Microbial Degradation of Plant Leachate Alters Lignin Phenols and Trihalomethane Precursors

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Although the importance of vascular plant–derived dissolved organic carbon (DOC) in freshwater systems has been studied, the role of leached DOC as precursors of disinfection byproducts (DBPs) during drinking water treatment is not well known. Here we measured the propensity of leachates from four crops and four aquatic macrophytes to form trihalomethanes (THMs)—a regulated class of DBPs—before and after 21 d of microbial degradation. We also measured lignin phenol content and specific UV absorbance ($SUVA_{254}$) to test the assumption that aromatic compounds from vascular plants are resistant to microbial degradation and readily form DBPs. Leaching solubilized 9 to 26% of total plant carbon, which formed 1.93 to 6.72 mmol THM mol C^{-1}. However, leachate DOC concentrations decreased by 85 to 92% over the 21-d incubation, with a concomitant decrease of 67 to 92% in total THM formation potential. Carbon-normalized THM yields in the residual DOC pool increased by 2.5 times on average, consistent with the preferential uptake of nonprecursor material. Lignin phenol concentrations decreased by 64 to 96% over 21 d, but a lack of correlation between lignin content and THM yields or $SUVA_{254}$ suggested that lignin-derived compounds are not the source of increased THM precursor yields in the residual DOC pool. Our results indicate that microbial carbon utilization alters THM precursors in ecosystems with direct plant leaching, but more work is needed to identify the specific dissolved organic matter components with a greater propensity to form DBPs and affect watershed management, drinking water quality, and human health.

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The concentration and composition of dissolved organic matter (DOM) in freshwater systems is of concern due to its role in the formation of disinfection byproducts (DBPs) during drinking water treatment. Reactions between chlorine and dissolved organic carbon (DOC) form a suite of compounds with potential human health impacts, including increased risk of cancers and birth defects (USEPA, 2006). Although a range of treatment technologies, including the use of ozone and chloramine, have been investigated as alternatives to chlorination, lowering the amount of precursor DOC in source waters has also received attention as a means of DBP reduction in finished drinking water. However, few studies have addressed both the source and persistence of DBP-precursors such as trihalomethanes (THMs) in aquatic environments (Engelage et al., 2009; Kraus et al., 2008; Reckhow et al., 2004).

Leaching of water-soluble compounds from fresh, senescent, and degraded plant biomass in contact with drainage water is a ubiquitous process in terrestrial and aquatic systems (Mann and Wetzel, 1996; Webster and Benfield, 1986; Carpenter, 1980). A number of short-term incubation studies have shown that DOM derived from fresh and senescent plant litter is a readily available and rapidly utilized carbon source for heterotrophic bacteria (Cleveland et al., 2004; Mann and Wetzel, 1996; Kaplan and Bott, 1983). The rapid DOC loss has been attributed to the preferential consumption of highly labile, low-molecular-weight compounds leached from fresh litter, with remaining plant secondary compounds more resistant to microbial degradation (Maie et al., 2006; Ibrahima et al., 1995). The propensity of DOM to form DBPs is also influenced by the physical and chemical properties of organic matter (Kanokkantapong et al., 2006; Chow et al., 2005) and is therefore likely altered by microbial degradation. However, there is a lack of data evaluating the extent to which degradation affects the propensity of DOM to form DBPs.

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Abbreviations: DBP, disinfection byproducts; DOC, dissolved organic carbon; DOM, dissolved organic matter; STHMFP, specific trihalomethane formation potential; THM, trihalomethane; THMFP, trihalomethane formation potential.

Efforts to understand the relationship between DOM composition and DBP precursors have largely focused on the aromatic moieties of terrestrially derived humic substances, which are in turn assumed to be derived largely from vascular plant tissues (Kraus et al., 2008; Pomes et al., 1999). Lignin phenols are generally thought to be representative of aromatic DOM from vascular plants (Ertel et al., 1984) and have been used more broadly to highlight changes in DOM composition (Hernes et al., 2007; Opsahl and Benner, 1998), source attribution (Kraus et al., 2008; Dalzell et al., 2007; Eckard et al., 2007), and changes in vascular plant content between discharge regimes (Hernes et al., 2008; Spencer et al., 2008; Dalzell et al., 2005). Several photodegradation studies have demonstrated preferential loss of dissolved lignin relative to bulk DOC and altered lignin composition (Hernes and Benner, 2003; Opsahl and Benner, 1998), but limited data exist to assess changes due to microbial degradation.

Here we report on a laboratory study assessing the impact of microbial degradation of plant leachates on DOC concentrations, composition, and reactivity. The specific goals of the study were (i) to determine the trihalomethane formation potential (THMFP) of DOC leached from four crops and four aquatic macrophytes; (ii) to assess the relationship between THMFP, DOM aromaticity, and lignin phenol content in plant leachates; and (iii) to quantify the loss of DOC and THMFP after 21 d of aerobic microbial degradation, as well as the loss of the expected THM-forming precursors within the pools of aromatic material and lignin phenols. Our results are critical to a better understanding of the role of microbial carbon utilization on THM precursor moieties in ecosystems affected by direct plant leaching.

Materials and Methods

Sample Collection and Leaching

Crop and aquatic plant biomass were collected from the Willow Slough watershed, a 415-km^2 intensive agricultural catchment in the Sacramento Valley of California (Fig. 1). The four common crop types collected for this study were tomato (*Lycopersicon esculentum* Mill.), alfalfa (*Medicago sativa*), white rice (*Oryza sativa*), and orchard grass (*Dactylis glomerata*), which collectively cover 45% of the watershed area during the growing season. The four aquatic macrophytes collected were tule (*Schoenoplectus acutus*), cattail (*Typha* spp.), swamp smartweed (*Polygonum hydropiperoides*), and creeping primrose (*Ludwigia peploides*). Four of the plant types were dicots (tomato, alfalfa, smartweed, and primrose), and four were monocots (rice, grass, cattail, and tule). Biomass samples were collected in September 2006 (middle to late growth stages for crops) by cutting at the soil or water surface and storing in plastic bags on ice in the dark until return to the laboratory. All samples were gently rinsed with organic-free deionized water, air-dried, and stored sealed in plastic bags before leaching.

Biomass was cut into <2.5-cm pieces that included leaves and stems, homogenized, and oven-dried at 60°C before leaching. Approximately 15 g of dried biomass from each plant type was leached in 900 mL of organic-free water in acid-washed, precombusted (460°C), amber glass bottles. After leaching for 4 h at room temperature (24°C) with constant stirring, leachates were filtered through precombusted 0.3-μm GF filters (Advantec MFS, Dublin, CA) and diluted with organic-free deionized water to 40 to 50 mg L^{-1} DOC for biodegradation experiments (started within 48 h).

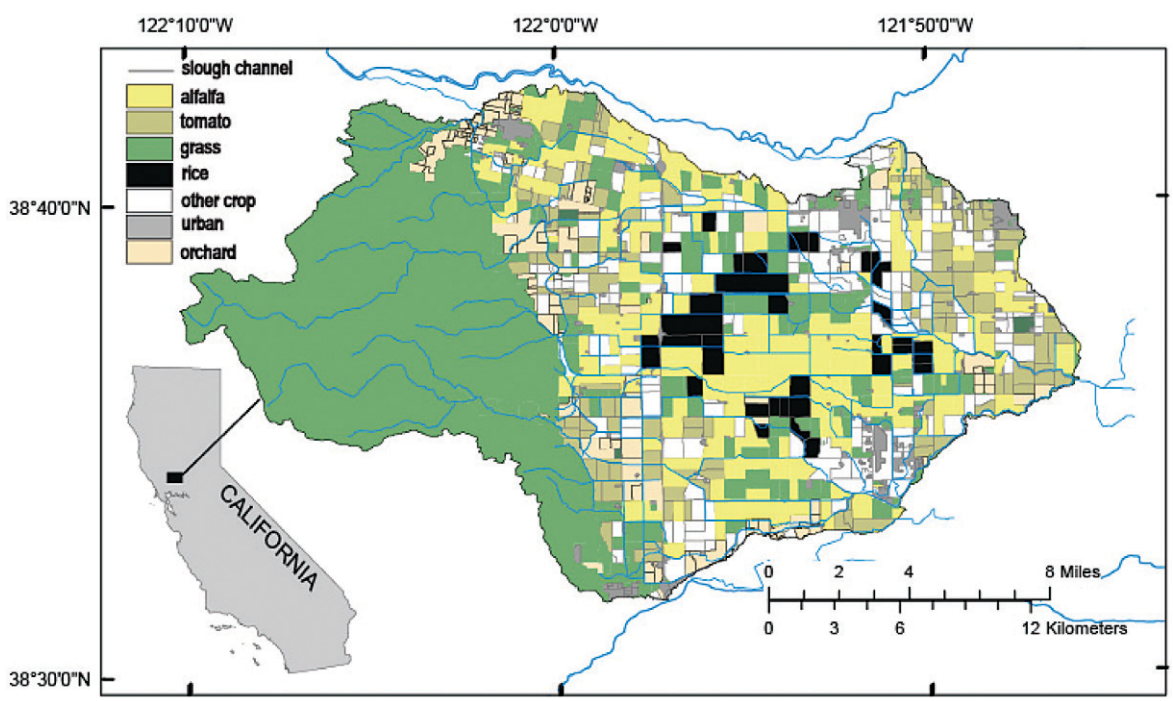

Fig. 1. Location of the Willow Slough watershed and dominant land use types.

Biodegradation Experiment

Plant leachates were inoculated with 3% (v/v) unfiltered surface water collected from the mouth of the Willow Slough watershed, homogenized, and distributed to triplicate 1-L amber bottles. The low concentrations of DOC in inoculum (2.4 mg L^{-1}) and low inoculum-to-sample ratio had no discernible effect on leachate DOC concentrations and composition. Samples were incubated in the dark at 22°C for 21 d and aerated continuously with filtered lab air to prevent anoxic conditions. Although condensation traps were used to allow air to escape while minimizing sample loss from evaporation, lost volume (typically <2% of sample volume) was replaced with organic-free water before sample collection.

Sample aliquots (100 mL) collected from gently shaken triplicate bottles at intervals of 0, 1, 4, 7, 15, and 21 d were syringe filtered through 0.2-μm, pre-rinsed, Whatman nylon capsule filters before analysis. Samples for DOC, total dissolved nitrogen, and THMFP (0 and 21 d only) were preserved by acidification to pH ~2 with high purity HCl, whereas absorbance spectra were run on unacidified samples within 24 h of being collected. Samples for lignin phenol analysis (0 and 21 d only) were frozen before analysis.

Sample Analysis

Dissolved organic carbon (DOC) and total dissolved nitrogen concentrations were measured on acidified samples with a Shimadzu TOC-V CHS analyzer with TNM-1 for total nitrogen analysis (Shimadzu Scientific Instruments, Columbia, MD). Absorption spectra of discrete samples were measured in the laboratory between 200 and 800 nm at constant temperature (25°C) in a 1-cm quartz cell using a spectrophotometer (Cary 300; Varian Inc., Palo Alto, CA) and are expressed as absorption coefficients ($a(\lambda)$, m^{-1}). Values for $SUVA_{254}$ were calculated by dividing UV absorbance measured at $\lambda = 254$ nm by DOC concentration and are reported in units of liters per milligram carbon per meter (Weishaar et al., 2003).

Trihalomethane formation potential was measured using a modified version of USEPA Method 502.2 (Crepeau et al., 2003). Acidified (pH <2) samples were adjusted to approximately pH 8.5 (mean, 8.48 ± 0.10; range, 8.36–8.74) with NaOH before dosing with chlorine as sodium hypochlorite (NaOCl). The dose of NaOCl was determined using a reactivity-based method as described by Crepeau et al. (2003) to leave a residual-free Cl_2 content of 2 to 4 mg L^{-1} at the end of the experiment. Dosed samples were incubated for 7 d at 25°C, quenched using sodium sulfite (Na_2SO_3) to neutralize the remaining free chlorine, and analyzed by purge and trap coupled to an HP 5890 gas chromatograph (Crepeau et al., 2003). Four THM species (chloroform [$CHCl_3$], bromodichloromethane [$CHCl_2Br$], dibromochloromethane [$CHClBr_2$], and bromoform $CHBr_3$]) were measured and summed for total THMFP. The specific THMFP (STHMFP) was calculated as the molar ratio of THM to DOC concentration (mmol THM mol C^{-1}).

Before cupric oxide (CuO) oxidation for lignin measurements, 200 to 250 mL of water was thawed, acidified to pH 2 to minimize precipitation, and rotary evaporated to ~3 mL. The concentrate was transferred to monel reaction vessels (Prime Focus, Inc., Seatle, WA) and dried under vacuum centrifugation in preparation for lignin analysis. Lignin phenols in freeze-dried samples were analyzed as in Hedges and Ertel (1982) with modifications specified by Hernes and Benner (2002) and Eckard et al. (2007). Lignin phenols were trimethylsilyl derivatized using bis-trimethylsilyltrifluoromethylacetamide, and separation of phenols was achieved using an Agilent 6890 gas chromatograph fitted with a DB5-MS capillary column (30 m, 0.25 mm inner diameter) (J&W Scientific, Santa Clara, CA) and interfaced to an Agilent 5973 mass selective detector. Quantification was achieved using selected ion monitoring with cinnamic acid as an internal standard following the five-point calibration scheme of Hernes and Benner (2002). At least one blank was run for every 10 sample oxidations performed, and all samples were blank-corrected due to the presence of trace amounts of contamination in the NaOH reagent. Blank concentrations of lignin phenols were low (40–55 ng) for the eight lignin phenols measured in this study (three vanillyl phenols [vanillin, acetovanillone, vanillic acid], three syringyl phenols [syringaldehyde, acetosyringone, and syringic acid], and two cinnamyl phenols [*p*-coumaric acid, ferulic acid]). Lignin concentrations are reported as the sum of eight syringyl, vanillyl, and cinnamyl concentrations (μg L^{-1}), as well as carbon-normalized lignin yields ($\Lambda 8$, mg 100 mg OC^{-1}) and carbon-normalized vanillyl yield (V, mg 100 mg OC^{-1}). Ratios of cinnamyl to vanillyl phenols (C/V), syringyl to vanillyl phenols (S/V), syringic acid to syringaldehyde ((Ad/Al)s), and vanillic acid to vanillin ((Ad/Al)v) are also reported.

The differences in DOC, $SUVA_{254}$, and THMFP between the initial samples and samples collected after 21 d were assessed by individual plant types using a one-way *t* test. Statistically significant differences are reported as $p < 0.05$ in the text and figures. All statistical analyses were conducted using S-Plus statistical software (Insightful Corp., Seattle, WA). Replicate lignin samples were not analyzed in our study, but lignin phenol concentrations are typically within 5% for replicate samples in our other recent studies of surface water and plant leachates (Spencer et al., 2009; Hernes et al., 2007). Differences in DOC concentrations over time were used to infer the percentage of bioavailable DOC over the 21-d study (del Giorgio and Davis, 2002).

Results and Discussion

Dissolved Organic Carbon Release from Plant Biomass Leaching

Our leaching experiment released 4 to 11% of the initial dry weight of the plant biomass (e.g., 40–114 mg C g^{-1} biomass) as DOC in 4 h, equivalent to 9 to 26% of the initial plant carbon content (Tables 1 and 2). Leaching of crop biomass over a 24-h period showed that 84 to 102% of the DOC leaching occurred within 4 h (data not shown), presumably due to the rapid release of soluble compounds such as simple carbohydrates and sugars (Sanderman and Amundson, 2003; Ibrahima et al., 1995; Benner et al., 1990). Although our results are from a laboratory study, Chow et al. (2009) also reported that 40 to 73% of DOC leaching losses from a field litter study in California woodlands occurred during the first rainfall event. The percentage of biomass leached as DOC was consistent with other

Table 1. Plant biomass carbon and nitrogen %, C/N ratios, isotopes ($\delta^{15}N$ and $\delta^{13}C$), and lignin parameters.

Type	Location	C	N	Atom C/N†	$\delta^{13}C$ (PDB‡)	$\delta^{15}N$ (air)	Λ8	C/V	S/V	(ad/al)v	(ad/al)s
		%			‰						
Alfalfa	crop	43.4	5.8	8.7	−28.14	−0.41	1.74	1.33	0.60	0.39	0.40
Grass	crop	41.3	2.1	23.3	−27.40	2.15	4.55	2.18	1.24	0.27	0.67
Rice	crop	36.3	1.5	28.3	−27.24	8.72	3.78	2.64	1.23	0.20	0.51
Tomato	crop	31.9	1.8	20.9	−29.30	8.06	1.28	0.84	1.54	0.30	0.23
Cattail	wetland	41.4	2.1	22.4	−29.42	8.43	7.93	1.02	1.42	0.30	0.25
Primrose	wetland	38.5	3.9	11.4	−29.59	8.45	0.27	1.02	1.13	0.39	0.38
Smartweed	wetland	37.2	3.7	11.9	−30.93	7.68	1.43	0.60	1.38	0.34	0.30
Tule	wetland	41.0	1.6	30.5	−28.13	7.48	7.75	4.82	1.80	0.19	0.20

† (Ad/Al)s, ratio of syringic acid to syringaldehyde; (Ad/Al)v, ratio of vanillic acid to vanillin; C/V, ratio of cinnamyl to vanillyl phenols; Λ8, carbon normalized lignin yields (mg 100 mg OC^{-1}); S/V, ratio of syringyl to vanillyl phenols.

‡ PDB, Pee Dee Belemnite.

Table 2. Plant leachate dissolved organic carbon concentration, composition, and reactivity on initial samples and degraded samples in our study.

Type	Day	DOC†		TDN		a254		SUVA		TTHMFP		STHMFP	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
		mg L^{-1}		mg L^{-1}		m^{-1}		L mg^{-1} m^{-1}		mg L^{-1}		mmol mol C^{-1}	
Alfalfa	0	41.64	na	5.42	na	68.37	na	0.71	na	1457.12	na	3.51	na
Grass	0	42.04	na	4.28	na	57.84	na	0.61	na	1194.25	na	2.86	na
Rice	0	41.51	na	1.38	na	42.39	na	0.43	na	829.50	na	1.93	na
Tomato	0	45.83	na	2.27	na	96.47	na	0.94	na	2999.88	na	6.71	na
Cattail	0	43.12	na	1.50	na	40.88	na	0.42	na	1002.12	na	2.38	na
Primrose	0	37.74	na	5.36	na	216.55	na	2.05	na	2021.88	na	4.42	na
Smartweed	0	44.62	na	6.63	na	76.87	na	0.88	na	1487.74	na	3.94	na
Tule	0	35.08	na	0.92	na	36.73	na	0.45	na	958.90	na	2.73	na
Alfalfa	21‡	3.41	0.45	4.49	0.28	13.00	0.39	1.68	0.27	119.96	14.90	3.47	0.13
Grass	21	3.31	0.11	2.54	0.21	18.80	1.40	2.47	0.24	153.23	11.45	4.32	0.29
Rice	21	3.44	0.42	0.47	0.12	22.49	1.52	2.86	0.16	269.67	13.27	7.90	1.18
Tomato	21	4.65	0.07	0.79	0.05	43.70	0.97	4.08	0.10	390.13	9.77	8.34	0.33
Cattail	21	2.90	0.41	0.44	0.24	14.57	1.08	2.20	0.24	211.57	33.59	7.14	1.68
Primrose	21	6.73	0.06	3.59	0.42	65.78	5.35	4.25	0.38	456.70	35.15	6.74	0.56
Smartweed	21	3.45	0.14	5.21	0.21	24.51	0.55	3.09	0.06	209.88	9.28	5.96	0.51
Tule	21	3.77	0.28	0.23	0.05	20.40	1.87	2.26	0.27	316.15	3.71	8.28	0.65

† DOC, dissolved organic carbon; STHMFP, specific trihalomethane formation potential; SUVA, specific UV absorbance; TDN, total dissolved nitrogen; TTHMFP, total trihalomethane formation potential.

‡ Values on Day 21 are triplicate means and SD.

plant solubility studies (Osborne et al., 2007; Cleveland et al., 2004; Webster and Benfield, 1986) and indicates a significant and rapid flux of plant-derived carbon to the dissolved phase on contact with water under our extraction regime. Differences in leaching between plants were not explicitly evaluated but likely reflect the complexity of leaf and support tissues as well as morphological and biochemical adaptations to inundation (Crawford, 1996).

Leached organic matter represents only a portion of the whole plant material because the hydrophilic components are more water soluble than the hydrophobic fraction (Benner et al., 1990). Plant leachates in this study exhibited a range of aromaticities as inferred from $SUVA_{254}$ values (Weishaar et al., 2003) with no systematic differences between crops and aquatic macrophytes but generally higher values in dicots (0.71–2.05 L mg C^{-1} m^{-1}) than monocots (0.43–0.61 L mg C^{-1} m^{-1}) (Table 2). The lignin content of plant leachates was less variable. For example, the vanillyl component of lignin (Table 3) showed carbon-normalized yields of 0.22 to 0.29 mg 100 mg OC^{-1} in leachates, with the exception of primrose (0.08 mg 100 mg OC^{-1}). For comparison, vanillyl phenols in surface water at the mouth of the Willow Slough watershed (the source of the plant materials) ranged from 0.07 to 0.62 mg 100 mg OC^{-1} (mean, 0.31 mg 100 mg OC^{-1}) across the hydrograph in 2006 (Hernes et al., 2008), indicating that Willow Slough DOC could largely reflect plant leachates during much of the year.

In our study, fresh leachates formed 1.93 to 6.71 mmol THM mol C^{-1} (Table 2), with >96% of the THM as chloroform ($CHCl_3$; data not shown) due in part to a lack of bromide in the samples. Similarly, Reckhow et al. (2004) and Chow et al. (2009) reported variability in DBP formation potentials in leachates from different plant types in forested and oak woodland systems. Combining the DOC yield from plant leaching and the fresh leachate THMFP in our study suggests that some plants contribute more THM precursor carbon per gram of dry biomass. For example, tomato and alfalfa formed 4.6 to 5.0 times more THM per gram of dry litter than the least reactive plant type (rice), whereas all other plants in our study formed 1.2 to 2.1 times more THM per gram of dry litter relative to rice. Bergamaschi et al. (1999) measured STHMFP values of

6.4 and 6.1 mmol mol C^{-1} in leachates from maize (*Zea maize* L.) and an aquatic bulrush (*Scirpus acutus*) using the same methods, equivalent to approximately 6.9 and 3.4 times higher reactivity compared with rice in our study. The difference in reactivity between plant types likely reflects a large number of structural and morphological differences between the plants but was not explicitly evaluated. The low concentrations of brominated THMs formed with leachates allows for a more direct comparison of DOM reactivity before and after biodegradation but may also explain some of the difference in STHMFP between our study and environmental samples.

Table 3. Lignin concentration and ratios from initial (Day 0) and biodegraded (Day 21) plant leachates.

Type	Day	Σ8†	Λ8	V	C/V	S/V	(Ad/Al)s	(Ad/Al)v
Alfalfa	0	410.2	0.98	0.29	1.61	0.83	0.99	0.52
Grass	0	335.5	0.81	0.24	1.45	0.88	1.01	1.23
Rice	0	370.7	0.86	0.29	1.08	0.93	1.03	0.89
Tomato	0	346.6	0.78	0.24	0.97	1.25	0.63	0.71
Cattail‡	0	nd	nd	nd	nd	nd	nd	nd
Primrose	0	112.8	0.25	0.08	1.05	0.87	0.74	1.31
Smartweed	0	267.9	0.71	0.21	1.33	1.08	1.02	1.01
Tule	0	245.2	0.70	0.21	1.08	1.26	0.55	0.71
Alfalfa	21	8.3	0.24	0.07	1.26	1.29	1.53	1.14
Grass	21	19.3	0.58	0.22	0.83	0.79	0.84	0.94
Rice	21	40.2	1.17	0.33	1.42	1.11	1.45	1.01
Tomato	21	39.4	0.85	0.26	1.03	1.22	0.80	0.69
Cattail	21	22.6	0.78	0.22	1.32	1.19	0.89	1.05
Primrose	21	17.9	0.27	0.09	1.01	0.96	0.48	0.96
Smartweed	21	8.1	0.23	0.09	0.80	0.90	0.80	1.50
Tule	21	43.9	1.16	0.33	0.76	1.74	0.73	0.64

† (Ad/Al)s, ratio of syringic acid to syringaldehyde; (Ad:Al)v, ratio of vanillic acid to vanillin; C/V, ratio of cinnamyl to vanillyl phenols; Σ8, sum of eight syringyl, vanillyl, and cinnamyl concentrations (μg L^{-1}); Λ8, carbon normalized lignin yields (mg 100 mg OC^{-1}); S/V, ratio of syringyl to vanillyl phenols; V, carbon normalized vanillyl yield (mg 100 mg OC^{-1}).

‡ For cattail, Day 0 sample lost during sample processing.

Effects of Biodegradation on Dissolved Organic Carbon Concentration and Lignin Phenols

Although leaching of plant litter produced high yields of DOC, time course samples showed that DOC concentrations decreased by 85 to 92% over the 21-d biodegradation study (Fig. 2). Previous studies of plant leachate DOC have also reported rapid utilization by heterotrophic bacteria (Mann and Wetzel, 1996; Findlay et al., 1986; Kaplan and Bott, 1983) and high overall consumption during incubation studies (Cleveland et al., 2004; Anesio et al., 2000) due to the preferential consumption of highly labile, low-molecular-weight compounds leached from fresh litter (Maie et al., 2006; Ibrahima et al., 1995; Benner et al., 1990). In comparison, DOM in surface waters is typically much more recalcitrant, with average DOC losses typically ranging from 9 to 37% during short-term microbial incubations of river and marsh waters (del Giorgio and Davis, 2002). Similarly, Sacramento–San Joaquin River Delta waters have low DOC bioavailability, with 10 to 12% DOC loss during short-term assays (Stepanauskas et al., 2005; Sobczak et al., 2002).

Compositional differences between the fresh leachate and the residual pool of DOM after biodegradation are evidenced by $SUVA_{254}$ and lignin phenol concentrations and composition. Decreases in lignin phenol concentrations (Σ_8) of 64 to 96% in plant leachates (Fig. 3a) were similar to the percentage loss of bulk DOC concentrations, while increased $SUVA_{254}$ after degradation (Table 2) reflects a relative increase in aromatic moieties. One possible explanation for the high lignin loss despite significant energetic costs to lignin decomposition is that it protects a substantial amount of simpler carbon (e.g., the lignocellulose complex) and nitrogen-rich DOM (Sanderman and Amundson, 2003). Lignin loss from leachates was also greater than the 16 to 21% observed in short-term incubations of surface water from the Mississippi River (Hernes and Benner, 2003; Opsahl and Benner, 1998), indicating that fresh plant leachate contains lignin that is more labile than found in highly processed riverine DOM. In addition, the leaching of highly labile, lignin-rich DOM has been observed for northern high-latitude catchments during spring flushing events that activate

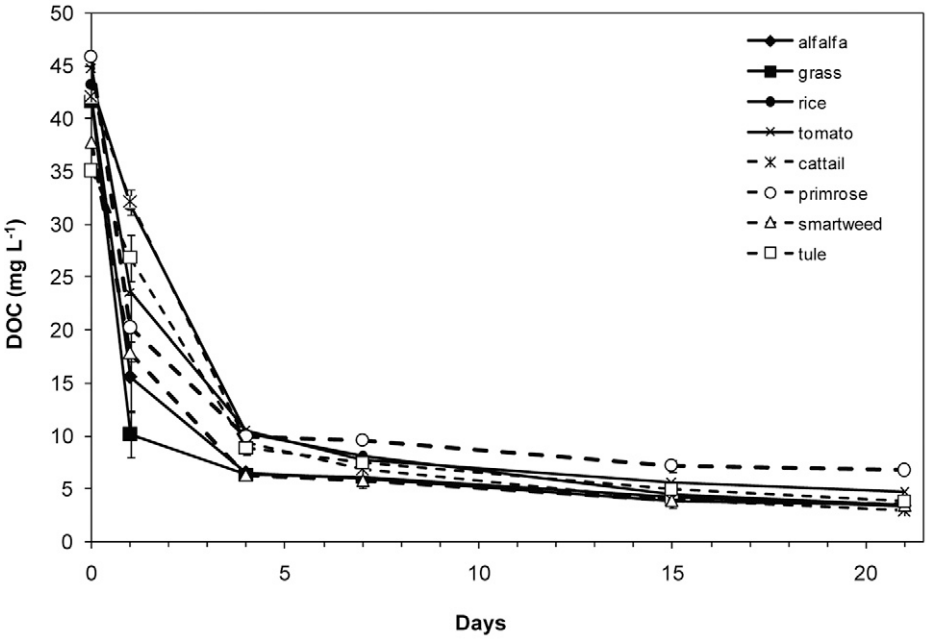

Fig. 2. Average dissolved organic C concentrations and standard deviation in aerobic plant leachate samples over 21 d of biodegradation (dark incubation at 22°C).

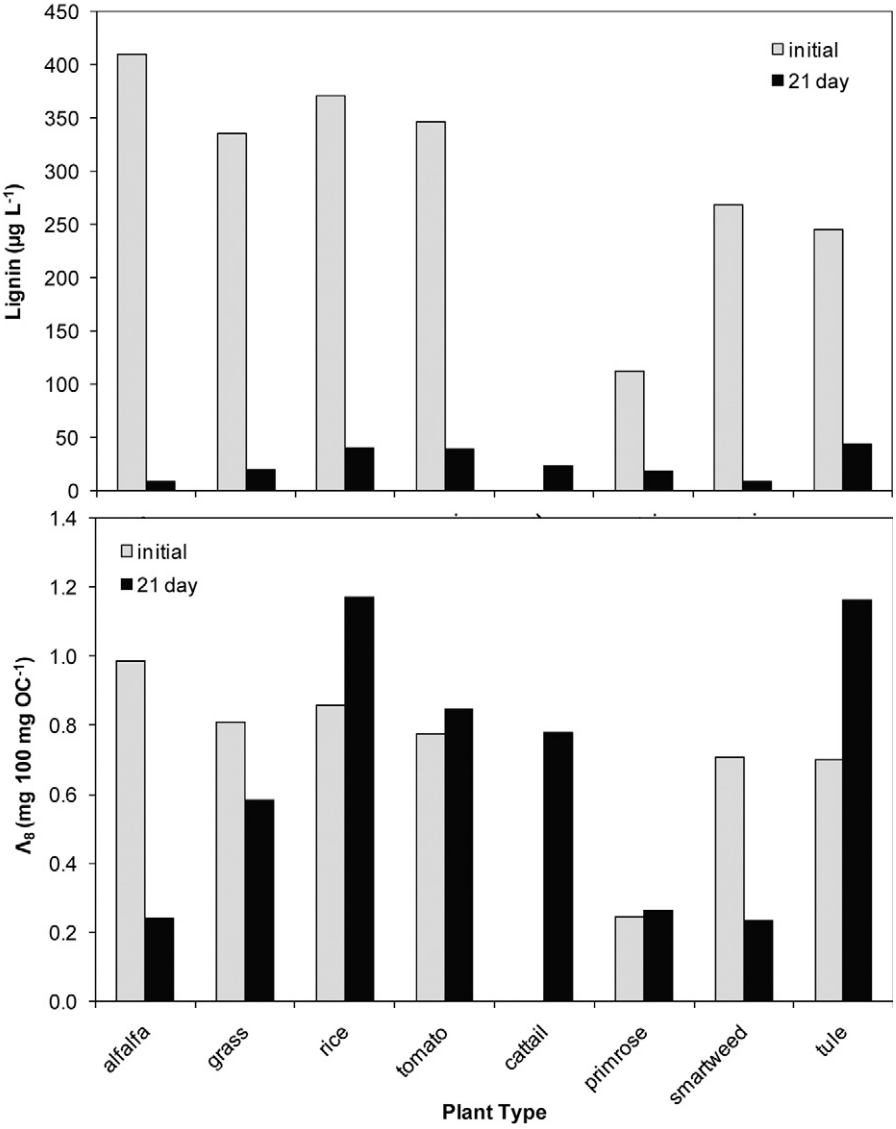

Fig. 3. (a) Lignin phenol concentrations (µg L^{-1}) in plant leachates at Day 0 and Day 21 and (b) carbon-normalized lignin phenol yield (Λ8, mg 100 mg OC^{-1}).

shallow or surface soil flow paths (Spencer et al., 2008; Holmes et al., 2008). For compounds other than lignin, another possible cause of the observed compositional changes on degradation is conversion to microbially produced DOM. However, a synthesis by del Giorgio and Cole (1998) reported median bacterial growth efficiencies of 0.3 (range, 0.04–0.92) on vascular plant–derived DOC, indicating that DOC loss in our experiment was likely due more to respiration than to bacterial production.

Previous studies have suggested that the distribution of lignin monomers and changes in Λ_8 and acid/aldehyde ratios can be used as indicators of the organic material source and relative diagenetic state (Opsahl and Benner, 1995; Hedges and Welicky, 1989). Although it is tempting to use these parameters to assess sources and composition of THM precursors, the lack of trends in Λ_8, acid/aldehyde ratios, C/V, and S/V (Fig. 3b and Table 3) suggest that the lignin content of leachates differs significant from source litter and is not consistently altered by microbial degradation. Average lignin ratios in degraded leachate (C/V, S/V, and (Ad/Al)v) were comparable with Willow Slough watershed surface water values reported in Hernes et al. (2008), indicating that biodegraded leachates are viable sources of surface water DOM. However, additional sources must be present or additional processing must occur. For example, direct linkages between plant leaching studies and lignin content or THM precursor formation in watersheds are influenced by interactions of plant-derived organic matter with soils (Hernes et al., 2007) and photolytic processes altering DOM concentration and composition (Hernes and Benner, 2003; Opsahl and Benner, 1998). The apparent discrepancy between increasing $SUVA_{254}$ values (Fig. 4) and the lack of an apparent trend in carbon-normalized lignin yields suggests that lignin was not a significant component of the pool of UV_{254} absorbing chromophores in all the samples or that minor structural changes shifted absorbing lignin compounds outside of our analytical capabilities in some samples.

Effects of Biodegradation on Trihalomethane Formation Potential

Understanding the role of biodegradation on DBP precursors is critical given that degraded leachates are often representative of the fraction of DOM entering rivers. The linkage between DOC loss and the 67 to 92% decrease in THMFP after biodegradation (Table 2) is supported by correlations between DOC concentration and THM formation reported in the literature (Kraus et al., 2008; Chow et al., 2007). However, the carbon-normalized THMFP yield (e.g., STHMFP) increased by 1.2 to 4.1 times after biodegradation for all samples except alfalfa, indicating that residual DOC formed on average 2.5 (±1.6) times more THM per mole of carbon than the DOC lost during the experiment (Fig. 4). In a similar study, Reckhow et al. (2007) found that STHMFP was 1.5 to 3 times higher after biodegradation of leachates from fresh maple, oak, and pine litter. Engelage et al. (2009) also found an increased propensity of wetland-derived DOC for form THMs after biodegradation but found no trend in agricultural runoff. The higher STHMFP of residual DOC is consistent with the preferential loss of nonprecursor material and a greater THM yield from compounds more resistant to microbial decay (Hua and Reckhow, 2007; Reckhow et al., 2004). However, the calculated STHMFP values for the DOC lost during the experiment (1.42–6.53 mmol mol C^{-1}; Fig. 4) indicate that labile compounds are also susceptible to halogenation and THM formation (Kanokkantapong et al., 2006; Pomes et al., 1999).

A comparison of our results to equivalent THM formation data showed that fresh plant leachates typically formed less THM per mole of carbon (1.93–6.72 mmol mol C^{-1}) than surface water from the Sacramento-San Joaquin Delta (7.0–12.7 mmol mol C^{-1}) (Kraus et al., 2008; Fleck et al., 2004). Similarly, surface water collected from the mouth of the Willow Slough watershed and used for inoculating leachates had a STHMFP of 7.69 mmol mol C^{-1} on the sampling date at the start of the experiment (data not shown). A similar observation of lower specific reactivity in fresh leachates relative to most natural waters was also observed by Chow et al. (2009) and Reckhow et al. (2004) in their respective studies. Our study showed that biodegradation of leachates resulted in STHMFPs (Fig. 5) closer to published surface water values, suggesting that DOM is a mixture of fresh and degraded material or that rapid changes in fresh vascular plant–derived DOM determine the THM precursor potential in surface waters.

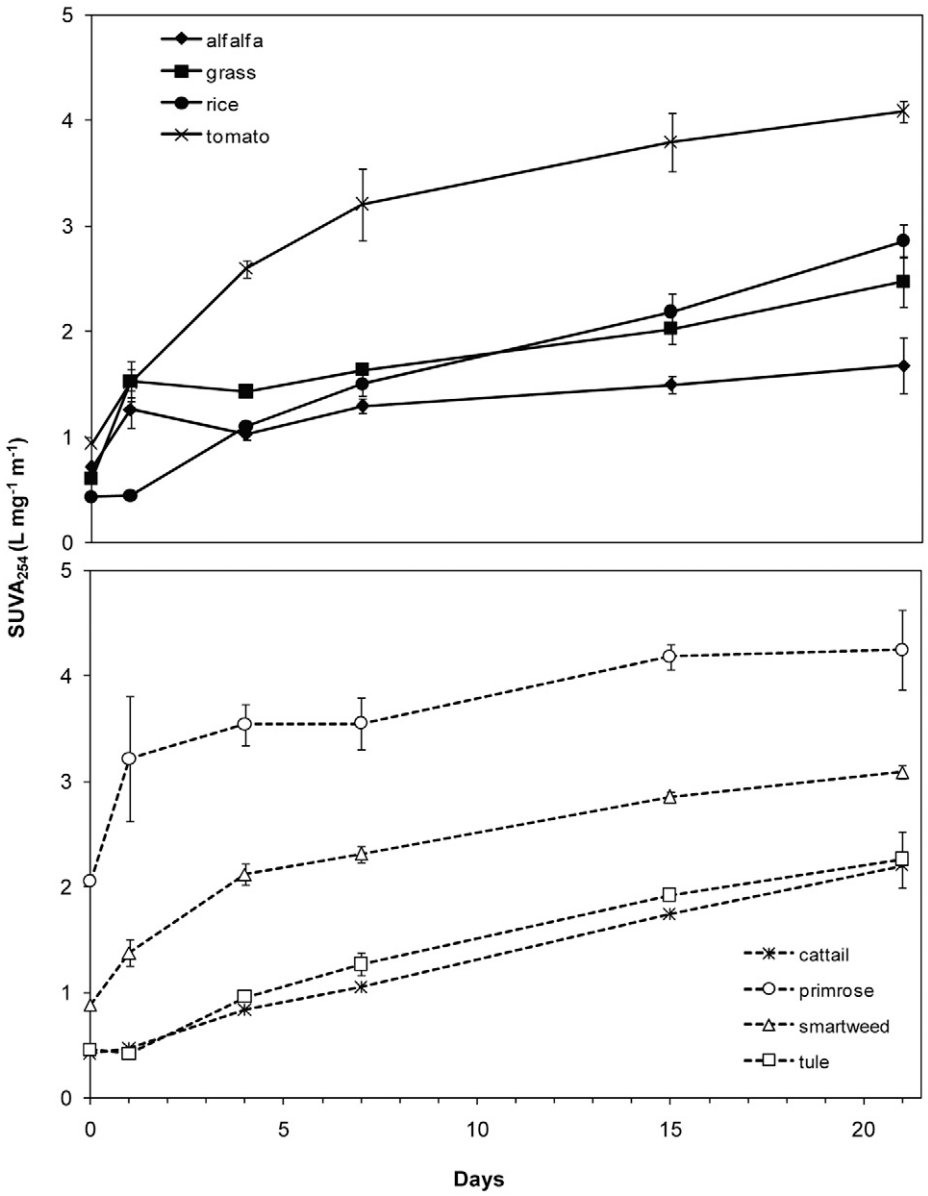

Fig. 4. Changes in specific UV absorbance (L mg C^{-1} m^{-1}) and standard deviation of plant leachates during the 21 d aerobic biodegradation study (dark incubation at 22°C).

Conclusions and Implications

Although our results show that labile, less aromatic DOM forms fewer THMs per unit carbon than recalcitrant DOM, the physical and chemical characteristics of DBP precursors are still not well understood (Chow et al., 2005). The lack of an increasing trend in Λ_8 after biodegradation despite increased STHMFP and increased $SUVA_{254}$ also indicates a disconnect between lignin yields and chlorine-reactive DOM moieties in our samples. Although the DBP precursors in this experiment are vascular plant derived, it appears that the leached precursors constitute a pool of vascular plant carbon that is not accurately represented by lignin phenols. Alternatively, some of the residual DOC could have been related to altered microbial compounds released during degradation (Kawasaki and Benner, 2006) but not explicitly addressed with our simple approach to calculating the STHMFP of lost DOM. In addition, our results highlight potential challenges of DOM source attribution using molecular-level lignin as a tracer when lignin lability is high and phase changes due to leaching and microbial degradation are not considered (Hernes et al., 2007; Dalzell et al., 2007).

The processes that were measured in this study—plant leaching of DOC followed by the microbial degradation of labile compounds—are fundamental ecosystem dynamics occurring in rivers and streams regardless of the type of heterotrophic microbes present. The leaching rates and the percentage loss or trajectories of DOC consumption may differ over space and time but not in the fundamental processes whereby microbes preferentially consume organic matter that is more labile and less likely to form THMs. Therefore, future work to identify the recalcitrant, vascular plant–derived DOM components with a greater propensity to form THMs and other DBPs is clearly needed.

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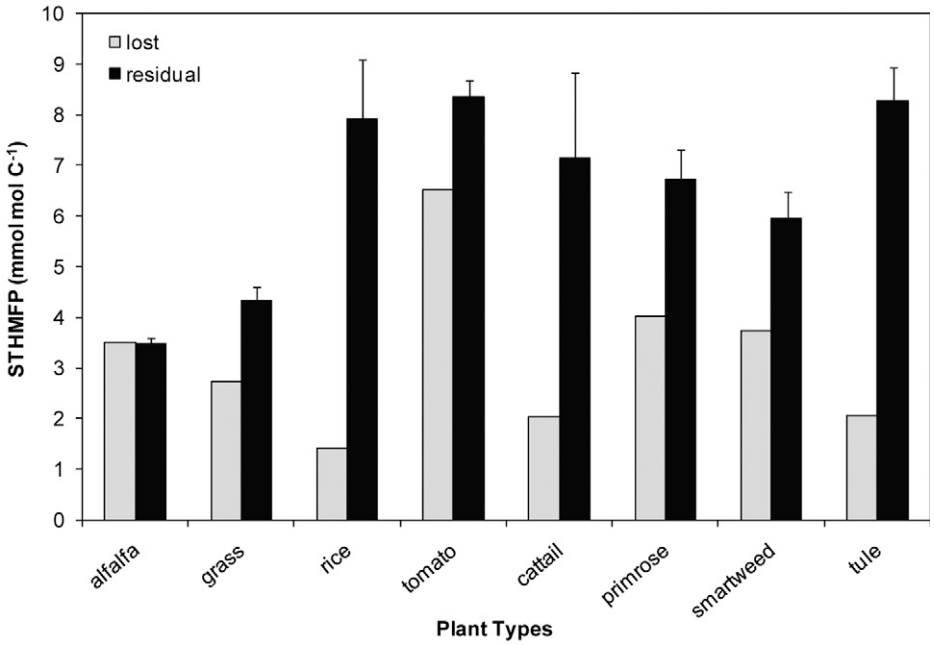

Fig. 5. Specific trihalomethane formation potential (STHMFP) of the residual dissolved organic C (DOC) at the end of the 21-d biodegradation experiment and the calculated STHMFP of the DOC lost to microbial uptake. All samples differ significant at $p < 0.05$ except for alfalfa ($p = 0.69$). Bars represent the standard deviations of triplicate samples.

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