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

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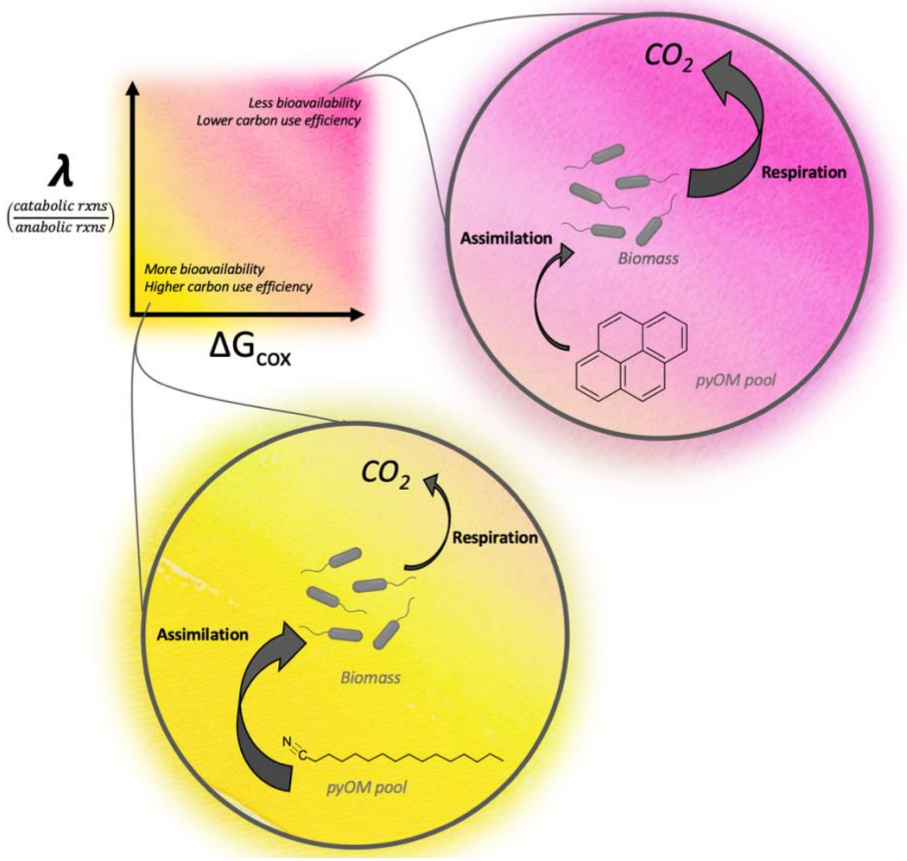
GRAPHICAL ABSTRACT



ABSTRACT

Pyrogenic materials generated by wildfires are transported across terrestrial landscapes into inland waters, creating many consequences for aquatic ecosystems. At least ~10% of dissolved organic matter pools may be comprised of pyrogenic organic matter (PyOM), but heterogeneous PyOM chemistry 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 from primary literature. This enabled us to systematically evaluate the bioavailability of a full spectrum of PyOM chemistries – which would be unfeasible with laboratory experiments – and compare it to measured natural organic matter (NOM) pools from global aquatic ecosystems. Overall, we found the range of predicted bioavailability of PyOM was similar to NOM. Thermodynamic properties and carbon use efficiencies of PyOM and NOM were statistically indistinguishable. Within PyOM, phenolic and BC molecules had lower metabolic efficiency than other PyOM and NOM compounds, and the impact of oxygen limitation on BC metabolism was lower than for other PyOM classes. In total, our work supports the recent paradigm sxhift 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 frequency, extent, and severity of wildfire activity in many regions has increased rapidly in response to pervasive hotter and drier conditions associated with climate change,[6-9] a trend that is expected to continue.[10] The impact of wildfires on river corridor biogeochemistry has received significant recent attention and is mediated by the loading of sediment and pyrogenic materials into waterways.[11, 12] In particular, pyrogenic organic matter (PyOM) is a complex continuum of materials generated by thermal alteration of biomass[13, 14] and can have substantial impacts on river corridor biogeochemistry due to the importance of organic matter as a carbon (C) and energy source in rivers. Globally, wildfires generate 116–385 Tg per year of the most commonly measured constituent of PyOM—black carbon (BC, defined herein as the condensed aromatic fraction of PyOM typically measured by benzene polycarboxylic acid (BPCA) method[11, 15]). This wildfire generated BC provides a continuous source of PyOM to inland waters that totals 300 to 500 giga-metric tons of C stored in sediments, soils, and waters.[16-20] Within river corridors, BC alone constitutes ~10% of dissolved organic C pools.[16] Given that organic matter drives biogeochemical cycles in most aquatic ecosystems, the loading of PyOM into river corridors has the potential to produce substantial impacts on ecosystem functions and downstream drinking water treatability.[21, 22]

Historically, PyOM has been considered refractory, passively transported and deposited throughout landscapes. Some estimates of PyOM residence times in aquatic landscapes extend thousands of years.[23-26] Yet, simple mass balances suggest depositional reservoirs, including soils and sediments, do not fully account for PyOM production via biomass burning.[27] This implies biotic and/or abiotic loss from source to sink.[28] This paradigm shift is highlighted by recent work that has shown PyOM may be more bioavailable than previously thought[29-31], and is also supported by biochar research highlighting a variety of reactivity of combustion by-products.[32, 33] PyOM may constitute a significant and unconstrained contributor to global biogeochemical cycles and climate feedbacks. To address this knowledge gap, we used a substrate-explicit model to predict 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. We hypothesized that the continuum of chemistries in PyOM would show roughly comparable bioavailability to diverse NOM pools.

We leverage a newly developed substrate-explicit model[34] that parameterizes OM-specific oxidative degradation pathways and reaction rates based on the thermodynamic properties of OM molecules. It relies on the elemental composition of individual OM molecules to provide a systematic way to formulate reaction kinetics and enable flexible application across environments, as it is agnostic of many factors that have complicated a universal understanding of OM bioavailability, including molecular structure (e.g., double boding and aromatic rings), priming dynamics, chemical inhibition, mineral-associations and physical protection, terminal electron acceptor concentrations, microbial community composition and accessibility, and abiotic reactions (reviewed in Arndt et al.[35]). The model requires two simple parameters (u_{max} and V_h, see Supporting Information), which focus on the chemical composition of OM as a governing factor in metabolism and overcome a key limitation in modelling aquatic OM decomposition, that variation in parameters across environments limits transferability of model predictions.[35] This ecosystem-agnostic approach allows us to examine the molecular bioavailability of individual OM molecules, and it provides a baseline for future work investigating pool-scale dynamics that require a broader range of parameters calibrated for environmental context and targeted laboratory experiments that a examine the bioavailability of a narrower range OM across environmental contexts. In total, our results support an emerging paradigm in wildfire science in which PyOM are more bioavailable than historically considered.

MATERIALS AND METHODS

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

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[36]), 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).[37, 38] 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.[34] 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[34, 39, 40], and its structure is consistent with Harvey et al.[41] 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.[42]

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[43] by Song et al.[34], as well as C use efficiency (CUE) as defined by Saifuddin et al.[44] 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.[34] 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.[42]; 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.[42] 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[45] (available at: doi:10.15485/1729719) and are discussed in more detail by Garayburu-Caruso et al.[42].

RESULTS AND DISCUSSION

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.[34, 39, 40, 42] In contrast to previous characterizations of PyOM bioavailability,[29, 30, 41, 46] 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.

Inferred Molecular Bioavailability of Pyrogenic Organic Matter.

Though previous work has shown that OM chemistry of sediment and surface waters is altered by wildfires,[11, 16, 47, 48] our results suggest that the chemically distinct pools of OM altered by pyrolysis 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.[44, 49, 50] 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,[11, 51, 52] 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 per mole of C to extant NOM pools. CUE is used in many microbially-explicit decomposition models to constrain organic matter bioavailability,[53-59] 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.[60]

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,[61] although they have also been reported to be bioavailable in soils and waters in recent years (e.g., [61, 62]). 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.[26, 36] Although the bioavailability of the phenols and BC classes is consistent with traditionally defined refractory PyOM pools, it is within the bioavailability range observed in NOM, and these compound classes represent only a small portion of the PyOM continuum.[11, 14] 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 NOM compounds. As a result, current understanding may substantially underestimate the size, reactivity, and hydrobiogeochemical role of PyOM.[11]

Inferred Metabolism of Pyrogenic Organic Matter.

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.[23-25] 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[28] 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.[63-66] 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.[67, 68] 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.

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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., [69, 70]). 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[71], 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;[71] as well as high n-alkene metabolism under anaerobic conditions (in particular by sulfate-reducing bacteria[72-75]), n-alkene metabolism in natural sediments,[75, 76] and a range in lipid reactivities at the sediment-water interface.[77] 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.[30] 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,[78] and could be potentially common enzymes utilized by aquatic microorganisms.[79] 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.[29, 30, 41, 46]

Conclusions.

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. 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. 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.[80] Furthermore, there has been no systematic evaluation of the bioavailability of different constituents within the heterogeneous pools that comprise the PyOM continuum.[81] 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 conceived of the manuscript and 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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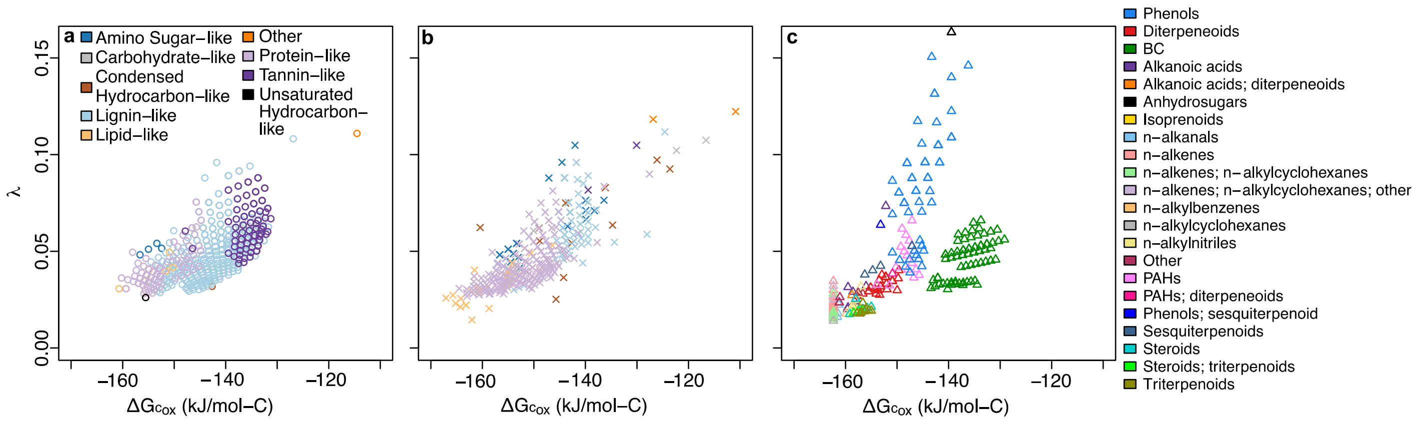

Figure 1. Comparison of PyOM energy content (ΔG_{Cox}) and metabolic efficiency (λ) to global NOM. Ubiquitous NOM molecules detected via FTICR-MS in global (**a**) surface water and (**b**) sediment are colored by inferred chemical class. (**c**) Representative PyOM molecules are colored by known chemical properties. Because PyOM molecules were derived from primary literature, we are able to assign chemical properties at higher resolution than inferred classes from measured NOM 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**).

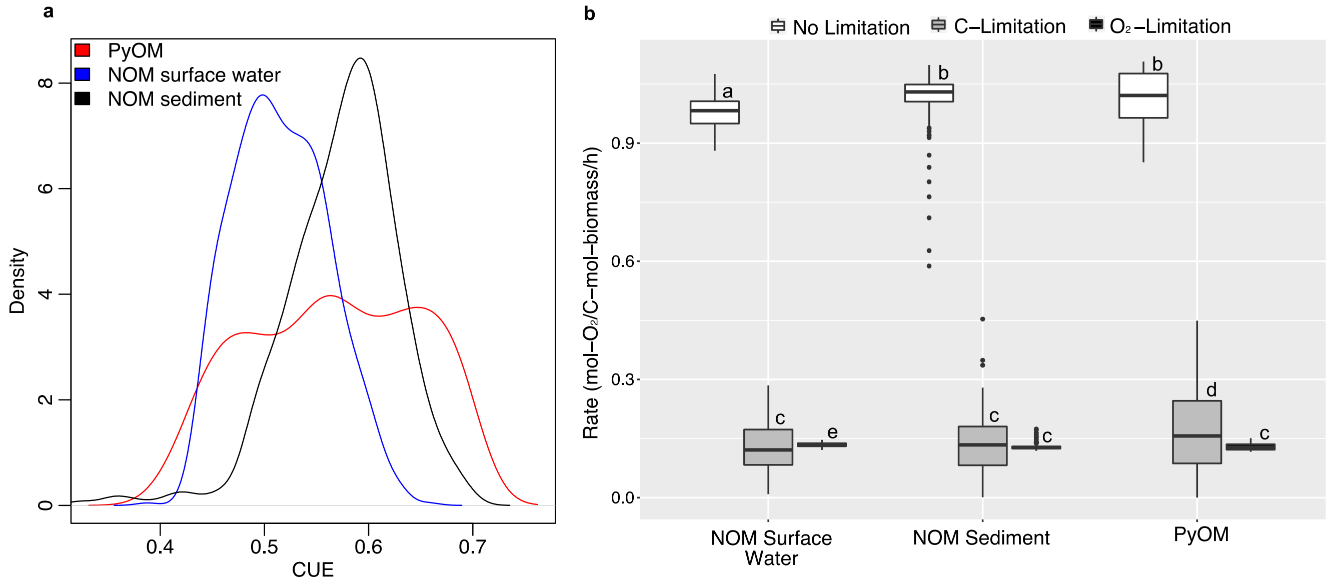

Figure 2. Carbon use efficiency (CUE) and predicted metabolism of NOM and PyOM. (**a**) shows the distribution of CUE in PyOM (red) and ubiquitous surface water (blue) and sediment (black) NOM molecules rates. It shows a probability density function (PDF) that reflects the relative likelihood that value 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 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

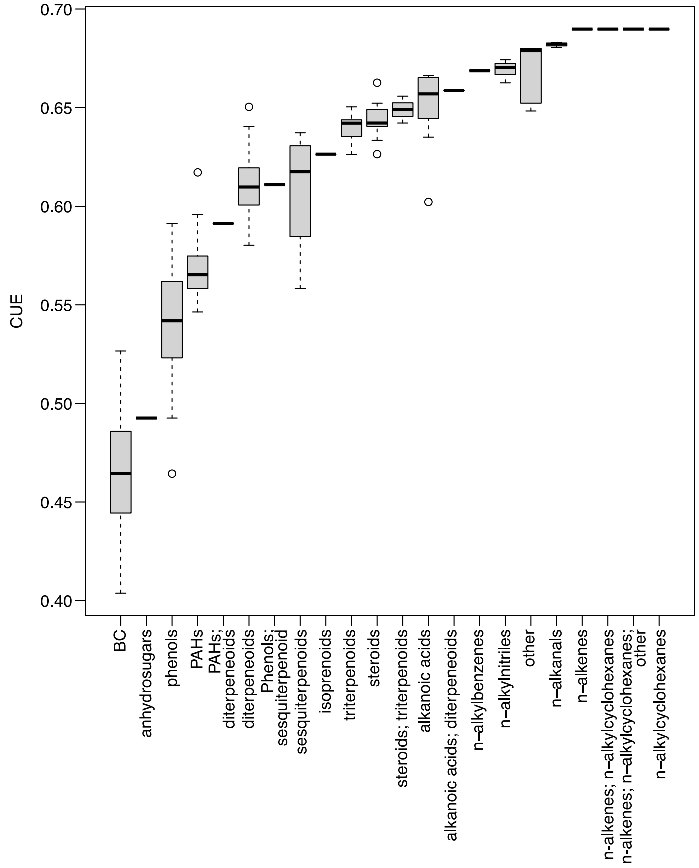

Figure S1. Carbon use efficiency (CUE) of PyOM, grouped by known chemical properties. High CUE values indicate more C incorporated into biomass vs. respired per unit C. Median values are denoted by a bar, the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles),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

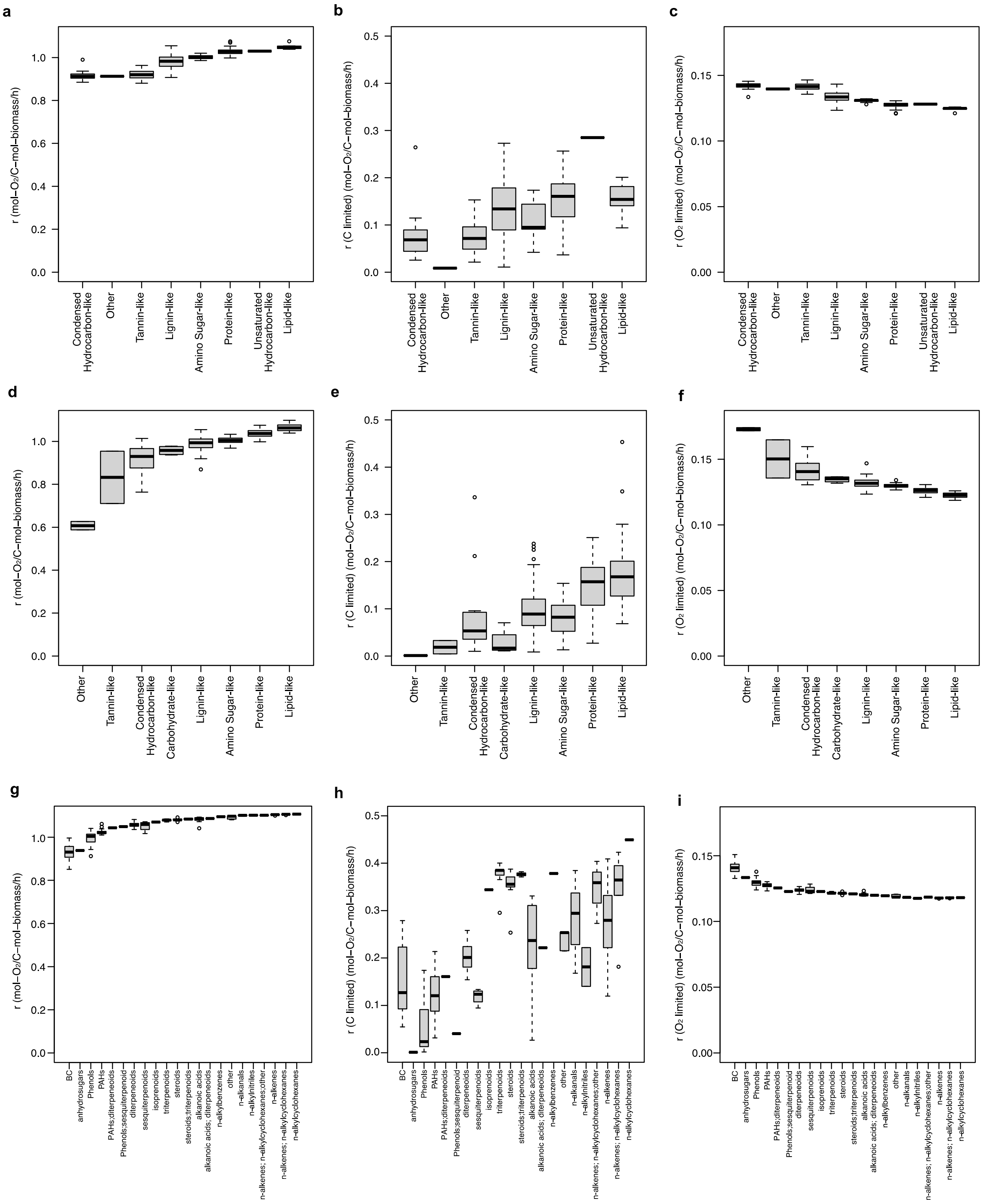

Figure S2. Predicted metabolism of ubiquitous (a–c) surface water NOM, (d–f) sediment NOM, and (g–i) PyOM molecules, grouped by inferred chemical class or known chemical properties. The first column shows predicted rates of aerobic metabolism without any elemental limitations.

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.

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