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Microbial Transformation of Dissolved Leaf Litter Organic Matter and Its Effects on Selected Organic Matter Operational Descriptors

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Changes in selected spectroscopic and chromatographic characteristics of water-soluble organic matter (WSOM) extracted from leaf litter and its ability to bind pyrene were monitored throughout 14 day microbial incubation experiments. To provide additional insight into the microbial transformation of the WSOM, incubation experiments were similarly conducted with controlled-composition mixtures of glucose and dissolved humic substances (HS) that were base extracted from the same leaf litter source. Microbial transformation increased the specific ultraviolet absorbance and number-average molecular weight of residual WSOM while polydispersity values decreased. Fluorescence measurements revealed loss of protein-like fluorescence and enhancement of fulvic- and humic-like fluorescence in residual WSOM. Overall, the incubation results suggest that nonaromatic and smaller sized carbon structures were being degraded while the microbial activity produced humic-like aromatic components in solution. Together, these changes resulted in enhanced pyrene binding by the altered WSOM. Consistent findings resulted from mixtures of glucose and the leaf litter HS. Changes in measured operational descriptors were more pronounced for mixtures containing a higher percentage of glucose, suggesting that utilization of labile constituents may be necessary for formation of unknown structures associated with high pyrene binding capabilities. Simple mass balance, end member mixing models often failed to predict changes in pyrene binding brought about by microbial transformation, suggesting that microbial utilization of labile constituents is not the predominant process governing the enhanced pyrene binding.

1. Introduction

Ubiquitous in natural waters, dissolved organic matter (DOM) is a heterogeneous mixture composed of carbohydrates, proteins, lignins, organic acids, and various other less characterized compounds such as humic substances (HS) (*1*). Temporal and spatial variations in DOM properties typically observed in aquatic environments are attributed to different sources of DOM, their respective mixing processes, and natural transformation processes such as mineral sorption, photodegradation, and biodegradation (*2*). Allochthonous DOM in surface waters is primarily derived from terrestrial carbon sources including plant- and soil-leached organic materials. Once such terrestrial carbon sources enter aquatic systems, they undergo various transformation processes that can alter the original DOM characteristics and reactivities. For example, recent studies have revealed the interactive roles and complex effects of natural transformation processes on DOM characteristics and reactivities such as enhanced bioavailability of plant-derived DOM upon photodegradation (*3, 4*), microbial degradation of lignin residues in DOM (*5*), and variations in DOM composition upon transformation by different microbial communities (*6*). Another excellent example is the Ogeechee River in Georgia, which has been the subject of many DOM studies over the past 20−25 years (*7*−*11*).

In aquatic environments, hydrophobic organic contaminants (HOCs) can partition to DOM, which alters HOC transport, bioavailability, toxicity, and ultimate fate. Selected physicochemical properties of DOM are known to be critical factors in determining the magnitude of HOC partitioning. For example, DOM having a higher molecular weight and more aromatic and/or aliphatic carbon tends to exhibit a higher organic carbon normalized binding coefficient (K_{oc}) for HOCs (*12*−*14*). Despite the numerous studies that have been conducted of HOC−DOM interactions, however, relatively little information is available to understand how natural transformation processes of DOM affect K_{oc} values. Previous studies often have utilized different sources of DOM to investigate correlations between DOM characteristics and HOC binding reactivities. Only a few studies, however, have examined the heterogeneous nature of single DOM sources and how natural alteration processes such as adsorption to mineral surfaces (*14, 15*) or photodegradation (*16*) may subsequently change HOC binding.

Despite our knowledge of the important role played by microorganisms in the transformation of DOM in natural waters, little attention has been paid to how microbial transformation affects HOC partitioning. In terms of bioavailability, DOM constituents can be classified broadly into two compartments depending on their general *labile* vs *refractory* characteristics. The refractory compartment is thought to be composed of HS constituents and/or very large macromolecules present within a DOM, whereas the biodegradable compartment is generally considered to be composed of smaller molecules or larger nonhumic DOM constituents (e.g., carbohydrates, proteins) (*1, 17*). Consequently, preferential utilization of the latter is expected during microbial degradation (*18*). Other studies have shown, however, that simple division of DOM into humic versus nonhumic fractions and/or high- versus low-molecular weight fractions cannot fully explain the changes that occur in the compositions and characteristics of DOM during microbial transformation reactions (*19*−*21*). Therefore, changes in DOM physicochemical properties due to microbial activity may be better explained as a result of more complicated processes—that is, a combination of preferential microbial utilization, enzymatic alteration, production of microbial and transformation products, etc. Additional factors that likely play a role in the microbial transformation of DOM are the original DOM source, concentration, and composition as well as the particular microorganism species and/or communities present (*20, 22, 23*).

The objectives of this study were to (1) monitor changes in physicochemical characteristics of a terrestrial source DOM

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TABLE 1. Effects of Microbial Transformation on Leaf Litter WSOM Characteristics

incubation time (days)	[DOC][a] (mg of C/L)	$SUVA_{280}$[b] [L/(mg of C m)]	MW_w	MW_n	polydispersity	FLF/PLF[c]	HLF/FLF[c]	FLF/DOC[b]	HIX[c]	$K_{oc} \times 10^{-3}$ [a] (mL/g of C)
0	30.8 (0.1)	1.33 (0.04)	2588	434	5.96	1.33 (0.04)	0.36 (0.01)	4.41 (0.09)	3.14 (0.09)	5.0 (0.7)
2	18.1 (0.1)	2.29 (0.06)	3263	705	4.62	1.74 (0.05)	0.43 (0.01)	6.46 (0.13)	4.39 (0.12)	13.0 (1.1)
5	15.5 (0.1)	2.42 (0.06)	2859	863	3.31	2.63 (0.07)	0.59 (0.02)	5.38 (0.11)	5.57 (0.16)	16.6 (1.2)
14	12.8 (0.1)	2.76 (0.05)	2597	1092	2.38	4.31 (0.12)	0.63 (0.02)	6.31 (0.12)	11.55 (0.33)	17.1 (1.2)

[a] Numbers in parentheses are standard errors based on triplicate sample measurements. [b] Numbers in parentheses are standard errors based on propagating corresponding value uncertainties. Uncertainties in UV absorption and fluorescence were based on the precision for each spectrometer. [c] Numbers in parentheses are standard errors based on propagating corresponding value uncertainties.

subjected to microbial transformation reactions, (2) examine the influence of microbial-induced changes of the DOM on its ability to bind pyrene, and (3) propose a dominant process controlling the changes in DOM binding of pyrene. To achieve the first two objectives, selected operational descriptors (*14, 24*) were measured prior to and during the microbial transformation of solutions containing DOM extracted from a leaf litter. For the last objective, we hypothesized that preferential microbiological utilization of the nonhumic components of leaf litter DOM would be the dominant process for transforming the DOM. Therefore, our expectation was that pyrene binding normalized by the amount of dissolved organic carbon (DOC) remaining in solution would be enhanced by the preferential removal of the nonhumic components, which were not expected to contribute greatly to the binding of pyrene. To rigorously test this hypothesis, separate microbial incubation experiments were conducted using varying mixtures of glucose and HS extracted by base from the same leaf litter source material. Examination of the operational descriptors was then conducted on these glucose−HS mixtures with and without microbial transformation.

2. Experimental Section

A detailed description of the experimental materials and methods is presented in the Supporting Information. Therefore, only a brief summary is presented here. Leaf litter was extracted separately with water and base and subsequently treated to produce working stock solutions of water-soluble organic matter (WSOM) and HS, respectively. Microbial transformation was conducted on WSOM samples (20−30 mg of C/L) to which inorganic nitrogen and phosphorus had been added to avoid nutrient limitations during the incubations. The microbial innoculum was isolated from a nearby river, and the samples were incubated in the dark at 25 °C for 14 days. Initial analyses of the leaf litter WSOM samples were conducted immediately after the inoculum was added (i.e., time zero). Thereafter, incubation flasks were sacrificed at appropriate sampling times to measure the pH, DOC concentration, ultraviolet (UV)−visible absorption spectrum, fluorescence spectra and excitation−emission matrix (EEM), apparent molecular weight (MW), and extent of pyrene binding on filtered subsamples. Separate control experiments were conducted using the same leaf litter WSOM but without the inoculum to confirm microbial transformation of the organic matter. To provide additional insight into microbial transformation of the leaf litter WSOM, the same incubation experiments were conducted with controlled-composition mixtures of glucose and the leaf litter HS described above.

3. Results and Discussion

3.1. Effects of Microbial Transformation on DOC Concentration, UV Absorption, MW, and Pyrene Binding of Leaf Litter WSOM. Inoculation of leaf litter WSOM with microorganisms resulted in a decrease in the concentration of DOC by ~60% and over a factor of 2 increase in specific ultraviolet absorbance (SUVA) by the end of the 14 day incubation, with most of the changes occurring within the first 2 days (Table 1 and Figure S3a in the Supporting Information). The increasing trend in SUVA is consistent with other previous incubation studies (*25, 26*). The dramatic and rapid changes in DOC concentration and SUVA may be due to the rapid utilization of labile, nonaromatic components of the leaf litter WSOM. Alternatively, it could be the result of rapid microbial transformation of labile compounds that leads to products containing more unsaturated carbon bonds that absorb UV light at 254 nm (*21, 27−29*). For example, Ogawa et al. (*21*) demonstrated that uncharacterized refractory DOM was produced from simple organic substrates such as glucose and glutamate within a 2 day incubation with marine bacteria. Microbial transformation has also been reported for HS, which is often assumed to be relatively resistant to enzymatic oxidation. Using microcosms, Moran and Hodson (*30*) demonstrated that bacterial utilization of aquatic HS resulted in a significant fraction of the total bacterial production. In addition, Hertkorn et al. (*20*) reported increased aromatic acid content in aquatic HS after 3 week microbial incubation studies.

The 14 day microbial incubation of leaf litter WSOM resulted in overall weight-average MW (MW_w) values that first increased but then decreased with time (Table 1). The high overall MW_w value observed after 2 days of incubation appears to have resulted primarily from unknown DOM components of very high MW (i.e., ~20000−30000) (Figure 1a). In contrast, there were consistent increases in the number-average MW (MW_n) and decreases in polydispersity with incubation time (Table 1, Figure S3b, Supporting Information). The relatively low initial MW_n value and high polydispersity of our leaf litter WSOM are consistent with reports of other plant-derived OM samples and lignin derivatives from plants (*31, 32*). The consistent change in molecular size as quantified by overall MW_n values likely reflects the rapid utilization of labile WSOM components, which are expected to be smaller and less aromatic than the more recalcitrant components. Conversely, the overall MW_w values are influenced predominantly by the larger, more recalcitrant structures and are less sensitive to the subtle changes brought about by microbial transformation. The relative difference in size exclusion chromatography (SEC) results before and after the 14 day incubation reveals that small (i.e., MW < 250) UV-absorbing components were preferentially removed from the leaf litter WSOM (Figure 1b). Therefore, the increase in bulk SUVA value with incubation time reported above appears to result from a depletion of the smaller WSOM components and the enrichment of UV-absorbing components with higher MWs. However, it is not clear yet if enrichment of UV-absorbing components resulted from condensation and/or polymerization of smaller sized molecules or from utilization of large, nonaromatic molecules present in the WSOM.

The results described above for our 14 day batch incubations are remarkably consistent with findings previously reported for the microbial processing of DOM in the Ogeechee

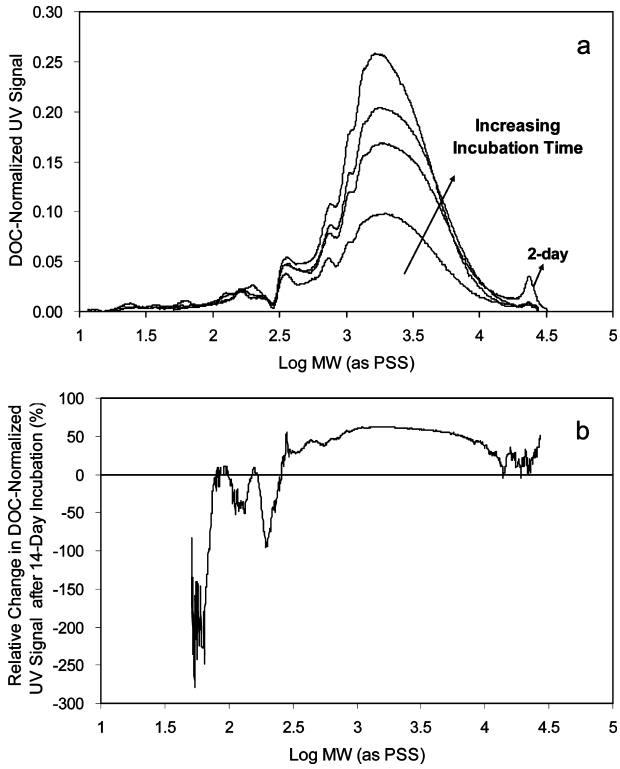

FIGURE 1. (a) Size exclusion chromatograms of leaf litter WSOM during 14 day microbial incubations. (b) Percentage change in the DOC-normalized UV signal (at 254 nm) with molecular weight after 14 day microbial incubation of the leaf litter WSOM.

River in Georgia. For example, Sun et al. (*9*) reported that microbial utilization of carbon decreased with downstream distance along the mainstem of the Ogeechee River. Elemental analysis of the DOM in their samples revealed increases in the O:C ratio and decreases in the H:C ratio with downstream distance. Sun et al. concluded that the decrease in downstream bioavailability of DOM in the Ogeechee River was due to selective degradation of aliphatic carbon in the riverine DOM. With regard to other operational descriptors of the Ogeechee River DOM, both Ryan (*10*) and McNaughton (*11*) reported increasing and then decreasing values of MW_w, increasing values of MW_n, and decreasing values of polydispersity with downstream distance along the mainstem of the river. Other changes in DOM characteristics reported with downstream distance in the Ogeechee River included increases in the percent humic acid and specific absorption coefficient (*10*) and increases in SUVA values (*11*).

Over the 14 day microbial incubation period, pyrene binding by the leaf litter WSOM increased by over 3-fold from its original value (Table 1, Figure S3b, Supporting Information). The same microbial transformation processes that led to an increase in SUVA with incubation time also appear to have contributed to enhancement in the K_{oc} value for pyrene, as evidenced by the very strong positive correlation between SUVA and K_{oc} ($r = 0.977$). A similar finding was previously reported by Guthrie et al. (*33*), who observed that noncovalent binding of pyrene was more evident in HS isolated from a sediment incubated with microorganisms for 60 days compared to the same sediment with no microbial activity. Guthrie et al. also reported that more aromatic and paraffinic structures, both of which are known to be highly associated with pyrene binding, were developed in the sedimentary HS after microbial incubation.

Due to the lack of a consistent trend for WSOM MW_w values with incubation time, no strong correlation was observed between MW_w and pyrene K_{oc} values. This finding contrasts with previous studies that fractionated or altered DOM by ultrafiltration (*14*), mineral adsorption (*15*), or photodegradation (*16*). For these previous *abiotic* studies, positive relationships were obtained between K_{oc} values and MW_w values of the separated or remaining DOM fractions. It seems intuitive to expect that microbial transformation of DOM would be more complex than abiotic processes which merely fractionate (e.g., ultrafiltration, adsorptive fractionation) or primarily degrade (e.g., photolysis) DOM constituents. However, further investigation is needed using a diverse range of microorganisms and DOMs with widely varying physicochemical characteristics to determine whether our present findings can be generalized.

3.2. Effects of Microbial Transformation on the Fluorescence of Leaf Litter WSOM. A three-dimensional fluorescence EEM provides an overall picture of the myriad of fluorescent moieties contained within a bulk DOM and therefore has been utilized frequently to monitor DOM structural and compositional changes (*26, 34*−*37*). Previous studies have recommended monitoring fluorescence at different spectral locations in an EEM to distinguish humic-like, fulvic-like, and protein-like fluorescence (*24, 27, 38*−*41*). Here, the three different fluorescence spectral regions were identified according to Baker (*38*). Thus, protein-like fluorescence (PLF) is represented by the intensity at an excitation wavelength of 275 nm and an emission wavelength of 350 nm, fulvic-like fluorescence (FLF) at 320−340 nm excitation and 410−430 nm emission wavelengths, and humic-like fluorescence (HLF) at 370−390 nm excitation and 460−480 nm emission wavelengths (Figure 2).

Substantial changes in fluorescence characteristics were observed with microbial incubation time for the leaf litter WSOM (Table 1, Figures 2 and S3c,d, Supporting Information). The PLF peak of the original WSOM decreased, while the FLF peak and particularly the HLF peak were both enhanced after the incubation. In addition, the location of the FLF peak was red-shifted. Previous reports have shown that fluorescence shifts to longer wavelengths are generally associated with enrichment of more condensed aromatic structures having electron-withdrawing substituents and/or more conjugation of aliphatic chains (*24, 42, 43*). Similar changes in fluorescence have been reported in other incubation studies using DOM from sources such as plants (*44*), wastewater treatment plant effluents (*26*), seawater (*36*), and algae (*34*). Despite the similarity of overall trends, however, the specific details of fluorescence changes are likely dependent on factors such as incubation conditions (*41, 42*), the types of substrate (*34*), and the type of DOM (*25*). For example, Elliott et al. (*35*) observed distinctive differences in fluorescence EEMs from a single type of bacterium grown under different temperatures.

More condensed aromatic structures may be formed upon utilization of labile compounds by microorganisms. For example, Ogawa et al. (*21*) demonstrated that utilization of simple labile substrates (glucose and glutamate) by marine bacteria produced uncharacterized, refractory organic materials that were resistant to further microbial degradation for over 1 year. However, it is unknown whether the reduction of PLF observed here is directly associated with the increase of FLF and the red-shifted fluorescence EEM. For example, Biers et al. (*34*) reported that fluorescent and chromophoric DOM was formed by the microbial degradation of amino-sugars whereas nonfluorescent refractory DOM was produced by microbial degradation of tryptophan, which is often considered the primary source of PLF. It cannot be ruled out that the disappearance of PLF observed here relates to rapid oxidation of tannin-like structures that generate fluorescence in the PLF region, because these structures are common components of organic materials from plant leachates (*45*).

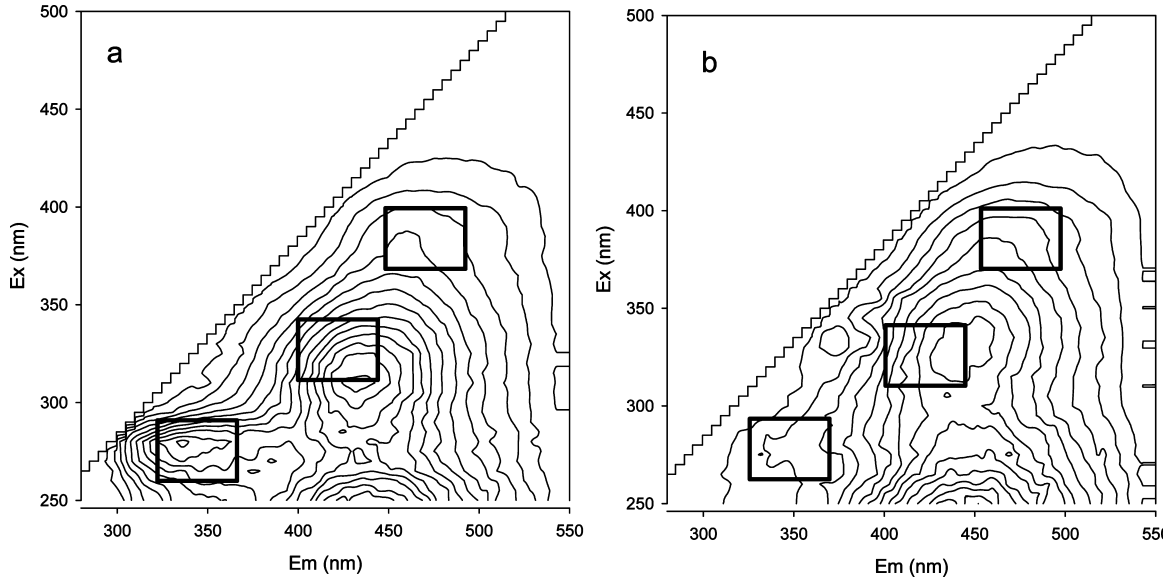

FIGURE 2. Fluorescence contour plots from the leaf litter WSOM: (a) prior to microbial transformation, (b) after the 14 day microbial incubation period. The boxes highlight the regions of PLF, FLF, and HLF, respectively, from left to right as described in the text.

A variety of humification indices (HIX) for DOM have been proposed based on intensities, regions, etc. in fluorescence emission or synchronous spectra. Many of these indices are based on observations of a shift in the maximum fluorescence peak to longer wavelengths for more humified DOM. Higher values of HIX reportedly are associated with a higher degree of aromaticity, lower rate of mineralization, and lower percentage of oxygen-containing functional groups for soil- and litter-derived DOM (*25*). Here, the HIX fluorescence quotient suggested by Zsolnay et al. (*46*) was used to monitor compositional changes within the leaf litter WSOM. This particular HIX is calculated from fluorescence spectra as the ratio of emission at 300−345 nm to that measured at 435−480 nm when using an excitation wavelength of 254 nm. After the 14 day microbial incubation, values of HIX for the WSOM increased from 3.1 to 11.6 (Table 1, Figures S3d, Supporing Information), consistent with the increase in SUVA.

The DOC-normalized FLF increased from 4.4 to 6.3 over the 14 day incubation period, although the increase was not a consistent trend with incubation time (Table 1, Figure S3d, Supporting Information). The overall increase in FLF/DOC suggests that microbial transformation reactions were producing fluorescent products and/or were preferentially removing nonfluorescent components in the WSOM.

3.3. Effects of Microbial Transformation on Glucose−HS Mixtures. Microbial transformation reactions were conducted with various glucose−HS mixtures and operational descriptors monitored with time in the same manner as described above for the leaf litter WSOM. Decreases in DOC concentration with time were more pronounced for the mixtures containing higher percentages of glucose (Table 2). For example, 85% of the initial DOC disappeared after 14 days for pure glucose solutions, whereas the decrease in DOC was only 15% for the leaf litter HS solutions. This dramatic difference clearly indicates the preferential utilization of glucose over HS by the microorganisms.

With the exception of MW_n and the ratio of HLF to FLF, values of the operational descriptors increased consistently with incubation time irrespective of the initial glucose−HS mixture ratio (Table 2). Values of MW_n tended to first increase and then decrease, while HLF/FLF values generally increased but not always in a consistent manner at intermediate times. Consistent with the findings above for leaf litter WSOM, the major changes in DOM-associated characteristics again occurred within the first 2 days followed by continued but smaller changes after that. As with DOC concentration, the relative rate of change in the operational descriptors was more pronounced for mixtures containing a higher percentage of glucose. For example, the relative difference in pyrene K_{oc} values before and after microbial incubation was 7% for the litter HS but was over 120% and 450% for the mixtures with 50% and 75% glucose, respectively. Note that the relative increase in the pyrene K_{oc} value could not be calculated for the pure glucose solution because the original solution did not quench the fluorescence of pyrene and thus its partitioning prior to microbial incubation could not be calculated. However, it can be inferred that the enhancement in K_{oc} was the highest for this particular starting solution.

The dramatic increase in the pyrene K_{oc} for the pure glucose solution after incubation can likely be attributed to production of hydrophobic DOM constituents by the microorganisms (*21*), which is consistent with our other spectroscopic and chromatographic results. For example, the DOC-normalized FLF of glucose solutions increased after incubation (Table 2), while SEC chromatograms revealed that UV-absorbing constituents were generated with MWs ranging from a few hundred up to as high as 12 000 (Figure S4a in the Supporting Information). The presence of readily biodegradable organic matter appears to be a prerequisite for the formation of the high-MW, UV-absorbing components because the new SEC peaks from glucose solutions were not observed in the leaf litter HS solutions (Figure S4d). Recall that all solutions received the same amounts of N and P, and thus, no solution would have been inorganic nutrient limited. Our incubation results for the glucose−HS mixtures are also consistent with previous studies that reported production of high-MW and humic-like DOM upon the bacterial utilization of labile compounds such as simple carbohydrates and amino acids (*27*−*29*).

It is important to note that the SUVA values measured for the glucose solutions were all relatively low, even after microbial incubation. This suggests that the products formed from glucose utilization had mostly nonaromatic carbon structures. The fact that these low-UV-absorbing constituents had K_{oc} values approaching those of the leaf litter HS further suggests that at least some of the glucose products had appreciable hydrophobicity, perhaps due to aliphatic carbon moieties which led to enhanced pyrene partitioning. Similar microbial products likely were also being generated in the other glucose−HS mixtures although the magnitude of change in the operational descriptors was clearly not as

TABLE 2. Effects of Microbial Transformation on Glucose−HS Mixtures

incubation time (days)	[DOC][a] (mg of C/L)	$SUVA_{280}$[b] [L/(mg of C m)]	MW_n	FLF/PLF[c]	HLF/FLF[c]	FLF/DOC[b]	$K_{oc} \times 10^{-3}$ [a] (mL/g of C)
			Glucose				
0	29.0 (0.5)	0.08 (0.00)	145	0.50 (0.00)	0.44 (0.05)	0.16 (0.00)	NA[d]
2	6.7 (0.2)	0.19 (0.00)	330	0.90 (0.03)	0.58 (0.02)	3.51 (0.08)	18.1 (0.9)
5	4.7 (0.0)	0.38 (0.01)	322	1.29 (0.04)	0.34 (0.01)	9.74 (0.20)	10.9 (2.9)
14	4.3 (0.1)	0.30 (0.01)	250	1.78 (0.05)	0.66 (0.02)	3.67 (0.08)	31.7 (3.9)
			Litter HS (25%)				
0	29.8 (0.6)	0.66 (0.02)	1214	1.36 (0.07)	0.46 (0.04)	0.99 (0.03)	7.5 (0.7)
2	8.3 (0.1)	2.68 (0.06)	1708	4.09 (0.21)	0.39 (0.06)	8.11 (0.18)	44.7 (1.8)
5	9.6 (0.1)	2.21 (0.05)	2046	3.23 (0.16)	0.44 (0.01)	6.68 (0.15)	47.5 (2.0)
14	8.6 (0.1)	2.32 (0.05)	2185	2.94 (0.15)	0.66 (0.02)	4.74 (0.11)	41.6 (1.4)
			Litter HS (50%)				
0	30.6 (0.6)	1.22 (0.03)	1568	1.48 (0.04)	0.48 (0.01)	1.78 (0.05)	18.4 (0.2)
2	14.0 (0.2)	3.09 (0.07)	1992	3.25 (0.09)	0.49 (0.01)	6.29 (0.14)	51.7 (2.0)
5	15.1 (0.2)	2.75 (0.06)	2138	4.29 (0.12)	0.56 (0.02)	5.46 (0.12)	50.8 (0.2)
14	14.0 (0.2)	2.98 (0.07)	1963	7.23 (0.21)	0.64 (0.02)	5.85 (0.13)	41.0 (0.3)
			Litter HS (100%)				
0	21.7 (0.6)	2.68 (0.09)	1880	1.93 (0.05)	0.43 (0.01)	2.67 (0.14)	46.6 (1.4)
2	18.5 (0.2)	3.06 (0.07)	1396	3.40 (0.10)	0.48 (0.01)	6.41 (0.15)	49.5 (1.5)
5	18.3 (0.3)	3.03 (0.07)	1507	4.75 (0.13)	0.61 (0.02)	4.58 (0.11)	54.3 (0.5)
14	18.5 (0.2)	3.23 (0.07)	1533	9.23 (0.26)	0.62 (0.02)	5.86 (0.14)	50.0 (1.6)
			Inoculum Only				
0	1.2 (0.1)	0.19 (0.01)	611	0.55 (0.02)	0.46 (0.01)	1.63 (0.16)	ND[e]
14	2.8 (0.1)	0.25 (0.02)	306	0.62 (0.02)	0.44 (0.01)	1.06 (0.06)	ND

[a] Numbers in parentheses are standard errors based on triplicate sample measurements. [b] Numbers in parentheses are standard errors based on propagating corresponding value uncertainties. Uncertainties in UV absorption and fluorescence were based on the precision for each spectrometer. [c] Numbers in parentheses are standard errors based on propagating corresponding value uncertainties. [d] NA = not applicable. Fluorescence of pyrene was enhanced, not quenched, in the 100% glucose solution prior to microbial transformation. [e] ND = not determined.

dramatic as for glucose alone (Table 2). However, alteration of HS structures has been reported previously (*20, 47*).

To further test the simple labile vs refractory two-component hypothesis above, predictions of the time-dependent DOC concentrations in the 75−25% and 50−50% glucose−HS mixtures were made using a mass-balance-based end member mixing model. As described in more detail in the Supporting Information, the model assumes glucose (labile) and leaf litter HS (refractory) are component end members whose microbial utilizations are independent of one another. Therefore, their respective changing DOC concentrations with time can be used together with an overall mass balance from the initial mixture composition to predict total DOC concentrations of the intermediate mixtures with incubation time. As shown in the Supporting Information (Figures S5a and S6a), generally good agreement was obtained between the predicted and measured DOC concentrations of the intermediate glucose−HS mixtures. The small differences observed (i.e., 1.5 and 0.5 mg of C/L for the mean difference and standard error, respectively, $n = 6$) suggest that the assumptions of the simple model were reasonable for explaining microbial utilization of glucose and HS in the sample mixtures. The successful predictions of DOC concentration also then enabled us to make estimates of the glucose and HS concentrations present in each mixture at different incubation times (Figures S5b and S6b).

On the basis of the relative success in predicting DOC concentrations and the subsequent compositions of glucose−HS mixtures with incubation time, an attempt was made to predict the operational descriptors of the same sample mixtures using mass balance. In other words, if the microbial-induced changes in the mixtures were primarily governed by utilization of the labile component of the mixture, then the measured values should approximate those predicted from the component ratios and end member properties. As described in the Supporting Information, various underlying assumptions can be made which lead to three slightly different predictive models for the time-dependent operational parameters. The simplest model essentially repeats the process described above for predicting DOC concentrations. A slightly more complex model accounts for the varying composition of the mixtures together with the regression equations shown in Table S1 in the Supporting Information. The third model essentially combines the features of the first two models.

Comparison of measured vs predicted operational descriptor values revealed that such simple mass-balance-based end member mixing models were less satisfactory for predicting SUVA, DOC-normalized FLF, MW_n, and pyrene K_{oc} at different incubation times than they were for predicting DOC concentration (Figures S5c−f and S6c−f, Supporting Information). These findings suggest that processes in addition to the simple utilization of glucose by microorganisms are important for the measured operational descriptors of sample mixtures. Similarly, the findings suggest that glucose and HS utilizations cannot be considered independent of one another, consistent with the previous report by Hertkorn et al. (*20*).

3.4. Predicting Pyrene Partitioning from other DOM Operational Descriptors Measured Prior to and during Microbial Transformation. Values of SUVA have been shown previously to be correlated with partitioning of HOCs to a variety of DOMs, as well as for constituents fractionated from a single DOM source (e.g., refs *14* and *15* and references therein). Therefore, despite the relatively limited range of values observed here from the different sample microbial incubations, we examined predictions of pyrene partitioning for both the WSOM samples and glucose−HS mixtures based on their SUVA values measured at different incubation times. As can be seen (Figure 3a), measured K_{oc} values for glucose−HS mixtures were not greatly different from those predicted from the regression developed between SUVA and

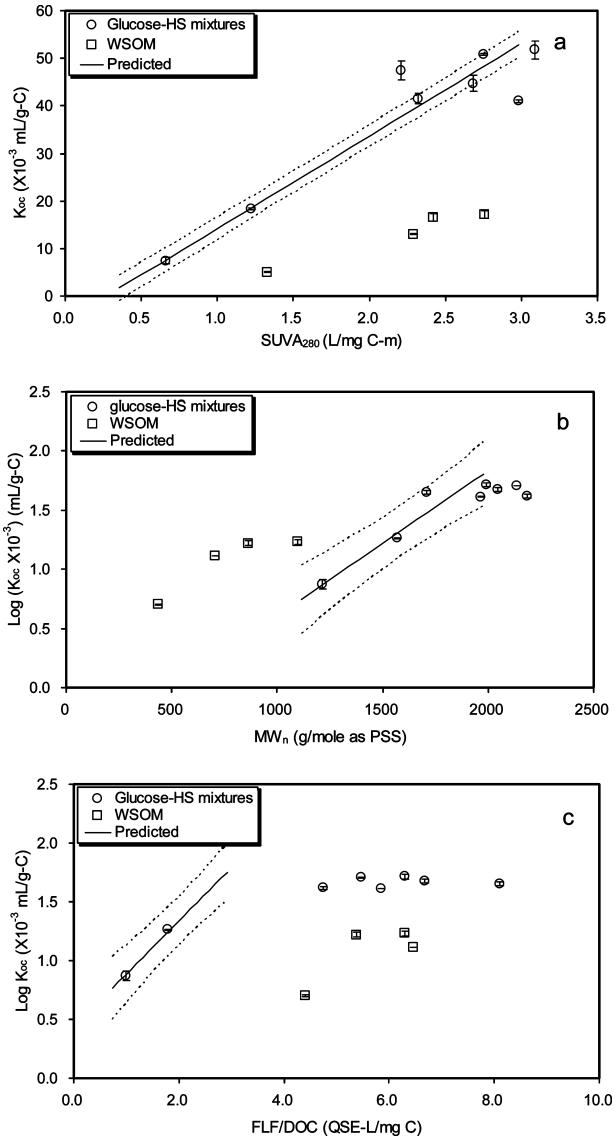

FIGURE 3. Prediction of pyrene K_{oc} values from other selected operational parameters measured at different incubation times using the regression equations shown in Table S1 (Supporting Information): (a) SUVA, (b) MW_n, (c) DOC-normalized FLF. Each prediction line shown spans the approximate range of operational parameter values used to derive the regression equations. The dotted lines indicate the 95% prediction intervals.

pyrene K_{oc} prior to microbial transformation (Table S1, Supporting Information). However, predicted K_{oc} values were more different for the WSOM samples. In all cases, pyrene partitioning to the leaf litter WSOM fell below the prediction line, which may reflect the relatively nonaromatic molecules produced from microbial transformation of glucose that were nevertheless capable of interacting with pyrene.

Predictions of pyrene partitioning based on measured MW_n also led to reasonably good results for the glucose−HS mixtures but less so for the WSOM samples (Figure 3b). In contrast with the SUVA-based predictions, the WSOM samples exhibited pyrene partitioning higher than that predicted by the regression from Table S1 (Supporting Information). Lastly, predictions of pyrene partitioning based on the DOC-normalized FLF regression line did not lead to good agreement with measured values (Figure 3c), with nearly all of the samples undergoing microbial transformation falling to the right of the prediction line and below its extrapolation. Therefore, on the basis of the present results, we conclude that FLF/DOC will be a less useful parameter for monitoring the microbial transformation of DOM with regard to its subsequent ability to interact with HOCs. However, further investigation is needed using a much wider range of microbial-altered DOM materials to determine whether the present findings can be generalized to other biogeochemical systems.

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Supporting Information Available

Detailed experimental description, representative experimental results for samples and controls, description of relationships among operational descriptors for glucose−HS mixtures prior to microbial transformation, description of the mass-balanced-based end member mixing models for intermediate glucose−HS mixtures, and comparison of predicted to measured results for DOC concentration and various operational descriptors as a function of microbial incubation time. This material is available free of charge via the Internet at http://pubs.acs.org.

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