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An Assessment of Dissolved Organic Carbon Biodegradability and Priming in Blackwater Systems

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Abstract Rivers deliver approximately 260 Tg of dissolved organic carbon (DOC) to the ocean annually, yet there is little evidence of terrigenous DOC (tDOC) in the ocean. While tDOC was historically believed to be stable and resistant to microbial degradation, it has recently been shown that freshwater systems mineralize more tDOC than originally thought. The priming effect is a possible mechanism by which inputs of biolabile DOC enhance the bioavailability of stable DOC components in aquatic systems, resulting in higher rates of microbial remineralization. Here we investigate tDOC biodegradability by conducting bioincubation experiments and utilizing ultrahigh resolution mass spectrometry to characterize the chemical composition of blackwater and leachate dissolved organic matter (DOM) samples. The role of priming in blackwater ecosystems was assessed through the inclusion of bioincubation treatments amended with a variety of simple biolabile OC substrates. Blackwaters lost 6.10 ± 3.85% DOC within 1 month, while leachates lost 38.10 ± 16.74% DOC. There were no significant differences between DOC remineralization in control and primed treatments, indicating that priming is not an important factor in the biodegradation of DOC in blackwater ecosystems. However, the proportion of biodegradable DOC and DOM composition were significantly correlated, mostly driven by the contribution of aliphatic compounds ($H/C \geq 1.5$, $O/C < 0.9$) that were abundant (9.3 ± 5.2%) in leachate DOM. The molecular signature of biodegraded leachate DOM resembled that of stable blackwater DOM, indicating that bioavailable DOM components leached from plant litter are rapidly utilized and stable DOM is exported downstream.

Key Points:
- The priming effect is unlikely to increase microbial remineralization of dissolved organic carbon in blackwater systems
- Minimal dissolved organic carbon degradation and compositional changes occurred in blackwaters while leachate dissolved organic matter was highly bioavailable
- Dissolved organic carbon biodegradability is controlled by chemical composition, particularly the contribution of aliphatic compounds

Supporting Information:
- Supporting Information S1

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(continued)

1. Introduction

Riverine transport of terrigenous dissolved organic carbon (tDOC) is an important link in the global carbon cycle, delivering approximately 260 Tg of dissolved organic carbon (DOC) to the ocean each year (Raymond & Spencer, 2015). Although the flux of tDOC exported from rivers is enough to account for the total turnover time of marine DOC (Hedges et al., 1997), biomarker and stable isotopic data show little evidence of tDOC in the ocean (Benner, 2002; Bianchi, 2011; Hedges et al., 1997). In addition, tDOC was historically thought to be stable and resistant to degradation by microbial communities, yet it has recently been shown that freshwater systems mineralize more DOC than originally thought (Bianchi, 2011; Cole et al., 2007; Ward et al., 2013). Inland waters are now generally accepted to be major sites of terrestrial carbon processing (Bianchi, 2011; Cole et al., 2007), contributing significant carbon dioxide (CO_2) flux to the atmosphere (Drake et al., 2017). As a result, in recent years, research in both soil and aquatic sciences has been directed toward possible mechanisms that contribute to the lack of tDOC in the ocean (Spencer et al., 2009; Stubbins et al., 2010; Ward et al., 2013).

A number of studies focused on soil organic carbon have highlighted that stable organic carbon substrates are degraded by microorganisms upon moderate inputs of biolabile substrates (Bingemann et al., 1953; Guenet et al., 2012; Kuzyakov, 2010), a process termed the priming effect (Bingemann et al., 1953). More recently, it has been suggested that priming may substantially influence carbon cycling in aquatic ecosystems (Bianchi, 2011; Guenet et al., 2014; Hotchkiss et al., 2014). Such studies have highlighted that the priming effect may play a role in altering decay rates of riverine DOC by enhancing the bioavailability of stable DOC components (Guenet et al., 2010). Thus, priming has the potential to increase the susceptibility of tDOC to remineralization as CO_2, contributing to the net heterotrophy of riverine systems and the fate of tDOC in the ocean (Battin et al., 2008; Bianchi, 2011). However, the priming effect

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has only recently received scrutiny and results of the limited studies to date have been highly discordant. While some studies have reported significant aquatic priming effects using various OC sources (Bianchi et al., 2015; Guenet et al., 2014; Hotchkiss et al., 2014; Steen et al., 2016), a nearly equal volume of literature argues that priming is not relevant in aquatic systems (Bengtsson et al., 2014; Blanchet et al., 2017; Catalán et al., 2015; Dorado-García et al., 2016). This ongoing debate highlights the need for more thorough investigations of the priming effect across multiple aquatic ecosystems.

Within freshwater ecosystems, blackwaters consist of a diverse mixture of organic substances and are ideal study sites for assessing biodegrability of tDOC and priming processes as they are rich in organic matter (i.e., high DOC concentrations), dominated by dissolved organic matter (DOM) that is highly aromatic in nature, and are historically believed to be a stable DOM pool (i.e., low reactivity, Hopkinson et al., 1998; Leff & Meyer, 1991; Moran et al., 1999; Sun et al., 1997). A large contributor to blackwater DOM is fresh allochthonous DOM leached from fallen vegetation that is rapidly utilized by heterotrophic bacteria, leaving refractory material behind (Cleveland et al., 2004; Pereira et al., 2014). Thus, vegetation leachates provide an opportunity to study biodegradability and priming of relatively bioavailable tDOC, while blackwaters provide an opportunity to study bioavailability and priming of biostable tDOC. Due to the characteristically high concentration and relatively low biolability of blackwater DOC, a priming effect could be easily detectable in blackwater systems since microbial interactions with the stable DOM pool would be more apparent. However, no experimental studies of the priming effect have been undertaken explicitly on blackwater ecosystems despite their importance in biogeochemical transformations of terrigenous DOM and potential to contribute CO_2 fluxes to the atmosphere (Battin et al., 2008; Drake et al., 2017; Tranvik et al., 2009) especially in the face of global change (Bianchi, 2011; Guenet et al., 2010; Mayorga et al., 2005).

This study investigates DOC biodegradability in blackwater ecosystems by conducting bioincubation experiments to measure the extent and rate of microbial utilization of DOC in five blackwater ecosystems and five vegetation leachates. Leachates were made from representative vegetation litter collected at each of the sites to mimic natural DOM leached from plant litter that is laterally transported to the blackwater system. To investigate the role of priming, we included treatments amended with a variety of simple biolabile organic carbon substrates. We determined the presence or absence of priming by comparing DOC loss through biodegradation in control and primed bioincubation samples and used a three-parameter exponential decay model to evaluate rates of DOC loss. The biodegradability assessment was complemented with characterization of background DOM composition using ultrahigh-resolution mass spectrometry. Specifically we applied electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR MS) to derive molecular signatures of DOM composition across blackwater sites and leachates to link initial DOM composition to biolability throughout the bioincubation experiments.

2. Materials and Methods

2.1. Site Description and Sample Collection

Sample sites include four blackwater ecosystems in northwest Florida, namely the Ochlockonee River, Sopchoppy River, Aucilla River, and Suwannee River, and a blackwater swamp in southwest Florida that is situated in the Corkscrew Regional Ecosystem Watershed (CREW, Figure 1). The climate in the sample region is subtropical to temperate and humid with warm temperatures year round. There are two distinct seasons, wet (May through October) and dry (November through April), based on average temperatures and precipitation (Black, 1993).

Statewide average monthly temperature and precipitation for the wet season are 25 °C and 142 mm (14–33 °C and 88–196 mm), respectively, while the cooler dry season experiences average temperature and precipitation of 14 °C and 108 mm (4–27 °C and 78–151 mm), respectively. The Suwannee River samples and Suwannee vegetation litter were collected during the wet season, while sampling for the other four sites occurred during the dry season (Table 1). Terrestrial blackwater ecosystems consisted of mixed pine flatwood and swamp communities dominated by sweetbay (*Magnolia virginiana)*, red maple (*Acer rubrum*), bald cypress (*Taxodium distichum)*, slash pine (*Pinus elliotti*), live oak (*Quercus virginiana*), and saw palmetto (*Serenoa repens*).

At each site, 4 L of blackwater were collected in 1-L acid-washed polycarbonate bottles and stored in the dark at 4 °C. The samples were filtered through precombusted (450 °C $>$ 5 hr) GF/F (0.7 μm) glass microfiber filters

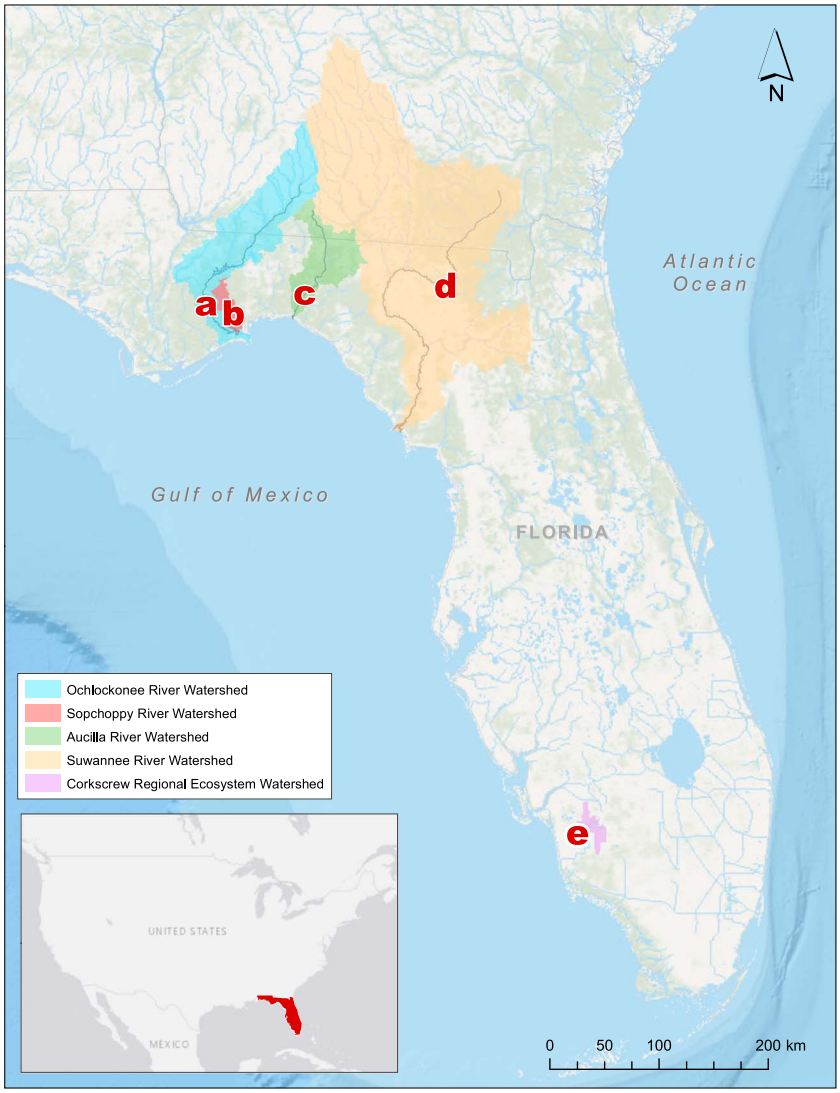

Figure 1. Blackwater sample site locations in Florida, United States of America. The (a) Ochlockonee River, (b) Sopchoppy River, (c) Aucilla River, and (d) Suwannee River are blackwater rivers in northwest Florida. The site in southwest Florida (e) is a blackwater swamp in the Corkscrew Regional Ecosystem Watershed (CREW).

with a peristaltic pump (Cole and Parmer, Masterflex L/S) upon return to the laboratory (within 3 hr of sample collection). Adjacent to where each blackwater sample was taken, a representative mix of the surface vegetation litter was collected from the ground just above the highest water line and stored in the dark at 4 °C until subsequent leaching.

Table 1
Site Information

Site	Site coordinates	Sampling date	Season
Suwannee River	30°19′38.3″N, 82°44′14.2″W	16 May	W
Sopchoppy River	30°10′13″N, 84°29′54″W	16 Nov	D
Aucilla River	30°15′18.6″N, 83°53′48.4″W	17 Jan	D
Ochlockonee River	30°10′37.6″N, 84°40′06.2″W	17 Mar	D
CREW freshwater swamp	26°18′57.0″N, 81°37′51.0″W	17 Apr	D

Note. W = wet season; D = dry season; CREW = Corkscrew Regional Ecosystem Watershed.

2.2. Vegetation Leachates

Prior to leaching, vegetation litter was dried at 50 °C for 24 hr. Fifty grams of dry material was added to 5 L of 0.001N $NaHCO_3$ and leached in the dark on a stir plate for 24 hr at 20 °C. Sodium bicarbonate ($NaHCO_3$) serves as a pH buffer and simulates the ionic strength of the sampled systems (Spencer et al., 2008; Wickland et al., 2007). Leachates were filtered through precombusted (450 °C > 5 hr) GF/F (0.7 μm) glass microfiber filters using a peristaltic pump (Cole and Parmer, Masterflex L/S).

2.3. Bioincubation Experiment Setup

For each of the five sample sites, two suites of bioincubation experiments were performed; one suite utilized the blackwater samples, and the other suite used the leachate samples. Following filtration, the same protocol was used for both the blackwater samples and the leachate samples. The DOC concentration of each sample type was obtained from a total organic carbon analyzer before amendment, as described in section 2.4. Bulk blackwater or leachate samples (3 L) were amended with a 10% inoculum (Fellman et al., 2010; Vonk et al., 2015) of the natural blackwater microbial community, obtained from filtering blackwater samples through precombusted (450 °C > 5 hr) GF/D (2.7 μm) glass microfiber filters (Guillemette & del Giorgio, 2011).

Two controls served as a reference for background microbial activity, an unamended control and a control amended with potassium nitrate (KNO_3) and sodium phosphate (Na_3PO_4) in a 106 C:32 N:2 P proportion. The proportion is representative of double Redfield molar ratios of nitrogen and phosphorus to ensure that these nutrients were not limiting factors during the bioincubations. Three treatment groups were amended with the same proportion of nitrate and phosphate along with a different biolabile carbon substrate (i.e., primers): acetate ($C_2H_9NaO_5$), glucose ($C_6H_{12}O_6$), or cellobiose ($C_{12}H_{22}O_{11}$). Primers were added in the amount of 0.5% C of the measured bulk DOC concentrations, calculated from relative molar ratios of carbon in each primer to carbon in the bulk blackwater or leachate batch. Catalán et al. (2015) tested the priming effect using a variety of primer concentrations (0.05%, 0.2%, 1%, and 5% C) and found no significant differences in DOC consumption between samples amended with different primer concentrations. We chose to amend our samples with 0.5% C primer additions, reflecting a median value of concentrations in their study.

Triplicates of control and treatment samples (3 × 30 mL) for each of five time points were partitioned into acid-washed, precombusted (550 °C > 5 hr) 40-mL amber glass vials with plastic lids sealed by Teflon-coated septa. The bioincubations were performed in the dark at room temperature (20 °C) over 28 days for the blackwater samples and 21 days for the leachate samples. Vials were opened and aerated every 7 days in order to maintain an oxygenated environment. At each time point, samples were refiltered through a precombusted (450 °C > 5 hr) GF/F (0.7 μm) glass microfiber filter and acidified to pH 2 to ensure no microbial activity. Acidified samples were stored in the dark at 4 °C until analysis.

2.4. DOC Analysis

DOC concentrations were obtained from a Shimadzu TOC-L CPH analyzer and were calculated as the mean of the three most consistent measurements of up to seven injections, with a maximum coefficient of variance of 2% and precision of < ±0.05 mg/L. DOC remineralization was calculated as the difference between the final and initial DOC concentrations as

$$\Delta\mathrm{DOC} = \mathrm{DOC}_{\mathrm{inital}} - \mathrm{DOC}_{\mathrm{final}} \quad (1)$$

and percent DOC loss as

$$\%\ \Delta\mathrm{DOC} = (\ \Delta\mathrm{DOC}/\mathrm{DOC}_{\mathrm{initial}}) * 100 \quad (2)$$

during the bioincubation experiments. These two metrics provided insight on the bioavailability of DOM pools at different sites (Guillemette & del Giorgio, 2011). More importantly, they reveal whether a priming effect was present based on significant differences between DOC remineralization in control and primer treatment groups. Furthermore, DOC data was fitted to a three-parameter reactivity exponential decay model to assess the rate of DOC consumption as

$$A\ (t) = A_\infty + b_0\left(e^{-kt}\right) \quad (3)$$

where $A\ (t)$ is the modeled value at time (t), A_∞ is the stable DOC component (mg/L) that remains at $t = \infty$, b is the biolabile DOC component (mg/L) at t = 0, e is the base of the natural logarithm, k is the decay rate ($mg{\cdot}L^{-1}{\cdot}d^{-1}$), and t is the time in days (Spencer et al., 2009).

Table 2
Initial DOC Concentrations for Each Site at the Start (t = 0) of the Bioincubation Experiments

Site	Initial DOC (mg/L)	ΔDOC (mg/L)	BDOC (%)
Suwannee River	42.74	1.26	2.95%
Sopchoppy River	13.37	1.59	11.91%
Aucilla River	18.63	1.30	6.95%
Ochlockonee River	21.37	1.37	6.43%
CREW freshwater swamp	30.05	0.68	2.26%
Suwannee leachate	11.78	1.02	8.64%
Sopchoppy leachate	28.57	13.47	47.14%
Aucilla leachate	28.80	13.36	46.39%
Ochlockonee leachate	16.38	6.62	40.41%
CREW leachate	76.95	36.89	47.94%

Note. Values are averages of all controls and treatments for each bioincubation experiment. ΔDOC is the amount of DOC loss over 28 or 21 days during blackwater and leachate bioincubations, respectively. Biodegradable dissolved organic carbon (BDOC) indicates the proportion of the DOC that is biolabile. DOC = dissolved organic carbon; CREW = Corkscrew Regional Ecosystem Watershed.

2.5. Molecular Characterization of Blackwater and Leachate DOM

DOM extracts of the initial and final unamended bioincubation samples were analyzed using ESI-FT-ICR MS to assess which compound classes were utilized by the microbial community. Samples were first concentrated by solid phase extraction (100 mg Bond Elut PPL) following the method described by Dittmar et al. (2008). Initial DOC concentrations were used to calculate the aliquot volume needed to achieve a target mass of 40 $\mu g{\cdot}C{\cdot}mL^{-1}$ after 1-mL methanol elutions. DOM extracts were stored at −20 °C before analysis on a 9.4 T FT-ICR MS at the National High Magnetic Field Laboratory (Tallahassee, Florida) with the instrument set to negative ion mode. The spectra produced from the FT-ICR MS analysis consist of thousands of individual molecular peaks and intensities that were evaluated in the mass range of 200–600 *m/z* (Koch & Dittmar, 2006). Molecular formulas calculated from *m/z* values were assigned in EnviroOrg(©) (Corilo, 2015) and only included elemental combinations of $C_{4\text{-}45}H_{4\text{-}200}N_{0\text{–}1}O_{0\text{-}25}S_{0\text{-}1}$ and mass errors less than 200 ppb, excluding noise (signals $>6\sigma$ root mean square baseline, O'Donnell et al., 2016). Molecular formulas were placed into compound classes based on their elemental stoichiometries (Santi-Temkiv et al., 2013) and modified aromaticity indices (AI_{mod}) (Koch & Dittmar, 2006). Compounds are classified as unsaturated phenolic low oxygen = $AI_{mod} < 0.5$, $H/C < 1.5$, $O/C < 0.5$; unsaturated phenolic high oxygen = $AI_{mod} < 0.5$, $H/C < 1.5$, $O/C \geq 0.5$; polyphenolic = AI_{mod} 0.50–0.67; condensed aromatic = $AI_{mod} > 0.67$; aliphatic = $H/C \geq 1.5$, $O/C < 0.9$, $N = 0$; and peptide-like = $H/C \geq 1.5$, $O/C < 0.9$ $N \geq 1$ (O'Donnell et al., 2016) using a script developed by Hemingway (2017). The contribution of compound classes to the overall DOM pool is based on relative abundance (i.e., peak intensity; the number of molecular formulas assigned to each compound class). Changes in relative abundance reflect shifts in the DOM pool due to microbial consumption and production of certain compound classes.

2.6. Statistical Analyses

Measuring microbial consumption of DOC over the bioincubation time series allows us to determine if additions of biolabile substrates significantly alter the quantity or turnover of tDOC respired by the microbial community in blackwater systems or leachates. One-way analysis of variance (ANOVA) followed by Dunnett's tests was conducted, comparing the nutrient-amended control and each nutrient-amended primer treatment group, where a significant difference ($p < 0.05$) in percent DOC consumed or decay rate (k) would indicate that a priming effect occurred. Additionally, one-way ANOVA tests comparing percent DOC loss and k values of the unamended control to the nutrient-amended control were conducted to detect any nutrient limitation effects.

3. Results

3.1. Biodegradation of Blackwater DOC

Background DOC concentrations of the blackwaters varied from 13.37 to 42.74 mg/L across sites (Table 2). Average DOC loss for all 28-day blackwater bioincubations, including control and treatment groups, was minimal and comparable across sites at 1.24 ± 0.34 mg/L (Table 2 and Figure 2). However, the proportion of DOC loss was more variable, ranging from 2.26 to 11.91% (Table 2 and Figure 2). Percent DOC loss is indicative of the biodegradable portion of DOC (BDOC), defined here as the proportion of DOC utilized within a 28-day time period (Guillemette & del Giorgio, 2011; Vonk et al., 2015; Wickland et al., 2007). The Sopchoppy River had the greatest proportion of BDOC at 11.91%, and the CREW freshwater swamp had the smallest biolabile pool at 2.26% (Table 2). The unsubstantial DOC loss in these blackwater systems is comparable to the lower range of BDOC values found in other studies that have conducted bioincubations in highly aromatic, organic-rich aquatic systems, such as ~1.2 to 3.2 mg/L (12.6% to 22.9%) BDOC seen in stream water derived from bog and forested wetlands in southeastern Alaska (Fellman, Hood, D'Amore, et al., 2009).

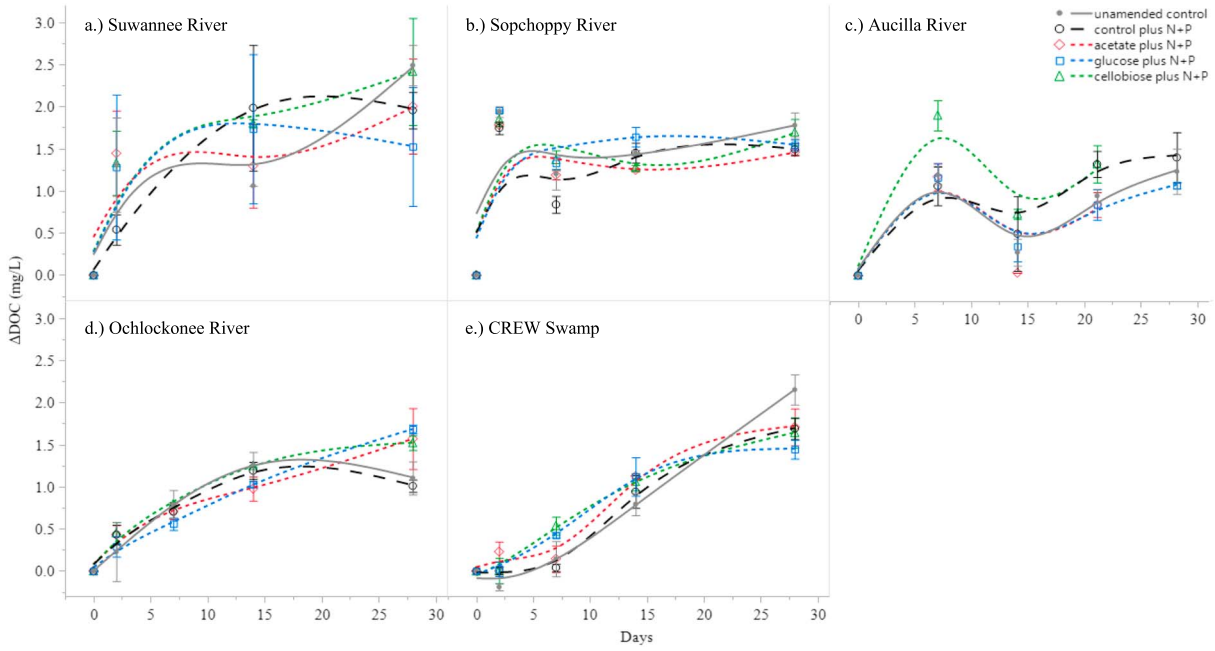

Figure 2. DOC consumption in the controls and primer treatment groups during 28-day blackwater bioincubations, where ΔDOC is calculated as $(DOC_{initial} - DOC_{final})/DOC_{initial}$. Points are averages of the three replicates for each control or treatment group with bars representing one standard error from the mean. CREW = Corkscrew Regional Ecosystem Watershed; DOC = dissolved organic carbon.

The single, three-parameter exponential decay model of microbial consumption of DOC over time estimated portions of DOC that were either reactive, *b*, or nonreactive, A_∞ (equation (3), Spencer et al., 2009). The nonreactive portion of blackwater DOC exceeded the reactive portion in all cases, and at least 85% of the blackwater DOC pools at all sites are considered nonreactive (Table 3). DOC decay rates (*k*) across sites and treatments ranged from 0.02 to 0.26 $mg{\cdot}L^{-1}{\cdot}d^{-1}$ (Table 3), which are consistent with stream DOC *k* values in the literature that typically range from 0.01 to 0.23 $mg{\cdot}L^{-1}{\cdot}d^{-1}$, as reviewed by Mineau et al. (2016). Overall, the decay model did not produce significant values for many of the blackwater sites (Table 3) due to the limited DOC degradation that occurred in these bioincubation samples (Table 2).

In our statistical analysis of the priming effect in blackwater systems, one-way ANOVA tests showed no significant differences in DOC consumption or decay rates (*k*) of the controls or the nutrient-amended control and nutrient-amended primer treatment groups (Table 4).

3.2. Biodegradation of Vegetation Leachate DOC

Initial DOC concentrations of the vegetation leachates ranged from 11.78 to 76.95 mg/L (Table 2). Average DOC loss from the 21-day leachate bioincubations varied from 1.02 mg/L in the Suwannee leachate to 36.89 mg/L in the CREW leachate, amounting to 38.10 ± 16.74% loss on average, a highly variable proportion of BDOC (Table 2 and Figure 3). Pellerin et al. (2010) conducted bioincubations of leached plant litter over 21 days resulting in 85–92% DOC loss, while Wickland et al. (2007) found that 11–90% of leachate DOC was mineralized depending on the initial plant species. Although the majority of DOC loss occurs within the first 2 weeks of our bioincubations, it is likely that leachate DOC biodegradation would have continued after 21 days (Figure 3) and is further evidenced by highly reactive DOC components described by the decay model (Table 3). Cleveland et al. (2004) found that in most cases 70% of DOC leached from plant litter was lost in the first 11 days, and less than 10% of the original DOC remained after 100 days.

The three-parameter exponential decay model estimated DOC decay rates ranging from 0.03 to 0.26 day^{-1} (Table 3), similar to the lower values for leaf leachate DOC *k* values in the literature that typically range from 0.02 to 0.98 day^{-1} (Mineau et al., 2016). With the exception of the Suwannee leachate, nonreactive and reactive DOC pools were comparable across blackwater sites and the reactive pools even exceeded the size of the nonreactive pools at some sites (Table 3). Leachate DOC remineralization in the nutrient-amended control and nutrient-amended primer treatments did not significantly differ (Table 4).

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Table 3

Three-Parameter Exponential Decay Model for Florida Blackwater River (_R) and Leachate (_L) DOC Values for the 28-Day Blackwater and 21-Day Leachate Bioincubation Experiments, Where A_{∞} = the Nonreactive DOC Component (mg/L), b = the Reactive DOC Component (mg/L), and k = the Decay Rate ($mg{\cdot}L^{-1}{\cdot}d^{-1}$)

		Suw_R	Sop_R	Auc_R	Och_R	CREW	Suw_L	Sop_L	Auc_L	Och_L	CREW_L
Unamendedcontrol	$A\infty$ = nonreactive	ns	11.61 ± 0.14	ns	20.15 ± 0.26	ns	ns	16.79 ± 0.61	15.46 ± 0.87	8.05 ± 0.45	49.06 ± 2.36
	b = reactive	ns	1.76 ± 0.18	ns	1.22 ± 0.31	ns	ns	11.32 ± 0.60	13.01 ± 0.88	8.28 ± 0.42	27.99 ± 2.24
	k = decay rate	ns	0.15 ± 0.04	ns	0.16 ± 0.11	ns	ns	0.13 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.11 ± 0.02
	r	ns	0.97 ± 0.22	ns	0.77 ± 0.43	ns	ns	0.99 ± 0.59	0.99 ± 0.77	1.00 ± 0.22	0.98 ± 1.95
	p	ns	<0.0001	ns	0.005	ns	ns	<0.0001	<0.0001	<0.0001	<0.0001
Controlplus N + P	$A\infty$ = nonreactive	ns	11.78 ± 0.13	17.13 ± 0.26	20.25 ± 0.13	ns	9.84 ± 1.18	14.67 ± 0.41	13.43 ± 0.29	9.33 ± 0.33	35.95 ± 0.82
	b = reactive	ns	1.61 ± 0.15	1.34 ± 0.36	0.86 ± 0.15	ns	1.70 ± 1.16	13.23 ± 0.50	14.74 ± 0.38	6.82 ± 0.31	40.74 ± 0.94
	k = decay rate	ns	0.13 ± 0.03	0.15 ± 0.11	0.14 ± 0.07	ns	0.03 ± 0.03	0.21 ± 0.02	0.13 ± 0.01	0.11 ± 0.01	0.17 ± 0.01
	r	ns	0.97 ± 0.17	0.77 ± 0.47	0.88 ± 0.19	ns	0.95 ± 0.12	0.99 ± 0.70	1.00 ± 0.48	0.99 ± 0.27	1.00 ± 1.19
	p	ns	<0.0001	0.0073	0.0002	ns	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Acetateplus N + P	$A\infty$ = nonreactive	ns	11.99 ± 0.04	ns	18.89 ± 1.98	28.78 ± 2.46	10.56 ± 0.24	14.55 ± 0.48	13.66 ± 0.27	8.49 ± 0.71	36.91 ± 0.88
	b = reactive	ns	1.38 ± 0.07	ns	2.26 ± 1.91	3.43 ± 2.39	1.19 ± 0.22	13.64 ± 0.60	15.20 ± 0.37	7.97 ± 0.67	39.33 ± 0.99
	k = decay rate	ns	0.25 ± 0.04	ns	0.03 ± 0.04	0.03 ± 0.03	0.10 ± 0.04	0.22 ± 0.03	0.15 ± 0.0100	0.10 ± 0.02	0.17 ± 0.01
	r	ns	0.99 ± 0.09	ns	0.87 ± 0.31	0.92 ± 0.35	0.94 ± 0.15	0.99 ± 0.86	1.00 ± 0.49	0.99 ± 0.46	1.00 ± 1.31
	p	ns	<0.0001	ns	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Glucoseplus N + P	$A\infty$ = nonreactive	ns	11.77 ± 0.07	ns	19.24 ± 0.39	30.32 ± 0.25	10.72 ± 0.12	15.34 ± 0.26	13.83 ± 0.27	9.20 ± 0.41	35.84 ± 1.07
	b = reactive	ns	1.60 ± 0.12	ns	2.29 ± 0.37	2.02 ± 0.24	1.26 ± 0.11	14.19 ± 0.34	15.49 ± 0.37	7.58 ± 0.40	40.77 ± 1.13
	k = decay rate	ns	0.26 ± 0.06	ns	0.06 ± 0.02	0.08 ± 0.03	0.13 ± 3.29	0.26 ± 0.02	0.15 ± 0.01	0.13 ± 0.02	0.16 ± 0.01
	r	ns	0.98 ± 0.14	ns	0.97 ± 0.18	0.96 ± 0.21	0.97 ± 0.12	1.00 ± 0.49	1.00 ± 0.50	0.99 ± 0.44	1.00 ± 1.53
	p	ns	<0.0001	ns	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cellobioseplus N + P	$A\infty$ = nonreactive	ns	11.80 ± 0.12	ns	19.85 ± 0.11	28.71 ± 2.75	10.78 ± 0.11	14.78 ± 0.42	14.02 ± 0.19	9.79 ± 0.38	36.44 ± 1.42
	b = reactive	ns	1.57 ± 0.17	ns	1.64 ± 0.12	3.25 ± 2.69	1.30 ± 0.12	14.24 ± 0.56	14.71 ± 0.28	7.25 ± 0.40	39.86 ± 1.57
	k = decay rate	ns	0.26 ± 0.09	ns	0.14 ± 0.03	0.02 ± 0.03	0.16 ± 0.04	0.25 ± 0.03	0.16 ± 0.01	0.16 ± 0.03	0.17 ± 0.02
	r	ns	0.96 ± 0.23	ns	0.97 ± 0.16	0.93 ± 0.26	0.97 ± 0.14	0.99 ± 0.84	1.00 ± 0.37	0.99 ± 0.51	0.99 2.06
	p	ns	<0.0001	ns	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note. Suw = Suwannee; Sop = Sopchoppy; Auc = Aucilla; Och = Ochlockonee; CREW = Corkscrew Regional Ecosystem Watershed; ns = not significant; DOC = dissolved organic carbon.

Table 4
P Values From One-Way ANOVAs Comparing Percent Change in DOC Over the Bioincubation Period for (a) the Nutrient-Amended Control Versus the Nutrient-Amended Primer Treatment Groups, Where $p < 0.05$ Represents a Priming Effect, and (b) the Unamended Control Versus the Nutrient-Amended Control, Where $p < 0.05$ Indicates a Nutrient Effect

		p values ($\alpha < 0.05$)			
		Blackwaters		Leachates	
		%ΔDOC	k	%ΔDOC	k
(a) Priming effect	control plus N + P versus				
	acetate plus N + P	1.0000	0.9970	1.0000	0.7695
	glucose plus N + P	0.9999	0.9947	1.0000	0.7695
	cellobiose plus N + P	0.9895	1.0000	1.0000	0.4860
(b) Nutrient effect	unamended control versus				
	control plus N + P	0.9986	0.9994	0.8578	0.8019

Note. DOC = dissolved organic carbon; ANOVA = analysis of variance.

3.3. Chemical Composition of Blackwater-Derived and Leachate DOM

ESI-FT-ICR MS analysis revealed that the blackwater and vegetation leachate DOM pools were dominated by unsaturated phenolics, the majority of which are high O/C compounds (Table 5, Figure 4, and Table S1). The next most abundant compound class was polyphenolics followed by condensed aromatics and aliphatics (Table 5, Figure 5, and Table S1). However, compared to the blackwater sites, leachates had a much higher proportion of aliphatic compounds (Table 5, Figure 5, and Table S1), which are inherently bioavailable to microbial communities (O'Donnell et al., 2016; Spencer et al., 2015; Sun et al., 1997). Loss of aliphatic compounds occurred in all blackwater and leachate bioincubations (Figure 5) but was exceptionally high in the Ochlockonee and Sopchoppy leachates (Figure 5b and Table S1). The relative abundance of polyphenolics

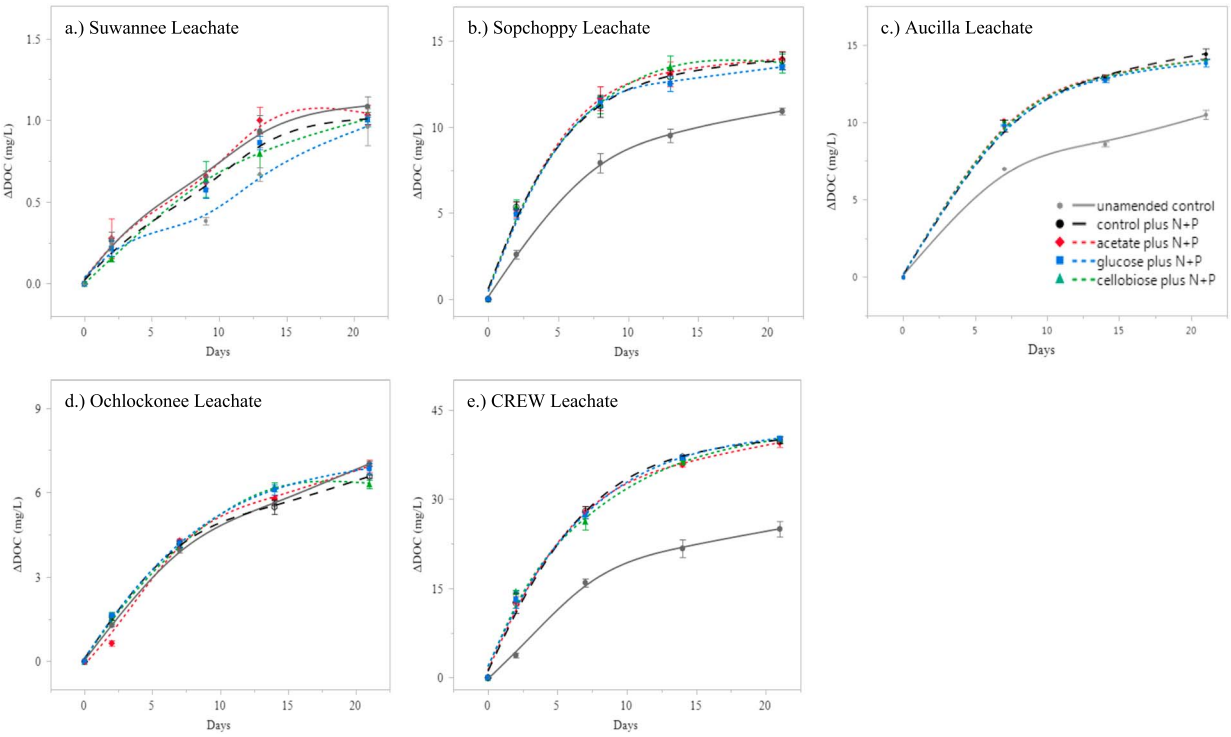

Figure 3. DOC consumption in the controls and primer treatment groups during 21-day plant litter leachate bioincubations, where ΔDOC is calculated as $(DOC_{initial} - DOC_{final})/DOC_{initial}$. Points are averages of the three replicates for each control or treatment group with bars representing one standard error from the mean. NB. Scale is different for each panel. CREW = Corkscrew Regional Ecosystem Watershed; DOC = dissolved organic carbon.

Table 5
Chemical Composition of Blackwater and Leachate DOM Pools

	Blackwater DOM		Leachate DOM	
Compound Class	Average %RA	Average #MF (n)	Average %RA	Average #MF (n)
Unsaturated phenolic high O/C	53.08 ± 4.49%	3785 ± 1398	40.37 ± 7.71%	3418 ± 641
Unsaturated phenolic low O/C	27.65 ± 5.43%	2897 ± 884	26.28 ± 4.84%	3246 ± 434
Polyphenolic	13.32 ± 1.50%	1486 ± 558	15.35 ± 3.13%	1229 ± 203
Condensed aromatic	3.62 ± 0.66%	664 ± 180	6.98 ± 1.94%	624 ± 181
Aliphatic	1.87 ± 0.35%	481 ± 105	9.29 ± 5.16%	1381 ± 604
Peptide like	0.12 ± 0.04%	80 ± 38	1.22 ± 1.00%	372 ± 157
Sugars	0.34 ± 0.12%	107 ± 54	0.50 ± 0.11%	171 ± 66

Note. Average %RA reflects initial average relative abundances of different compound classes in blackwater and leachate DOM samples at $t = 0$, indicated by the percent of molecular formulas assigned to each compound class. Average #MF is the average number of molecular formulas that ionized in the blackwater or leachate DOM samples during FT-ICR MS analysis. DOM = dissolved organic matter; DOC = dissolved organic carbon; FT-ICR MS = Fourier transform ion cyclotron resonance mass spectrometry.

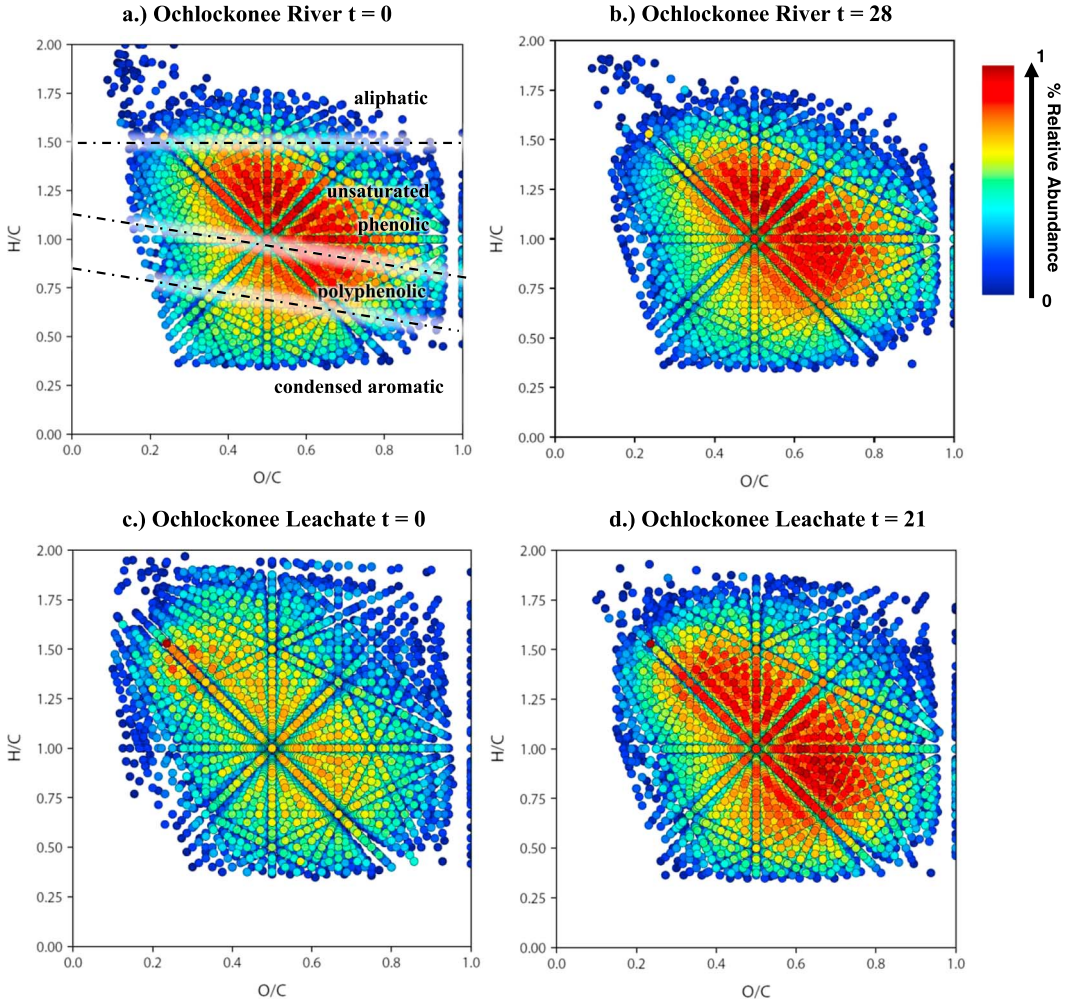

Figure 4. van Krevelen diagrams of elemental DOM composition determined by FT-ICR MS analysis. Plots include initial and final control samples for the Ochlockonee River and leachate. Figure 4a shows the stoichiometric boundaries of formula assignment for different compound classes in van Krevelen space. The color scale represents a gradient of increasing relative abundance (percent of molecular formulas assigned). DOM = dissolved organic matter; FT-ICR MS = Fourier transform ion cyclotron resonance mass spectrometry.

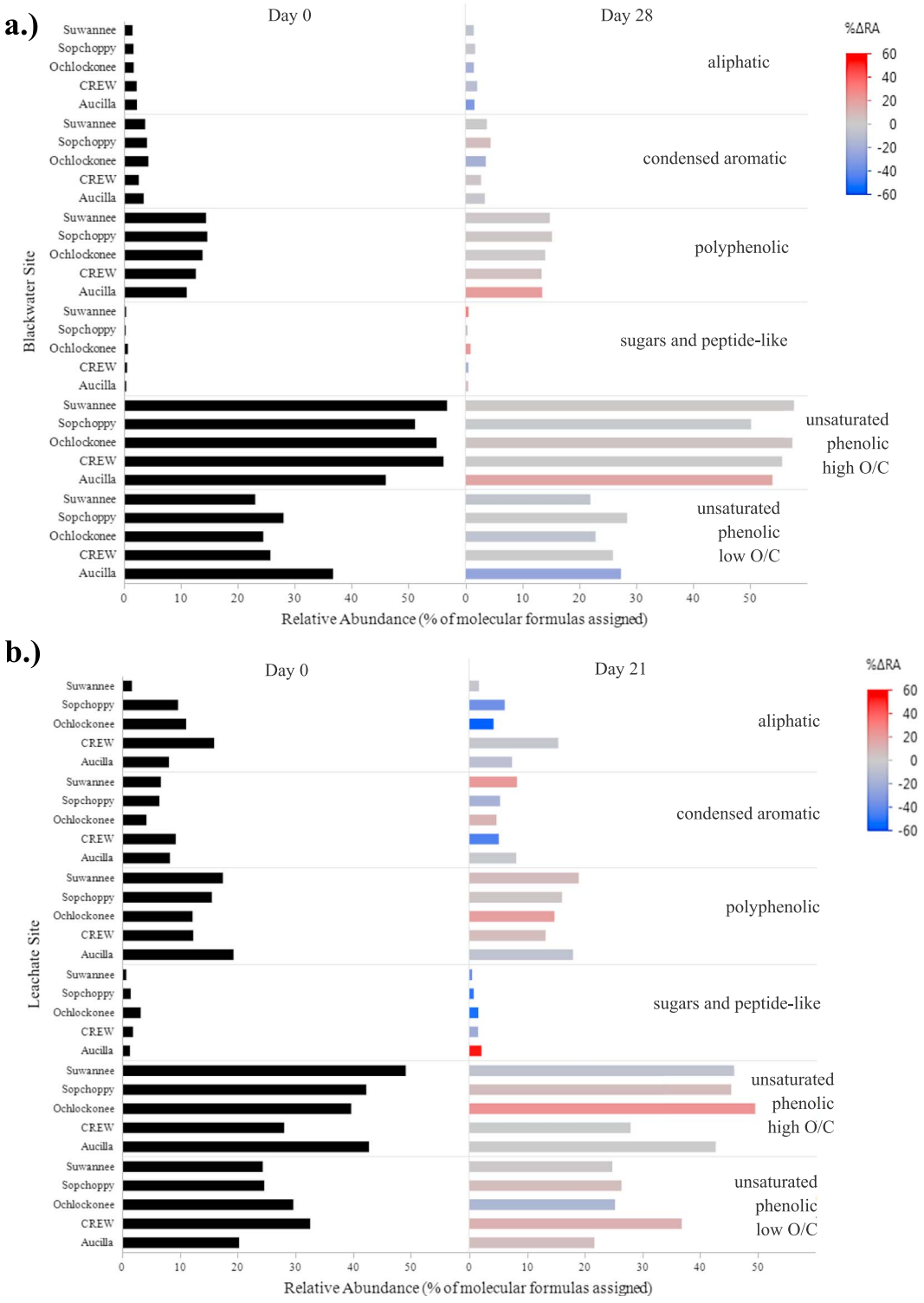

Figure 5. Chemical composition of blackwater DOM in (a) five blackwater bioincubations and (b) five leachate bioincubations. Bars represent the relative abundances (RA) of different compound classes in start and end control samples determined by FT-ICR MS, indicated by the percent of molecular formulas assigned to each compound class. The color gradient in the right column represents the change in relative abundance of compounds, where red indicates a relative increase and blue indicates a relative decrease. Sugars and peptide-like compound classes are combined. DOM = dissolved organic matter; FT-ICR MS = Fourier transform ion cyclotron resonance mass spectrometry.

increased for all sites except the Aucilla leachate (Figure 5b and Table S1). Sugars and peptide-like compounds made up only a small proportion of the DOM pool in blackwater (<1%) and leachate (<2%) samples, and changes in relative abundances were negligible (Figure 5 and Table S1).

3.4. Differences in Blackwater Versus Leachate DOC

Leachate BDOC values (i.e., the proportion of DOC loss) exceeded blackwater BDOC values at each corresponding site (Table 2). On average, blackwater bioincubations showed DOC loss of 6.10 ± 3.85%, while leachate bioincubations showed much greater loss at 38.10 ± 16.74% (Table 2). Leachate DOC was therefore much more bioavailable than blackwater-derived DOC. For instance, only 6.95% of DOC was utilized in the Aucilla River bioincubation, yet 46.39% of DOC was utilized in its associated leachate bioincubation (Table 2). Furthermore, leachate bioincubations only lasted for 21 days compared to 28 days for the blackwater bioincubations and may have experienced DOC loss during the 24-hr leaching period, thus providing comparatively conservative estimates of leachate BDOC. It is also evident that leachate DOC is more bioavailable from the three-parameter decay model, which revealed that leachates had much larger reactive pools relative to blackwater reactive pools (Table 3) in conjunction with a much larger contribution of energy-rich aliphatic molecules determined by FT-ICR MS (Table 5 and Figure 5).

4. Discussion

4.1. Lack of Evidence for a Priming Effect

While there is substantial evidence for priming in soils, our work indicates that priming appears to play a limited role with respect to microbial transformation of DOC in blackwater systems. Experimental evidence presented here shows that DOC biodegradation in treatment groups amended with biolabile primers did not significantly differ from the control in both blackwater and leachate incubations (Figures 2 and 3, and Table 4). While we took a pragmatic approach to studying the priming effect by conducting simple biodegradation experiments with extensive replication and treatments amended with simple biolabile compounds, we did not distinguish decay rates of biolabile and stable DOC. The definition of priming is widely accepted to be the biodegradation of stable DOC upon moderate biolabile inputs (Bingemann et al., 1953; Guenet et al., 2012; Kuzyakov, 2010), thus reactivity continuum modeling (Koehler et al., 2012) and isotopic labeling (Bengtsson et al., 2014; Hotchkiss et al., 2014; Kuzyakov et al., 2000) can be useful for assessing degradation dynamics of reactive and stable DOC separately. However, Bengtsson et al. (2014) conducted priming experiments using stream biofilm microcosms with a ^{13}C-labeled allochthonous OC source in combination with different autochthonous inputs and still found no evidence of priming.

Other studies in aquatic ecosystems have also failed to find a priming effect, for example, Catalán et al. (2015) tested for a priming effect in lakes of different trophic states using the same primers as in our investigation but did not observe significant differences in DOC consumption. Degradation of riverine DOC by coastal marine bacterial communities was also unaffected by inputs of biolabile DOC over a 42-day incubation (Blanchet et al., 2017). When studied in humic boreal lake mesocosms using sucrose as a primer, remineralization and microbial abundance and production were unresponsive to various concentrations of primers (Dorado-García et al., 2016). Despite the complexity of different primer compounds added and variety of aquatic ecosystems studied, a clear priming effect could not be detected in any of these cases, highlighted by a recent meta-analysis by Bengtsson et al. (2018) showing an overall nonsignificant effect of priming across aquatic ecosystems.

Ultimately, priming effects are governed by microbial activity (Blagodatskaya & Kuzyakov, 2008). It has been shown that bacterial growth efficiency may be altered by the presence of multiple DOC sources of mixed quality (Fonte et al., 2013), raising questions about how priming may change the proportion of DOC allocated to biomass versus respiration. Additionally, changes in bacterial community composition and bacterial community activity can occur in response to biolabile DOC inputs (Fonte et al., 2013). Converging DOM and microbial perspectives is critical to our understanding of the fate of tDOC. We argue that the underlying microbial mechanisms of DOM transformation should be the focus rather than the presence or absence of priming, since priming appears to be quantitatively unimportant in blackwaters and many other aquatic systems (Bengtsson et al., 2014, 2018; Blanchet et al., 2017; Catalán et al., 2015; Dorado-García et al., 2016).

Based on comparisons of DOC biodegradation between the unamended and nutrient-amended controls, we found that the alleviation of inorganic nutrient limitation might enhance DOC degradation. DOC consumption in the unamended control diverged from the nutrient-amended samples in several of the leachate bioincubations (Figure 3), indicative of nutrient limitation at those sites. However, all leachate bioincubations were pooled to increase statistical power, and thus, the quantity and rate of turnover was not significantly different between unamended and nutrient-amended controls, as nutrient limitation was only apparent at some sites. It is important to highlight that increased DOC turnover as a result of inorganic nutrient additions should not be interpreted as a priming effect; it is simply the product of nutrient limitation in the system of study. For instance, Steen et al. (2016) describe priming as a change in remineralization of a degraded OM source due to additions of nutrients or biolabile OC. Although the results showed increased remineralization for one of the biolabile OC plus phosphorus additions (Steen et al., 2016), the control was not nutrient amended and thus a priming effect may potentially be due to phosphorus limitation. Aquatic studies of priming stemmed from substantiated evidence for priming of soil organic matter, particularly in agricultural experiments that included inorganic nutrient limitation as a priming effect (Bingemann et al., 1953; Kuzyakov et al., 2000). As we shift our focus from priming of soil organic matter in the terrestrial environment to DOM in aquatic systems, we must also adjust our definition of priming due to obvious differences in OM processing, substrate availability, nutrient exchange, microbial and decomposer communities, and OM residence time that are vastly different between soils and aquatic media.

Additionally, the time scale over which the priming effect operates becomes important when considering how priming ultimately alters CO_2 fluxes from aquatic ecosystems as a result of DOC remineralization. A real priming effect occurs when OM remineralization is higher than ambient rates and results in decomposition of refractory OM (Jenkinson et al., 1985) as opposed to apparent priming, in which microbial biomass turnover is affected but not OM decomposition (Blagodatskaya & Kuzyakov, 2008; Kuzyakov, 2010). In other words, if DOC turnover is altered by the input of biolabile DOC over a short period (hours to days) but is unchanged in the long term (weeks to months), then a priming effect is only apparent. Previous experiments have described the priming effect as *transient* because, after month-long microcosms, the amount of DOC consumed was the same in all treatments and the control (Abbott et al., 2014; Steen et al., 2016). As seen in our experiments, the amount of DOC consumed is the same in the nutrient amended control, and nutrient and primer-amended experiments after 3 to 4 weeks (Figures 2 and 3, and Table 4) when it is assumed the vast majority of the BDOC has been respired (Vonk et al., 2015; Wickland et al., 2007). Thus, it appears that priming may be of limited importance in organic-rich blackwater systems.

The limited studies and conflicting results put forward to date demonstrate the need to bring clarity to several problems concerning the priming effect in the aquatic sciences including: (1) absence of a clear universal definition, (2) attribution of priming as a misnomer for other biogeochemical mechanisms (e.g., nutrient effects), (3) need for a relevant time scale, (4) lack of understanding behind the drivers of priming, (5) disconnect between the geochemical DOM perspective and microbial perspective of priming, and (6) failure to distinguish major differences between terrestrial and aquatic priming. Reaching a consensus on these issues and how we interpret the results of aquatic priming studies is ultimately critical to our understanding of the priming effect and how it influences the fate of tDOC.

4.2. Controls on the Bioavailability of Blackwater DOC

Inorganic nutrient availability appeared to impact microbial consumption of DOC in some treatments (Figure 3); however, other abiotic factors may be driving some of the observed patterns in DOC bioavailability, including residence time and seasonality. Residence time is an important control on biogeochemical cycling (Battin et al., 2008; Casas-Ruiz et al., 2017), resulting in a decrease in DOC remineralization along the inland water continuum (Catalán et al., 2016) where biodegradation of riverine DOM is highest in headwater streams (Mann et al., 2015; Spencer et al., 2015). In this study it seems likely that the blackwater samples had been extensively altered through exposure to microbial degradation prior to collection, providing a source of degraded and thus stable DOM, evidenced by low BDOC values (Table 2) and large nonreactive DOC pools (Table 3). This is further supported based on known characteristics of blackwater systems, which previous studies have shown typically have a substantial contribution of biostable DOC (Amon & Benner, 1996; Sun et al., 1997).

Contrary to the stable nature of blackwater DOM, leachates simulated a relatively fresh DOM source (releasing up to ~77 mg·C·L^{-1} in 24 hr) and contained a greater proportion of BDOC (Table 2). Leachate DOC is known to be more bioavailable than riverine DOC and is quickly consumed by riverine heterotrophic bacteria (Mineau et al., 2016; Sun et al., 1997). The high bioavailability of leachate DOC is evidenced by higher decay rates on average compared to blackwater samples (Tables 2 and 3) and large relative contributions of energy-rich aliphatic compounds (Table 5), which were selectively utilized (Figure 5b). Thus, in blackwater systems, fresh inputs of DOM from leached terrestrial litter may be rapidly utilized and stable material is exported downstream (Raymond & Bauer, 2001; Sun et al., 1997), resulting in higher aromatic content (Spencer et al., 2008; Wickland et al., 2007) and decreased biomineralization rates with increasing residence time (Catalán et al., 2016; Guillemette & del Giorgio, 2011; Koehler et al., 2012).

Differences in bioavailability between sites may also be due to seasonality and timing of storm events as Suwannee samples were collected in the wet season and samples from the remaining sites were collected in the dry season. The Suwannee River had the highest DOC concentration of all the sites (Table 2), yet only ~3% was biolabile (Table 2). The Suwannee River DOC concentration is also much higher than that of its associated leachate, compared to other sites where leachate DOC concentration exceeded blackwater DOC concentrations (Table 2). This suggests that Suwannee litter was already extensively leached and degraded in the wet season prior to collection, which is further evidenced by the minimal loss of DOC compared to the other leachates (Table 2 and Figure 3) and having the smallest reactive DOC pool of all the leachates (Table 3). It is likely that runoff from previously leached surface soil layers and plant litter released refractory DOM, resulting in higher DOC concentrations and lower BDOC values in the wet season (Tables 1 and 2, Moore et al., 2011). Similarly, temperate and arctic aquatic environments experience decreasing BDOC after spring thaw into summer and the autumn rainy season as terrestrial DOM from vegetation and soils are continuously exposed to leaching and biodegradation (Fellman, Hood, Edwards, et al., 2009; Striegl et al., 2005; Vonk et al., 2015; Wickland et al., 2012).

In contrast, samples that were collected during the dry season have much higher leachate DOC concentrations and proportions of BDOC than their associated blackwater samples (Tables 1 and 2). For instance, the CREW site had the lowest proportion of blackwater BDOC and highest proportion of leachate BDOC of all the sites (Table 2). The Ochlockonee River is the only case in which blackwater DOC collected in the dry season was higher in concentration than its associated leachate (Table 1), but this could have been due to a recent storm event as large amounts of terrestrial OM from surrounding catchments can be mobilized during stormflows, increasing DOC concentrations (Fellman, Hood, Edwards, et al., 2009; Pereira et al., 2014). However, the Ochlockonee leachate still had a greater proportion of BDOC than the Ochlockonee River (Table 2). Overall, while blackwater DOC and wet-season leachate DOC were fairly resistant to degradation, the leachate DOC in the dry season was highly bioavailable (Tables 1 and 2, and Figure 3).

Compositionally, the blackwater DOM during the wet season was indistinguishable from blackwater DOM during the dry season because it is all from the same terrestrial source and likely highly biodegraded before collection. However, the leachate from the wet season (e.g., Suwannee leachate) shows paucity in aliphatic compounds in comparison to leachates from the dry season that have high relative abundances of aliphatic molecular formulas (Figure 5b), further supporting the conclusion that the vegetation had been degraded and leached prior to collection.

Results from this and other recent studies that highlight priming may not be important with respect to impacting degradation of tDOC, suggesting that other mechanisms are crucial for explaining the observed lack of tDOC in the ocean (Bengtsson et al., 2018; Benner, 2002; Bianchi, 2011). For example, photomodification of DOM that can result in compositional changes (Spencer et al., 2009; Stubbins et al., 2010), as well as may cause the resultant DOM to become more susceptible to microbial utilization, has been shown to be important for answering the question of the fate of tDOC in the ocean (Cory et al., 2013; Moran et al., 2000; Rossel et al., 2013). Several studies have shown that some fractions of DOM are lost upon irradiation especially colored, highly aromatic molecules characteristic of blackwater DOM (Spencer et al., 2009; Stubbins et al., 2010). While it is possible that photodegradation is directly responsible for the lack of tDOC in coastal oceans (Bianchi, 2011) either through direct remineralization or by increasing the bioavailability of DOM, it can also modify terrestrial DOM signatures so that it resembles marine DOM further complicating this conundrum (Rossel et al., 2013; Spencer et al., 2009; Stubbins et al., 2010).

4.3. Selective Microbial Utilization of Specific DOM Compounds

DOM is a complex mixture of poorly characterized molecular compounds (Koch et al., 2005; Koehler et al., 2012; Nebbioso & Piccolo, 2013). As chemical composition plays a substantial role in the bioavailability of aquatic DOM pools (Kellerman et al., 2015; Sun et al., 1997), the extreme mass accuracy of FT-ICR MS analysis provides unparalleled insights into the composition of natural DOM and is currently the only method that allows us to obtain an ultrahigh resolution of the individual compounds in a given DOM pool (D'Andrilli et al., 2015; Nebbioso & Piccolo, 2013). Therefore, we applied FT-ICR MS to investigate the relationship between background DOM composition (i.e., initial and final control bioincubation samples) and biolability in blackwater systems.

Aromatic compounds, including polyphenolics and condensed aromatics, are abundant in our sample set (Tables 5 and S1, and Figure 5) and are typical of blackwater DOM (Kellerman et al., 2018; Stubbins et al., 2010; Wagner et al., 2015). Polyphenolics increased in relative abundance throughout time in both blackwater and leachate bioincubations (Figures 4 and 5, and Table S1) consistent with other studies that show these compounds are biorefractory (Kim et al., 2006; O'Donnell et al., 2016). The largest source of polyphenolic compounds is vascular plant inputs (Kellerman et al., 2015), which produce DOM typically resistant to biodegradation and are often used as a biomarker for terrestrially derived DOM (Spencer et al., 2009; Ward et al., 2013). Condensed aromatics, also characteristic of blackwater DOM, lacked clear patterns of microbial utilization but were relatively biostable (Figure 5). Condensed aromatics have historically been associated with black carbon (Hockaday et al., 2006), formed as a combustion product of terrestrial material, but it has recently been reported that they may even form as a product of nonpyrogenic lignin oxidation (Waggoner et al., 2015). However, condensed aromatics are difficult to measure with negative ESI-FT-ICR MS because they do not ionize as well as other compounds (Hodgkins et al., 2016). Thus, changes in relative abundance of condensed aromatic compounds may be due to their failure to ionize or reduced competition for charge as other compounds (e.g., aliphatics) are lost (Stubbins et al., 2010).

Energy-rich aliphatic compounds were preferentially utilized by the blackwater microbial community in both blackwater and leachate bioincubations at all sites, evidenced by a decrease in the percent of aliphatic molecules assigned compared to other compounds (Figures 4 and 5). This is consistent with other studies of blackwaters and fresh DOM leachates that observed selective utilization of aliphatics (O'Donnell et al., 2016; Spencer et al., 2015; Sun et al., 1997). Leachate DOM had a greater relative abundance of aliphatic compounds, 9.29% on average compared to only 1.87% in blackwaters (Tables 5 and S1 and Figure 5) along with higher BDOC (Table 2) as leachates provide fresh, minimally processed DOM whereas blackwater samples are more degraded. These findings agree with other studies that have linked the bioavailability of DOM to the contribution of aliphatic molecules in freshwater DOM (O'Donnell et al., 2016; Spencer et al., 2015; Sun et al., 1997). Additional evidence for the positive relationship between percent BDOC and relative abundance of aliphatics is shown in Figure 6. The proportion of BDOC and commonly assigned molecular formula (present in $\geq$50% of samples) was significantly correlated (Figure 6a; $p < 0.05$). This correlation was mostly driven by leachate DOM composition as the correlation increases in strength when these variables are plotted for leachates alone (Figure 6b). The strong positive correlation between percent BDOC and leachate DOM composition was mostly related to the relative abundance of aliphatics (H/C $\geq$ 1.5, O/C $<$ 0.9; Figure 6b), which were also preferentially utilized in the bioincubation experiments (Figure 5). Contrarily, less than 10% of common formulae in blackwater DOM were correlated to BDOC, highlighting its stable nature (Figure 6c).

Sugars and peptide-like compounds covaried and contributed minimally to the composition of DOM at all sites (Tables 5 and S1), and therefore these classes were combined for simplicity in Figure 5. These compounds did not show strong changes in relative abundance over the bioincubation period in blackwater DOM (Figure 5a and Table S1). However, leachate DOM had a considerably larger contribution of peptides than blackwater DOM samples (Tables 5 and S1), and these compounds were selectively utilized in most leachate samples (Figure 5b). While proteins and carbohydrates (e.g., peptide-like compounds and sugars respectively) are considered biolabile compounds and are inherently prone to biodegradation, there is also evidence that they can accumulate through microbial synthesis during DOM degradation (Guillemette & del Giorgio, 2012; Kalbitz et al., 2003) that may explain the contrasting changes in relative abundance.

Overall, microbial utilization of blackwater and leachate DOM from these systems tends to follow a degradation pathway that results in H/C and O/C ratios that are lower and higher, respectively (Figure 4), as seen in

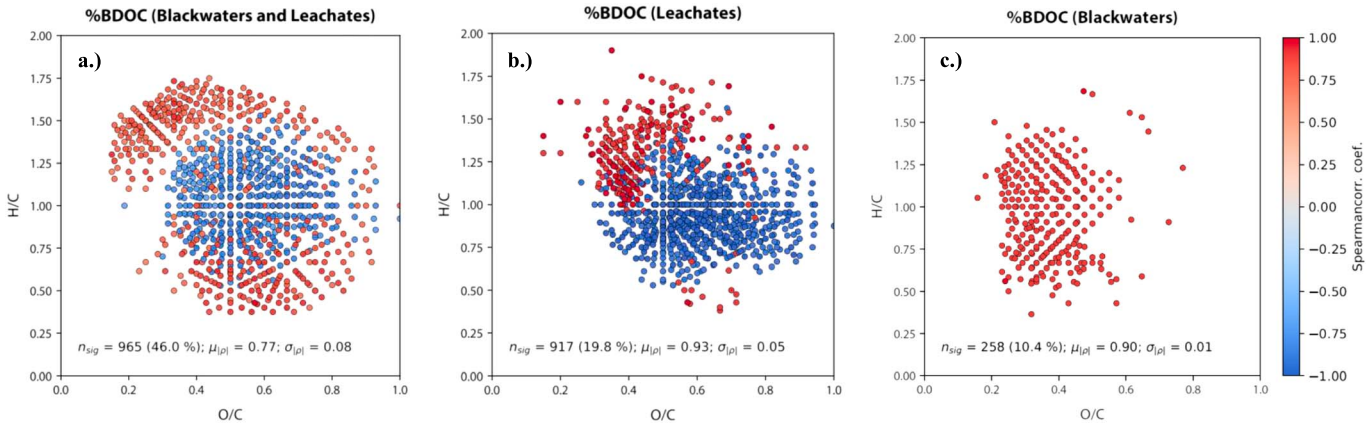

Figure 6. DOM composition as a function of percent biodegradable dissolved organic carbon (BDOC) for (a) all unamended blackwater and leachate samples, (b) leachates only, and (c) blackwaters only, plotted in van Krevelen space. The colors represent the Spearman rank correlation coefficient between %BDOC and the relative intensity of each molecular formula as measured by FT-ICR MS. Red formulae have a higher relative abundance when %BDOC is high, while blue formulae have a higher relative abundance when %BDOC is low. Displayed formulae were present in all samples and were significantly correlated with %BDOC ($p < 0.05$). DOM = dissolved organic matter; FT-ICR MS = Fourier transform ion cyclotron resonance mass spectrometry.

other studies (D'Andrilli et al., 2015; Kim et al., 2006; Spencer et al., 2015; Sun et al., 1997). Minor changes in DOM composition of the blackwater samples along with negligible DOC loss over the bioincubation period reflect the biostable nature of blackwater DOM, while leachate DOM was relatively bioavailable. Significant correlations existed between DOM composition (e.g., relative peak intensity of common compounds assigned through FT-ICR MS) and proportion of BDOC in blackwater systems (i.e., blackwaters and vegetation leachates; Figure 6a), mostly driven by aliphatics (H/C $\geq$ 1.5, O/C $<$ 0.9) and DOM released from leachates (Figure 6b). The molecular signature of leachate DOM begins to resemble that of blackwater DOM by the end of the bioincubation experiments (Figure 4, Rossel et al., 2013; Spencer et al., 2015). Thus, it seems apparent that in blackwater systems, the most bioavailable forms of DOM leached from plant litter are rapidly utilized and stable material is exported downstream (Meyer et al., 1987). Our work has shown that leachate DOM contains a large contribution of aliphatic compounds that are highly bioavailable (Figures 5 and 6, and Table 5), supporting the current paradigm that rivers may actively transform fresh inputs of organic matter from the terrestrial environment (Battin et al., 2008; Bianchi, 2011; Cole et al., 2007; Mayorga et al., 2005).

Acknowledgments

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