar, T.; Yager, P. L.; Krusche, A. V.; Richey, J. E., Degradation of terrestrially derived macromolecules in the Amazon River. *Nature Geoscience* **2013,** *6*, (7), 530-533.

59. Bushnell, L.; Haas, H., The utilization of certain hydrocarbons by microorganisms. *Journal of Bacteriology* **1941,** *41*, (5), 653.

60. Pozdnyakova, N. N., Involvement of the ligninolytic system of white-rot and litter-decomposing fungi in the degradation of polycyclic aromatic hydrocarbons. *Biotechnology research international* **2012,** *2012*.

61. Rabus, R.; Boll, M.; Heider, J.; Meckenstock, R. U.; Buckel, W.; Einsle, O.; Ermler, U.; Golding, B. T.; Gunsalus, R. P.; Kroneck, P. M., Anaerobic microbial degradation of hydrocarbons: from enzymatic reactions to the environment. *Journal of molecular microbiology and biotechnology* **2016,** *26*, (1-3), 5-28.

62. Coates, J. D.; Woodward, J.; Allen, J.; Philp, P.; Lovley, D. R., Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. *Applied and Environmental Microbiology* **1997,** *63*, (9), 3589-3593.

63. Knicker, H., “Black nitrogen”–an important fraction in determining the recalcitrance of charcoal. *Organic Geochemistry* **2010,** *41*, (9), 947-950.

64. de la Rosa, J. M.; Knicker, H., Bioavailability of N released from N-rich pyrogenic organic matter: an incubation study. *Soil Biology and Biochemistry* **2011,** *43*, (12), 2368-2373.

65. Wiesenberg, G. L.; Schwarzbauer, J.; Schmidt, M. W.; Schwark, L., Source and turnover of organic matter in agricultural soils derived from n-alkane/n-carboxylic acid compositions and C-isotope signatures. *Organic Geochemistry* **2004,** *35*, (11-12), 1371-1393.

66. Smittenberg, R. H.; Hopmans, E. C.; Schouten, S.; Hayes, J. M.; Eglinton, T. I.; Sinninghe Damsté, J., Compound-specific radiocarbon dating of the varved Holocene sedimentary record of Saanich Inlet, Canada. *Paleoceanography* **2004,** *19*, (2).

67. Knicker, H.; Hilscher, A.; De la Rosa, J.; González-Pérez, J. A.; González-Vila, F. J., Modification of biomarkers in pyrogenic organic matter during the initial phase of charcoal biodegradation in soils. *Geoderma* **2013,** *197*, 43-50.

68. Grossi, V.; Cravo-Laureau, C.; Guyoneaud, R.; Ranchou-Peyruse, A.; Hirschler-Réa, A., Metabolism of n-alkanes and n-alkenes by anaerobic bacteria: A summary. *Organic Geochemistry* **2008,** *39*, (8), 1197-1203.

69. Colby, J.; Stirling, D. I.; Dalton, H., The soluble methane mono-oxygenase of Methylococcus capsulatus (Bath). Its ability to oxygenate n-alkanes, n-alkenes, ethers, and alicyclic, aromatic and heterocyclic compounds. *Biochemical Journal* **1977,** *165*, (2), 395-402.

70. Wilkes, H.; Buckel, W.; Golding, B. T.; Rabus, R., Metabolism of hydrocarbons in n-alkane-utilizing anaerobic bacteria. *Journal of molecular microbiology and biotechnology* **2016,** *26*, (1-3), 138-151.

71. Mbadinga, S. M.; Wang, L.-Y.; Zhou, L.; Liu, J.-F.; Gu, J.-D.; Mu, B.-Z., Microbial communities involved in anaerobic degradation of alkanes. *International Biodeterioration & Biodegradation* **2011,** *65*, (1), 1-13.

72. Yongdong, Z.; Yaling, S.; Zhengwen, L.; Xiangchao, C.; Jinlei, Y.; Xiaodan, D.; Miao, J., Long-chain n-alkenes in recent sediment of Lake Lugu (SW China) and their ecological implications. *Limnologica* **2015,** *52*, 30-40.

73. Canuel, E. A.; Martens, C. S., Reactivity of recently deposited organic matter: Degradation of lipid compounds near the sediment-water interface. *Geochimica et cosmochimica acta* **1996,** *60*, (10), 1793-1806.

74. Bacik, J. P.; Jarboe, L. R., Bioconversion of anhydrosugars: emerging concepts and strategies. *IUBMB life* **2016,** *68*, (9), 700-708.

75. Suciu, L. G.; Masiello, C. A.; Griffin, R. J., Anhydrosugars as tracers in the Earth system. *Biogeochemistry* **2019,** *146*, (3), 209-256.

76. Abiven, S.; Santin, C., From Fires to Oceans: Dynamics of Fire-Derived Organic Matter in Terrestrial and Aquatic Ecosystems. *Frontiers in Earth Science* **2019,** *7*, 31.

77. Zimmerman, A. R.; Mitra, S., Trial by fire: on the terminology and methods used in pyrogenic organic carbon research. *Frontiers in Earth Science* **2017,** *5*, 95.