Supporting Information

Growing Algae Alter Spectroscopic Characteristics and Chlorine Reactivity of Dissolved Organic Matter from Thermally-Altered Forest Litters

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MATERIALS AND METHODS

Analyses of Water Chemistry and DOM spectroscopic characteristics

Characteristics of water extracts and algal solution, including pH, specific conductivity, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), ammonium (NH_4^+), and nitrate/nitrite (NO_x^-), were analyzed using standard methods adopted by Wang et al.[1] The pH and specific conductivity were measured using an Accumet XL60 dual channel pH/Ion/Conductivity meter. The DOC and TDN were determined by a Shimadzu TOC/TN analyzer (SM 5310B). The NH_4^+ and NO_x^- were measured using a Systea® Easychem™ discrete analyzer (EPA 350.1-01 and 353.2-01). Dissolved organic nitrogen (DON) was calculated by subtracting dissolved inorganic N (NH_4^+-N + NO_x^--N) from TDN.

DOM was characterized by UV-VIS spectrometry (Shimadzu UV-1800). Specific UV absorbance at 254 nm ($SUVA_{254}$ in L mg-C^{-1} m^{-1}), an indicator for aromaticity, was calculated by normalizing UV absorbance at 254 nm to DOC level. The E2/E3 ratio, an optical index which is inversely correlated with molecular size of aquatic humic substance, was calculated as absorbance at 254 nm divided by absorbance at 365 nm.[2] Additionally, DOM was also characterized by 3D spectrofluorometry (Shimadzu Spectrofluorometer RF5301). The fluorescence scans [excitation wavelength (Ex): 220-450 nm; emission wavelength (Em): 280-550 nm] for DOM were conducted with 5-nm slits for both excitation and emission. Fluorescence excitation-emission matrices (EEMs) from 3-D spectrofluorometry were analyzed by fluorescence regional integration (FRI).[3] The raw EEM was corrected for instrument-dependent effects, inner-filter effects, and Raman effects, and standardized to Raman's units (normalized to Raman peak at Ex 350 nm).[4] FRI can be used to quantify the fluorescent DOM by dividing EEM into five operationally-defined regions [I: tyrosine-like (Ex: 200-250 nm; Em:

280-330 nm); II: tryptophan-like (Ex: 200-250 nm; Em: 330-380 nm); III: fulvic acid-like (Ex: 200-250 nm; Em: 380-550 nm); IV: soluble microbial byproduct-like (250 nm < Ex < 400 nm; Em: 280-380 nm); and V: humic acid-like (250 nm < Ex < 400 nm; Em: 380-550 nm)].[3] The percent fluorescent response in each region ($P_{i,n}$ for the proportion of area-normalized volume in region i to the entire region) was calculated. Three fluorescence spectroscopic indices were used to describe DOM characteristics.[5] The humification index (HIX), an index of humic substance content, was determined as the area under the emission spectra 435–480 nm divided by the sum of peak areas 300–345 nm, at Ex 254 nm.[6] The fluorescence index (FI), an index of degradation degree of DOM, was calculated as the ratio of Em at 470 and 520 nm, at Ex 370 nm.[7] The freshness index (β/α), an index for the contribution of recently produced autochthonous DOM, was calculated as the ratio of Em at 380 nm divided by the Em maximum between 420 and 435 nm, at Ex 310 nm.[8] β and α peaks present the abundance of marine humic-like and terrestrial humic-like components, respectively.

Analyses of Disinfection Byproducts

The samples were diluted with Milli-Q water to a DOC concentration of 3 mg/L, buffered by H_3BO_3/NaOH solution to pH 8.0, and chlorinated with freshly prepared NaOCl/H_3BO_3 solution (pH 8.0) in 64-mL incubation tubes at 25 ^{o}C in dark for 24 hours without headspace.[9] The chlorine concentration added to the sample was calculated according to the equation $[Cl_2] = [3 \times (DOC) + 7.6 \times (TDN)]$.[1] After reaction, the residual chlorine concentration was measured using a Pocket Colorimeter™ II Filter Photometer (Hach Company). The residual chlorine was quenched by a 10% Na_2SO_3 solution and DBPs were extracted and quantified by GC-ECD (Agilent 7890) following EPA method 551.1.[1] Chlorine reactivity of DOM was expressed as specific chlorine demand. Specific chlorine demand (SCD) was calculated by dividing chlorine demand by DOC concentration (mg-Cl_2/mg-DOC), where chlorine demand was the difference in chlorine concentration added and residual chlorine concentration. We quantified four trihalomethanes (THMs; including trichloro-, dichlorobromo-, dibromochloro-, and tribromo- methanes), four haloacetonitriles (HANs; including trichloro-, dichloro-, bromochloro-, and dibromo- acetonitriles), chloral hydrate (CHD), and three haloketones (HKs; including 1,1-dichloro-2-, 1,1,1-trichloro-2-, 1,2,3-trichloro- propanones). The MRLs for all the above DBP species were approximately 0.1-0.3 μg/L. The DOM reactivity in DBP formation potential was expressed as specific DBP-FP (μg-DBP/mg-DOC), which was calculated by dividing the DBP concentration with the initial DOC concentration. The proportion of total halogen positions with bromine-substituted atoms (bromine incorporation factor, BIF) was calculated for trihalomethanes using the equation of study by Huang et al.[10]

RESULTS AND DISCUSSION

Water Quality and Algal Growth

Composition of cultural medium substantially affects algal growth as well as chemistry of algal solution.[11,12] Noticeably, BG11 medium has been extensively used for culturing algae to study DBP formation from algae-produced organic matters.[13–15] However, original BG11 medium consists of 6 mg/L of citric acid and 6 mg/L ferric ammonium citrate.[16] Citric acid has been identified as a precursor for formations of trihalomethanes and haloacetic acids during water chlorination.[17,18] In order to prevent uncertainty in the analyses of algae-produced organic matters and related DBP formation potential, in this study we minimized DOC concentration in the medium.

For the control, measured DOC and TDN concentrations in the medium were 0.7 ± 0.0 mg-DOC/L and 11.8 ± 0.2 mg-DTN/L (Table 1), mainly contributed from Na_2EDTA and $NaNO_3$ in the composition (Table S1). OD_{680} values in the control consistently increased from 0.06 to 1.00 for *P. subcapitata* and from 0.05 to 0.37 for *M. aeruginosa*, indicating the increases of algal biomass over time (Figure 1A). Concomitantly, DOC concentrations increased from 0.7 ± 0.0 to 2.4 ± 0.1 and 11.6 ± 0.2 mg/L for *P. subcapitata* and *M. aeruginosa*, respectively (Figure 1B), indicating the presence of algae-produced organic matter. Also, TDN concentrations decreased from 11.8 ± 0.2 to 0.5 ± 0.0 and 1.3 ± 0.0 mg/L for *P. subcapitata* and *M. aeruginosa*, respectively (Figure 1C), indicating the uptake of nitrate by algae. These results demonstrated that both algal species were able to exponentially grow in this medium during the experiment.

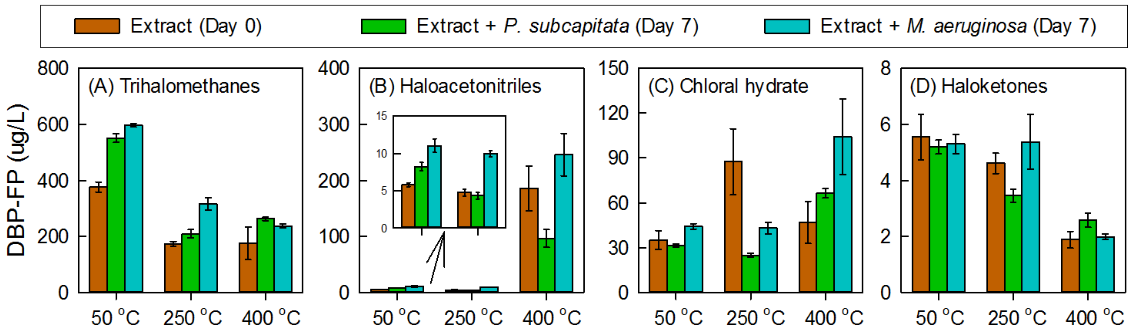

Figure S1. Disinfection byproduct formation potential (DBP-FP) of thermally-altered extracts before (Day 0) and after (Day 7) inoculations with *P. subcapitata* and *M. aeruginosa*. Error bars represent the standard deviation.

Table S1. The composition of algal culture medium.

Chemical	Stock solution (g/L)	Final concentration (mg/L)
Macronutrients		
$NaNO_3$	7.5	75
K_2HPO_4	2	20
$MgSO_4·7H_2O$	7.5	75
$CaCl_2·2H_2O$	3.6	36
Na_2CO_3	2	20
Micronutrients		
$Na_2EDTA·2H_2O$	2	2
H_3BO_3	2.86	2.86
$FeCl_3·6H_2O$	1	1
$MnCl_2·4H_2O$	1.81	1.81
$ZnSO_4·7H_2O$	0.22	0.22
$Na_2MnO_4·2H_2O$	0.39	0.39
CuSO4·5H2O	0.079	0.079
$Co(NO_3)_2·6H_2O$	0.0494	0.0494

Table S2. Total dissolved nitrogen (TDN), dissolved inorganic nitrogen (NH_4^+-N and NO_x^--N), and dissolved organic nitrogen (DON) of litter extracts before (Day 0) and after (Day 7) inoculations with *P. subcapitata* and *M. aeruginosa*.

	Extract + Algae (50 °C)	Extract + Algae (250 °C)	Extract + Algae (400 °C)
P. subcapitata			
Day 0			
TDN (mg/L)	13.1 ± 0.3	10.5 ± 0.1	11.6 ± 0.2
NH_4^+-N (mg/L)	0.3 ± 0.2	0.6 ± 0.3	0.4 ± 0.0
NO_x^--N (mg/L)	11.0 ± 0.0	14.5 ± 1.3	11.6 ± 1.0
DON (mg/L)	1.8 ± 0.5	ND	0.2 ± 0.3
Day 7			
TDN (mg/L)	0.7 ± 0.0	0.5 ± 0.0	1.9 ± 0.3
NH_4^+-N (mg/L)	0.1 ± 0.1	0.9 ± 0.1	0.6 ± 0.1
NO_x^--N (mg/L)	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.5
DON (mg/L)	0.5 ± 0.1	ND	0.9 ± 0.7
M. aeruginosa			
Day 0			
TDN (mg/L)	12.4 ± 0.3	9.3 ± 0.0	13.6 ± 0.2
NH_4^+-N (mg/L)	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
NO_x^--N (mg/L)	11.3 ± 0.1	12.5 ± 0.2	14.4 ± 1.0
DON (mg/L)	0.8 ± 0.2	ND	ND
Day 7			
TDN (mg/L)	1.1 ± 0.1	1.1 ± 0.1	1.9 ± 0.1
NH_4^+-N (mg/L)	0.1 ± 0.0	0.6 ± 0.4	0.5 ± 0.0
NO_x^--N (mg/L)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
DON (mg/L)	1.0 ± 0.1	0.5 ± 0.4	1.4 ± 0.1

ND: Not detectable

Table S3. Correlation coefficients (R^2) for specific DBP formation potential and DOM spectroscopic index (n =9).

	STHM-FP			SHAN-FP			SCHD-FP			SHK-FP		
	Before inoculation	After inoculation with Ps*	After inoculation with Ma#	Before inoculation	After inoculation with Ps*	After inoculation with Ma#	Before inoculation	After inoculation with Ps*	After inoculation with Ma#	Before inoculation	After inoculation with Ps*	After inoculation with Ma#
SUVA	-0.31	-0.01	-0.07	0.99	0.91	0.89	-0.11	0.90	0.78	-0.76	-0.65	-0.34
HIX	-0.39	-0.15	-0.14	0.99	0.97	0.99	-0.08	0.99	0.96	-0.96	-0.84	-0.46
E2/E3	-0.68	-0.14	-0.09	0.90	0.96	0.87	0.00	0.98	0.95	-0.49	-0.86	-0.33
FI	-0.72	-0.18	-0.17	0.86	0.93	0.96	0.00	0.97	0.89	-0.72	-0.85	-0.50
β/α	0.08	-0.04	0.13	-0.83	-0.70	-0.92	0.38	-0.65	-0.87	0.93	0.39	0.46
$\%P_{I,n}$	0.74	0.37	0.13	-0.85	-0.88	-0.93	-0.01	-0.92	-0.89	0.42	0.90	0.46
$\%P_{II,n}$	0.39	0.29	-0.11	-0.98	-0.91	-0.38	0.06	-0.96	-0.42	0.73	0.86	0.00
$\%P_{III,n}$	-0.44	-0.26	-0.09	0.99	0.93	0.96	-0.04	0.97	0.91	-0.67	-0.87	-0.40
$\%P_{IV,n}$	0.34	0.26	0.29	-0.99	-0.93	-0.94	0.09	-0.96	-0.85	0.75	0.85	0.62
$\%P_{V,n}$	-0.72	-0.36	-0.01	0.86	0.87	0.78	0.00	0.92	0.79	-0.45	-0.85	-0.23

Ps* and Ma# represent *P. subcapitata* and *M. aeruginosa*, respectively.
Highlight values with different colors indicate significant correlation ($P < 0.05$) for a specific DBP species.
Negativity symbols indicate negative correlations.

Table S4. Correlation coefficients (R^2) for DBP formation potential and DOM spectroscopic index (n = 9).

	THM-FP			HAN-FP			CHD-FP			HK-FP		
	Before inoculation	After inoculation with Ps*	After inoculation with Ma#	Before inoculation	After inoculation with Ps*	After inoculation with Ma#	Before inoculation	After inoculation with Ps*	After inoculation with Ma#	Before inoculation	After inoculation with Ps*	After inoculation with Ma#
SUVA	-0.29	-0.01	-0.32	0.99	0.92	0.89	-0.25	0.97	0.72	-0.80	-0.29	-0.46
HIX	-0.35	-0.16	-0.43	0.99	0.97	0.99	-0.21	0.96	0.93	-0.90	-0.58	-0.58
E2/E3	-0.66	-0.15	-0.31	0.89	0.96	0.88	-0.02	0.96	0.97	-0.81	-0.56	-0.43
FI	-0.70	-0.19	-0.48	0.86	0.93	0.96	-0.01	0.92	0.84	-0.98	-0.63	-0.64
β/α	0.06	-0.03	0.44	-0.84	-0.72	-0.91	0.56	-0.81	-0.80	0.57	0.06	0.62
%$P_{I,n}$	0.72	0.38	0.46	-0.84	-0.87	-0.93	0.00	-0.81	-0.81	0.78	0.80	0.63
%$P_{II,n}$	0.37	0.30	0.00	-0.98	-0.90	-0.37	0.17	-0.86	-0.32	0.85	0.73	0.04
%$P_{III,n}$	-0.42	-0.27	-0.39	0.99	0.93	0.95	-0.14	0.89	0.84	-0.83	-0.70	-0.56
%$P_{IV,n}$	0.31	0.27	0.56	-0.99	-0.92	-0.94	0.22	-0.88	-0.84	0.81	0.69	0.70
%$P_{V,n}$	-0.70	-0.37	-0.25	0.86	0.86	0.78	-0.00	0.80	0.68	-0.81	-0.79	-0.41

Ps* and Ma# represent *P. subcapitata* and *M. aeruginosa*, respectively.
Highlight values with different colors indicate significant correlation ($P < 0.05$) for a specific DBP species.
Negativity symbols indicate negative correlation.

REFERENCES

(1) Wang, J. J.; Dahlgren, R. A.; Erşan, M. S.; Karanfil, T.; Chow, A. T. Wildfire altering terrestrial precursors of disinfection byproducts in forest detritus. *Environ. Sci. Technol.* **2015**, *49* (10), 5921–5929.

(2) Peuravuori, J.; Pihlaja, K. Molecular size distribution and spectroscopic properties of aquatic humic substances. *Anal. Chim. Acta* **1997**, *337* (2), 133–149.

(3) Chen, W.; Westerhoff, P.; Leenheer, J. a; Booksh, K. Fluorescence excitation - Emission matrix regional integration to quantify spectra for dissolved organic matter. *Environ. Sci. Technol.* **2003**, *37* (24), 5701–5710.

(4) Murphy, K. R.; Butler, K. D.; Spencer, R. G. M.; Stedmon, C. a; Boehme, J. R.; Aiken, G. R. Measurement of Dissolved Organic Matter Fluorescence in Aquatic Environments: An Interlaboratory Comparison RID B-8217-2009 RID B-5841-2008. *Environ. Sci. Technol.* **2010**, *44* (24), 9405–9412.

(5) Fellman, J. B.; Hood, E.; Spencer, R. G. M. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnol. Oceanogr.* **2010**, *55* (6), 2452–2462.

(6) Ohno T. Fluorescence Inner - Filtering Cerrection for Determining the Humification Index of Dissolved Organic Matter. *Environ. Sci. Technol.* **2002**, *36* (4), 742–746.

(7) Cory, R. M.; Mcknight, D. M. Fluorescence Spectroscopy Reveals Ubiquitous Presence of Oxidized and Reduced Quinones in Dissolved Organic Matter Fluorescence Spectroscopy Reveals Ubiquitous Presence of Oxidized and Reduced Quinones in Dissolved Organic Matter. *Environ. Sci. Technol.* **2005**, *39* (21), 8142–8149.

(8) Wilson, H. F.; Xenopoulos, M. a. Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nat. Geosci.* **2008**, *2* (December 2008), 37–41.

(9) Summers, R. S.; Hooper, S. M.; Shukairy, H. M.; Solarik, G.; Summers, R. S.; Hooper, S. M.; Owen, D. Assessing DBP yield : uniform formation conditions. *J. Am. Water Work. Assoc.* **1996**, *88* (6), 80–93.

(10) Huang, J.; Graham, N.; Templeton, M. R.; Zhang, Y.; Collins, C.; Nieuwenhuijsen, M. A comparison of the role of two blue-green algae in THM and HAA formation. *Water Res.* **2009**, *43* (12), 3009–3018.

(11) Kilham, S. S.; Kreeger, D. A.; Lynn, S. G.; Goulden, C. E.; Herrera, L. COMBO: A defined freshwater culture medium for algae and zooplankton. *Hydrobiologia* **1998**, *377*, 147–159.

(12) Huang, W.; Chu, H.; Dong, B. Characteristics of algogenic organic matter generated under different nutrient conditions and subsequent impact on microfiltration membrane fouling. *Desalination* **2012**, *293*, 104–111.

(13) Fang, J.; Ma, J.; Yang, X.; Shang, C. Formation of carbonaceous and nitrogenous disinfection by-products from the chlorination of *Microcystis aeruginosa*. *Water Res.*

2010, *44* (6), 1934–1940.

(14) Liao, X.; Liu, J.; Yang, M.; Ma, H.; Yuan, B.; Huang, C.-H. Evaluation of disinfection by-product formation potential (DBPFP) during chlorination of two algae species — Blue-green *Microcystis aeruginosa* and diatom *Cyclotella meneghiniana*. *Sci. Total Environ.* **2015**, *532*, 540–547.

(15) Zhou, S.; Shao, Y.; Gao, N.; Deng, Y.; Li, L.; Deng, J.; Tan, C. Characterization of algal organic matters of *Microcystis aeruginosa*: Biodegradability, DBP formation and membrane fouling potential. *Water Res.* **2014**, *52*, 199–207.

(16) Stanier, R. Y.; Kunisawa, R.; Mandel, M.; Cohen-Bazire, G. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev.* **1971**, *35* (2), 171–205.

(17) Chowdhury, S.; Al-hooshani, K.; Karanfil, T. Disinfection byproducts in swimming pool: Occurrences, implications and future needs. *Water Res.* **2014**, *53*, 68–109.

(18) Kanan, A.; Karanfil, T. Formation of disinfection by-products in indoor swimming pool water: The contribution from filling water natural organic matter and swimmer body fluids. *Water Res.* **2011**, *45* (2), 926–932.