5-2017

Alterations of Disinfection Byproduct Formation Following Exposures of Algae to Wildfire Ash Solutions and Copper Algaecide

Kuo-Pei Tsai
Clemson University

ALTERATIONS OF DISINFECTION BYPRODUCT FORMATION FOLLOWING EXPOSURES OF ALGAE TO WILDFIRE ASH SOLUTIONS AND COPPER ALGAECIDE

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Wildlife and Fisheries Biology

---

by
Kuo-Pei Tsai
May 2017

Accepted by:
Dr. Alex T. Chow, Committee Chair
Dr. Tanju Karanfil
Dr. Elizabeth Carraway
Dr. Nishanth Tharayil

ABSTRACT

Previous studies demonstrated that wildfires can alter spectroscopic characteristics of terrestrial dissolved organic matter (DOM) and potentially affect disinfection byproduct (DBP) formation during drinking water disinfection processes (e.g., chlorination and chloramination). Elevated levels of nutrient due to wildfire ash input into stream waters will likely cause algal blooms. Thus, wildfires can also elevate DOM levels as a result of ash input and excessive algal growth in source waters, and consequently impacting DBP formation in finished water. However, it is unclear whether characteristics of thermally-altered DOM (TA-DOM) are altered by biogeochemical processes (e.g., transformed by growing algae) before entering water treatment facilities. In addition, little information is available regarding how quality and quantity of overall allochthonous and autochthonous DOM as well as associated DBP formation are changed during an entire algal life cycle. When resource water is impeded by algae and require immediate restoration, copper algaecides are usually applied to control their growth. Previous studies suggest that Cu^{2+} can promote reactivity of DOM in DBP formation. However, it is unclear that how DBP formation is changed after treatment of fire-induced algal bloom by copper algaecide. To answer these questions, three independent experiments were designed, and the results are reported as three chapters in this dissertation (CHAPTER TWO, THREE, and FOUR).

In the first experiment, freshwater green algae *Pseudokirchneriella subcapitata* and blue-green algae *Microcystis aeruginosa* were separately incubated in the mixture of cultural medium and pine (*Pinus palustris*) litter-derived TA-DOMs (50 °C, 250 °C, and 400 °C) over 7 days to demonstrate the effects of algal growth on alterations in SDBP-FP. TA-DOM optical characteristics and SDBP-FP were quantified by absorption and

fluorescence spectroscopy and chlorination-based DBP-FP experiments. After the inoculation with *P. subcapitata*, TA-DOM aromaticity (indicated by $SUVA_{254}$) increased from 1.19 to 1.90 L/mg/m for 50 °C-extract but decreased from 4.95 to 3.75 L/mg/m for 400 °C-extract. The fraction of tyrosine-like components decreased from 25.9 to 9.3% for 50 °C-extract but increased from 0.9 to 1.3% for 400 °C-extract. Same patterns were also observed for *M. aeruginosa*. Growing algae generally increased chlorine reactivities and formations of trihalomethanes, haloacetonitriles, chloral hydrate, and haloketones. Our data suggest that the biodegradable dissolved organic carbon in TA-DOM decreases as fire intensity (i.e., temperature) increases. Post-fire algal blooms can increase chlorine reactivity of fire-affected terrestrial DOM for DBP formation.

In the second experiment, *Microcystis aeruginosa* was cultured in the medium containing low and high concentrations [10% and 65% (v/v)] of black and white ash water extracts (BE and WE) to study dynamic changes of carbonaceous, nitrogenous, and oxygenated DBP precursors during algal growth. DOM was characterized by absorption and fluorescence spectroscopy and chlorination/chloramination-based DBP formation experiments. In the treatment with 10% BE, the amount of C-DBP precursors decreased from 6.8 to 3.0 mmol/mol-C at lag-exponential phase then increased to 4.2 mmol/mol-C at death phase. The same trend was observed for O-DBP precursors. However, these dynamic changes of C- and O-DBP precursors exhibited opposite patterns in 65% extracts. Similar patterns were also observed in the WE treatments. On the other hand, N-DBP precursors continuously declined in all treatments. These results indicate that postfire ash loading and algal bloom stage may significantly affect DBP formation in source water.

In the third experiment, *Microcystis aeruginosa* was cultured in the medium containing black/white ash extracts (BE and WE) to study DBP concentrations before and after 4-days exposures to 0.5 and 1.0 mg-Cu/L. Algal population was indicated by optical density at 680 nm (OD_{680}). DOM was characterized by absorption and fluorescence spectroscopy and chlorination/chloramination-based DBP formation experiments. In the end of experiment, OD_{680} and DOM in the treatments were lower than control. N-nitrosodimethylamine concentrations in both treatments were 4-6 times higher than control, but haloacetonitrile concentrations in the treatments and control revealed no significant difference, regardless of type of ash solution. The results may serve to support risk evaluations of algal population and DBP concentration when wildfire-induced *M. aeruginosa* bloom is left untreated and when it is treated by copper algaecide.

DEDICATION

To all the people who care about this planet and environmental health.

ACKNOWLEDGMENTS

Especially thanks to my family for their unconditional support.

TABLE OF CONTENTS

Table of Contents (Continued) Page

LIST OF TABLES

LIST OF FIGURES

List of Figures (Continued)

Figure Page

ORGANIZATION OF DISSERTATION

This dissertation consists of five chapters, including introduction and summary. Chapters II, III, and IV are comprised of three independent manuscripts formatted for publication in peer-reviewed journals.

Chapter I: Introduction

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

*Tsai, K.P.; Chow, A. T. Growing Algae Alter Spectroscopic Characteristics and Chlorine Reactivity of Dissolved Organic Matter from Thermally-Altered Forest Litters. *Environ. Sci. Technol.* 2016, *50* (15), 7991–8000.

Chapter III: Dynamic Changes of Disinfection Byproduct Precursors following Exposures of *Microcystis aeruginosa* to Wildfire Ash Solutions

(In review by Environmental Science & Technology)

Chapter IV: Control Wildfire-Induced *Microcystis aeruginosa* blooms by Copper Sulfate: Trade-Offs between Reducing Algal Organic Matter and Promoting Disinfection Byproduct Formation

(In preparation)

Chapter V: Environmental Significance and Implications

CHAPTER ONE

INTRODUCTION

Wildfire can affect stream water quality by elevating dissolved organic matter (DOM) concentrations released from burned materials and potentially increase treatment costs for drinking water supply.[1,2] DOMs are important precursors of hazardous disinfection byproducts (DBPs) formed during drinking water chlorination/chloramination processes,[3] and DBP formations are highly related to DOM composition and chemical characteristics.[4] With respect to potential impacts of wildfire on drinking water quality, wildfire could increase chlorine reactivity of terrestrial DOM and thus DBP formations during water chlorination.[5,6] During transportation of fire-affected terrestrial DOMs from forested watersheds to water treatment plants, various biogeochemical processes (e.g., biotransformation) can further alter their chlorine reactivities. To better understand impacts of wildfire on drinking water quality, a comprehensive knowledge on physical, chemical, and biological processes that may occur during DOM transport is essential. Due to global warming and increasing human activities in forested watersheds such as forest fertilization practices in pine plantations, elevation of nutrients after wildfire events concomitant with occurrences of algal blooms in downstream waterbodies will likely happen more frequently.[7–9] In addition to utilizing inorganic nutrients and carbon dioxide, freshwater algae can also use DOM as carbon and nitrogen sources.[10,11] Accordingly, it is expected that growing algae will potentially alter chemistry of fire-affected DOM.

Wildfire can significantly change chemical structure of forest detritus with subsequent effects on the chemical reactivity of fire-affected terrestrial DOM.[6,12] Wang et

al.[5] demonstrated that chlorine reactivity of thermally-altered DOM (TA-DOM) for the formation of trihalomethanes, chloral hydrate, and haloketones decreased with increasing of combustion temperature from 50 to 400 °C. However, chlorine reactivity for the formation of haloacetonitriles as well as formation of brominated DBPs was elevated as combustion temperature increased. Those data suggest that chemical reactivity of TA-DOM is temperature dependent. Regarding biological reactivities of burned materials, it has been well studied that biochar degradability is related to charring temperature.[13–15] To determine degradability of DOM, biodegradable dissolved organic carbon (BDOC) has been used as an indicator of the fraction of labile compounds in DOM, where the loss of DOC after incubations of DOM with microorganisms was regarded as BDOC.[16–18] It is expected that biodegradation of TA-DOM would substantially alter DOM composition and characteristics and consequently affect chlorine reactivity in DBP formation.[19–22] Simultaneous consumption and production of DOM by growing algae would lead to either more or less DBP precursors compared with original TA-DOM. The net effects appear to be related to the original DOM characteristics as well as algal species.[23] Among all regulated DBPs in the U.S., bromine-containing DBPs usually reveal more toxic effects on testing animals than chlorine-containing DBPs, and the toxicity is correlated with the number of bromine atoms.[24] To minimize formation of Br-DBPs in finished waters, it is important to understand the sources of Br-DBP precursors and their fate during water transportation.

Algae are prevailing producers of autochthonous DOM in freshwaters,[25] and the level of DOM released from algae is usually proportional to their population.[26] Thus, increases

of DOM in fire-impacted source water can be attributed to both allochthonous inputs from ash and autochthonous productions from algae. Regardless of wildfires, successions of harmful algal bloom, especially *Microcystis aeruginosa*, often occur in reservoirs causing serious threat to drinking water quality due to DOM and toxins released from algal cells;[27] and excess inputs of nutrient and organic matter in waters are important factors for stimulating its population growth.[28,29] DOMs released from both ash and *M. aeruginosa* are DBP precursors. To understand how postfire elevated DOM affects DBP-FP in drinking water supply, it is essential to obtain comprehensive knowledge on the changes in concentration and composition of DOM during algal blooms in the absence and presence of ash-contaminated solutions.

The amounts of ash-derived DOM and nutrient entering downstream source water could be changed by environmental variables such as postfire precipitation, snowmelt, and hydrologic conditions.[30,31] Black and white ashes are commonly observed in burned forest watersheds. Generally, black ash is produced at relatively lower burning temperature and contains higher amount of organic compounds.[32,33] Regarding quality and quantity of DOMs released from black and white ashes, Wang et al.[6] demonstrated that concentrations of DOC in black and white ash solutions were not statistically different, but black ash solution contained significantly less aromatic carbon content than white ash solution. Variations in water quality caused by wildfires would lead to changes in algal population.[34] Ecotoxicological studies found that exposures of freshwater algae to DOM released from wildfire ash or biochar elicited different degrees of impact on its population, depending on the DOM concentration and composition.[35–37] Using optical

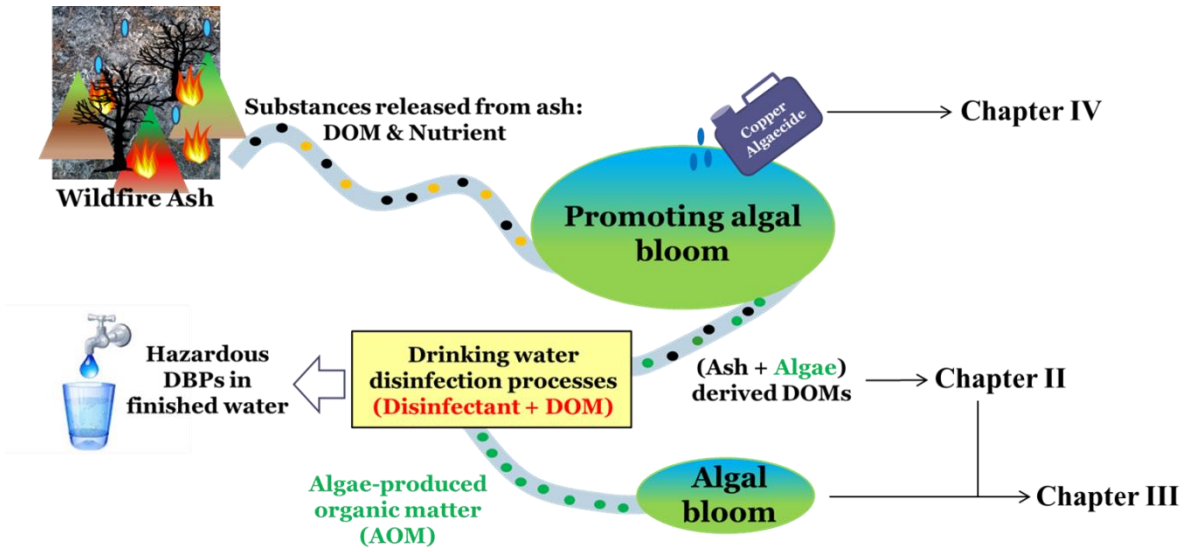
indices (e.g., $SUVA_{254}$, HIX, and FI) to examine AOM characteristics, Huang et al.[38] observed that changes in nitrogen and phosphorus concentrations in the cultural medium altered *M. aeruginosa* AOM aromaticity, molecular weight, protein and chlorophyll concentrations. Moreover, AOM quality and quantity vary at different algal growth stages.[39–42] Henderson et al.[40] reported that the AOM released by *M. aeruginosa* at exponential phase contained greater amount of aromatic compounds than at stationary phase. These studies suggest that concentration and composition of ash solution can play crucial roles in affecting *M. aeruginosa* population and associated AOM quality and quantity during its life cycle (i.e., from lag to death phase), which would consequently lead to substantial changes in DBP precursor in a DOM pool.

Elevated levels of nutrient (e.g., phosphorous, nitrogen, carbon) and DOM due to inputs of wildfire ash in stream waters will likely cause excessive growth of algae.[1,43] In addition, blooms of toxin-producing algae (e.g., *M. aeruginosa*) can exacerbate postfire drinking water quality.[44] In order to control abundance of noxious algae in drinking water resources, copper-based algaecides are extensively applied.[45,46] The amount of algae-produced organic matter (AOM) usually increases with increasing algal population. Following exposure of algae to copper algaecides, concentration of AOM and associated DBP precursors may not increase owing to inhibition of algal growth.[47] Application of copper sulfate ($CuSO_4$) for controlling noxious algae has been practiced for decades.[48] Noticeably, previous studies have showed that soluble copper may enhance DBP formation by complexing with DOM and catalyzing haloform formation.[49–52] Thus, persistence of copper in the water column following application of copper sulfate may

lead to increase in DBP-FP during water disinfection. In order to ensure drinking water safety after wildfires, minimizing AOM level and associated DBP precursors is particularly important in situations where algal bloom is ongoing. However, it is unclear whether controlling wildfire-induced algal blooms by copper sulfate would face the trade-offs between reduction of AOM associated DBP precursors versus promotion of DBP formation. Therefore, it is needed to investigate alterations of DOM and DBP concentration following exposures of algae to copper sulfate during postfire algal blooms.

Both autochthonous and allochthonous DOMs released from algae and ash contain several metal-binding functional groups (e.g., carboxyl, carbonyl, phenolic, and alcohol groups) with various binding capacity.[53] Importantly, binding capacity of DOM and copper ions relates to DOM functional groups and copper concentration, which is critical for copper to act as a catalyst for DBP formation.[54,55] Regarding catalytic effect of copper concentration on DBP-FP, Zhang and Andrews[51] found that during DOM chloramination, as Cu^{2+} concentrations increased from 0 to 1 mg/L, N-nitrosodimethylamine (NDMA) concentrations proportionally increased from 31 to 104 ng/L. In addition, previous studies showed that effects of Cu^{2+} on DOM chlorine/chloramine reactivities in DBP formation were related to DOM properties. During chloramination of a series of concentrations of Suwannee River DOM (SR-DOM) spiked with 1 mg-Cu/L as $CuSO_4$, Zhang and Andrews[51] found that NDMA-FP significantly decreased as SR-DOM concentration increased. On the contrary, Barnes et al.[56] reported that concentrations of chloroform formed during chlorination were positively correlated with concentrations of humic acid. Moreover, it was documented that influence of Cu^{2+} on promoting THMs

formation was more remarkable than HAAs during the chlorination of humic acid.[55] It suggests that catalytic effect of copper on DBP-FP is also DBP species dependent. Exposures of algae to copper can significantly impact algal populations as well as AOM quantity and quality,[47] which would consequently affect Cu-AOM complexation. Hence, it is expected that controlling wildfire-induced noxious algal blooms by copper sulfate affect DBP formation during subsequent water disinfection process.



The overall concept and related chapters of this dissertation is illustrated in the scheme above. Three major study questions were answered in Chapter II, III, and IV, respectively. Question 1: How is postfire overall DOM reactivity for DBP formation changed during algal growth? Question 2: How is the pattern of DOM reactivity for DBP formation affected by ash color and ash solution concentration during an entire algal life cycle? Question 3: How are algal population, DOM, and subsequent DBP formation changed following copper algaecide application for controlling fire-induced algal bloom?

SPECIFIC OBJECTIVES

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

The specific objectives were to: (1) measure water quality of three TA-DOMs (50, 250, and 400 °C) inoculated with algae and respective algal growth over 7 days; (2) compare biodegradability of TA-DOMs by measuring spectroscopic characteristics; (3) compare chlorine reactivities of TA-DOMs before and after inoculations with algae and associated DBP formation; (4) assess alterations of Br-trihalomethanes by growing algae.

Chapter III: *Dynamic Changes of Disinfection Byproduct Precursors following Exposures of Microcystis aeruginosa to Wildfire Ash Solutions*

The specific objectives were to: (1) compare *M. aeruginosa* population and growth rate in the absence and presence of black and white ash water extracts with low and high concentrations; (2) evaluate alterations of DOM spectroscopic characteristics; (3) assess DOM reactivities and DBP-formation at lag, exponential, stationary, and death phases; and (4) identify correlations among specific DBP formation and DOM optical indices.

Chapter IV: *Control Wildfire-Induced Microcystis aeruginosa blooms by Copper Sulfate: Trade-Offs between Reducing Algal Organic Matter and Promoting Disinfection Byproduct Formation*

The specific objectives were to: (1) measure DOM concentration and population of *M. aeruginosa* following 4-day exposures of 0, 0.5, and 1 mg-Cu/L as copper sulfate; (2) demonstrate changes of DOM spectroscopic characteristics in the absence and presence of copper sulfate; and (3) identify differences in DOM reactivity for DBP formation without and with exposures to copper sulfate.

REFERENCES

(1) Bladon, K. D.; Emelko, M. B.; Silins, U.; Stone, M. Wildfire and the future of water supply. *Environ.Sci.Technol.* **2014**, *48* (16), 8936–8943.

(2) Spencer, C. N.; Gabel, K. O.; Hauer, F. R. Wildfire effects on stream food webs and nutrient dynamics in Glacier National Park, USA. *For. Ecol. Manage.* **2003**, *178* (1–2), 141–153.

(3) Kraus, T. E. C.; Anderson, C. a; Morgenstern, K.; Downing, B. D.; Pellerin, B. a; Bergamaschi, B. a. Determining sources of dissolved organic carbon and disinfection byproduct precursors to the McKenzie River, Oregon. *J. Environ. Qual.* **2010**, *39* (6), 2100–2112.

(4) Kitis, M.; Karanfil, T.; Wigton, A.; Kilduff, J. E. Probing reactivity of dissolved organic matter for disinfection by-product formation using XAD-8 resin adsorption and ultrafiltration fractionation. *Water Res.* **2002**, *36* (15), 3834–3848.

(5) Wang, J. J.; Dahlgren, R. A.; Chow, A. T. Controlled Burning of Forest Detritus Altering Spectroscopic Characteristics and Chlorine Reactivity of Dissolved Organic Matter: Effects of Temperature and Oxygen Availability. *Environ. Sci. Technol.* **2015**, *49* (24), 14019–14027.

(6) 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.

(7) Bisson, P.A., Ice, G.G., Perrin, C.J., Bilby, R. E. Effects of forest fertilization on water quality and aquatic resources in the Douglas-fir region. In *Forest Fertilization: Sustaining and Improving Nutrition and Growth of Western Forests. Institute of Forest Resources*; Chappel, H.N. Wettman, G.F. Miller, R. E., Ed.; College of Forest Resources, University of Washington, Seattle, WA, 1992; pp 179–193.

(8) Fox TR, Allen HL, Albaugh TJ, Rubilar R, C. C. Forest Fertilization and Water Quality in the United States. *Better Crop.* **2007**, No. 91, 7–9.

(9) Anderson, C. W. Ecological effects on streams from forest fertilization; literature review and conceptual framework for future study in the western Cascades. *Water-Resources Investig. Rep.* **2002**.

(10) Markou, G.; Vandamme, D.; Muylaert, K. Microalgal and cyanobacterial cultivation: The supply of nutrients. *Water Res.* **2014**, *65*, 186–202.

(11) Subashchandrabose, S. R.; Ramakrishnan, B.; Megharaj, M.; Venkateswarlu, K.; Naidu, R. Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. *Environ. Int.* **2013**, *51*, 59–72.

(12) Quill, E. S.; Angove, M. J.; Morton, D. W.; Johnson, B. B. Characterisation of dissolved organic matter in water extracts of thermally altered plant species found in boxironbark forests. *Aust. J. Soil Res.* **2010**, *48* (8), 693–704.

(13) Bruun, S.; Jensen, E. S.; Jensen, L. S. Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration. *Org. Geochem.* **2008**, *39* (7), 839–845.

(14) Hilscher, A.; Knicker, H. Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28month microcosm experiment. *Org. Geochem.* **2011**, *42* (1), 42–54.

(15) Harvey, O. R.; Kuo, L. J.; Zimmerman, A. R.; Louchouarn, P.; Amonette, J. E.; Herbert, B. E. An index-based approach to assessing recalcitrance and soil carbon sequestration potential of engineered black carbons (biochars). *Environ. Sci. Technol.* **2012**, *46* (3), 1415–1421.

(16) Fellman, J. B.; D'Amore, D. V.; Hood, E.; Boone, R. D. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. *Biogeochemistry* **2008**, *88* (2), 169–184.

(17) Hur, J.; Lee, M. H.; Song, H.; Schlatman, M. A. Microbial transformation of dissolved organic matter from different sources and its influence on disinfection byproduct formation potentials. *Environ. Sci. Pollut. Res.* **2013**, *20* (6), 4176–4187.

(18) Kalbitz, K.; Schmerwitz, J.; Schwesig, D.; Matzner, E. Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma* **2003**, *113* (3–4), 273–291.

(19) Beggs, K. M. H.; Summers, R. S. Character and chlorine reactivity of dissolved organic matter from a mountain pine beetle impacted watershed. *Environ. Sci. Technol.* **2011**, *45* (13), 5717–5724.

(20) Hertkorn, N.; Claus, H.; Schmitt-Kopplin, P.; Perdue, E. M.; Filip, Z. Utilization and transformation of aquatic humic substances by autochthonous microorganisms. *Environ. Sci. Technol.* **2002**, *36* (20), 4334–4345.

(21) Hur, J.; Park, M.-H.; Schlautman, M. A. Microbial transformation of dissolved leaf litter organic matter and its effects on selected organic matter operational descriptors. *Environ. Sci. Technol.* **2009**, *43* (7), 2315–2321.

(22) Pellerin, B. a; Hernes, P. J.; Saraceno, J.; Spencer, R. G. M.; Bergamaschi, B. a. Microbial degradation of plant leachate alters lignin phenols and trihalomethane precursors. *J. Environ. Qual.* **2010**, *39* (3), 946–954.

(23) Guillemette, F.; del Giorgio, P. A. Simultaneous consumption and production of fluorescent dissolved organic matter by lake bacterioplankton. *Environ. Microbiol.* **2012**, *14* (6), 1432–1443.

(24) Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; DeMarini, D. M. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutat. Res. - Rev. Mutat. Res.* **2007**, *636* (1–3), 178–242.

(25) Leenheer, J. J. a.; Croué, J. J.-P. Peer reviewed: characterizing aquatic dissolved organic matter. *Environ. Sci. Technol.* **2003**, *37* (1), 18A–26A.

(26) Hoehn, R. C.; Barnes, D. B.; Thompson, B. C.; Randall, C. W.; Grizzard, T. J.; Shaffer, P. T. B. Algae as sources of trihalomethane precursors. *J. Am. Water Work. Assoc.* **1980**, *72* (6), 344–350.

(27) Paerl, H. W.; Paul, V. J. Climate change: Links to global expansion of harmful cyanobacteria. *Water Res.* **2012**, *46* (5), 1349–1363.

(28) Burkholder, J. M.; Glibert, P. M.; Skelton, H. M. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* **2008**, *8* (1), 77–93.

(29) Paerl, H. W.; Scott, J. T.; McCarthy, M. J.; Newell, S. E.; Gardner, W. S.; Havens, K. E.; Hoffman, D. K.; Wilhelm, S. W.; Wurtsbaugh, W. A. It Takes Two to Tango: When and Where Dual Nutrient (N & P) Reductions Are Needed to Protect Lakes and Downstream Ecosystems. *Environ. Sci. Technol.* **2016**, *50* (20), 10805–10813.

(30) Wang, J. J.; Dahlgren, R. A.; Erşan, M. S.; Karanfil, T.; Chow, A. T. Temporal variations of disinfection byproduct precursors in wildfire detritus. *Water Res.* **2016**, *99*, 66–73.

(31) Writer, J. H.; Hohner, A.; Oropeza, J.; Schmidt, A.; Cawley, K.; Rosario-Ortiz, F. L. Water treatment implications after the High Park Wildfire in Colorado. *J. Am. Water Works Assoc.* **2014**, *106* (4), 85–86.

(32) Bodí, M. B.; Martin, D. A.; Balfour, V. N.; Santín, C.; Doerr, S. H.; Pereira, P.; Cerdà, A.; Mataix-Solera, J. Wildland fire ash: Production, composition and eco-hydro-geomorphic effects. *Earth-Science Rev.* **2014**, *130*, 103–127.

(33) Pereira, P.; Úbeda, X.; Martin, D. A. Fire severity effects on ash chemical composition and water-extractable elements. *Geoderma* **2012**, *191*, 105–114.

(34) Neary, D. G. .; Ryan, K. C. .; DeBano, L. F. Wildland Fire in Ecosystems, effects of fire on soil and water. *USDA-FS Gen. Tech. Rep.* **2005**, *4* (September), 250.

(35) Silva, V.; Pereira, J. L.; Campos, I.; Keizer, J. J.; Gonçalves, F.; Abrantes, N. Toxicity assessment of aqueous extracts of ash from forest fires. *Catena* **2015**, *135*, 401–408.

(36) Smith, C. R.; Buzan, E. M.; Lee, J. W. Potential impact of biochar water-extractable substances on environmental sustainability. *ACS Sustain. Chem. Eng.* **2013**, *1* (1), 118–126.

(37) Smith, C. R.; Hatcher, P. G.; Kumar, S.; Lee, J. W. Investigation into the Sources of Biochar Water-Soluble Organic Compounds and Their Potential Toxicity on Aquatic Microorganisms. *ACS Sustain. Chem. Eng.* **2016**, *4* (5), 2550–2558.

(38) 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.

(39) Leloup, M.; Nicolau, R.; Pallier, V.; Yéprémian, C.; Feuillade-Cathalifaud, G. Organic matter produced by algae and cyanobacteria: Quantitative and qualitative characterization. *J. Environ. Sci.* **2013**, *25* (6), 1089–1097.

(40) Henderson, R. K.; Baker, A.; Parsons, S. A.; Jefferson, B. Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. *Water Res.* **2008**, *42* (13), 3435–3445.

(41) Gough, R.; Holliman, P. J.; Cooke, G. M.; Freeman, C. Characterisation of algogenic organic matter during an algal bloom and its implications for trihalomethane formation. *Sustain. Water Qual. Ecol.* **2015**, *6*, 11–19.

(42) Myklestad, S. M. Dissolved Organic Carbon from Phytoplankton. *Mar. Chem.* **2000**, *5*, 112–144.

(43) Smith, H. G.; Sheridan, G. J.; Lane, P. N. J.; Nyman, P.; Haydon, S. Wildfire effects on water quality in forest catchments: A review with implications for water supply. *J. Hydrol.* **2011**, *396* (1–2), 170–192.

(44) Otten, T. G.; Paerl, H. W. Health Effects of Toxic Cyanobacteria in U.S. Drinking and Recreational Waters: Our Current Understanding and Proposed Direction. *Curr. Environ. Heal. reports* **2015**, *2* (1), 75–84.

(45) Hoko, Z.; Makado, P. K. Optimization of algal removal process at Morton Jaffray water works, Harare, Zimbabwe. *Phys. Chem. Earth* **2011**, *36* (14–15), 1141–1150.

(46) McKnight, D. M.; Chisholm, S. W.; Harleman, D. R. F. CuSO4 treatment of nuisance algal blooms in drinking water reservoirs. *Environ. Manage.* **1983**, *7* (4), 311–320.

(47) Tsai, K.-P. Effects of two copper compounds on Microcystis aeruginosa cell density, membrane integrity, and microcystin release. *Ecotoxicol. Environ. Saf.* **2015**, *120*, 428–435.

(48) Haughey, M. A.; Anderson, M. A.; Whitney, R. D.; Taylor, W. D.; Losee, R. F. Forms and fate of Cu in a source drinking water reservoir following CuSO4 treatment. *Water Res.* **2000**, *34* (13), 3440–3452.

(49) Blatchley, E. R.; Margetas, D.; Duggirala, R. Copper catalysis in chloroform formation during water chlorination. *Water Res.* **2003**, *37* (18), 4385–4394.

(50) Fu, J.; Qu, J.; Liu, R.; Qiang, Z.; Liu, H.; Zhao, X. Cu(II)-catalyzed THM formation during water chlorination and monochloramination: A comparison study. *J. Hazard. Mater.* **2009**, *170* (1), 58–65.

(51) Zhang, H.; Andrews, S. A. Factors affecting catalysis of copper corrosion products in NDMA formation from DMA in simulated premise plumbing. *Chemosphere* **2013**, *93* (11), 2683–2689.

(52) Zhao, Y.; Yang, H.; Liu, S.; Tang, S.; Wang, X.; Xie, Y. F. Effects of metal ions on disinfection byproduct formation during chlorination of natural organic matter and surrogates. *Chemosphere* **2016**, *144*, 1074–1082.

(53) Aiken, G. R.; Hsu-kim, H.; Ryan, J. N. Influence of Dissolved Organic Matter on the Environmental Fate of Metals, Nanoparticles, and Colloids. *Environ. Sci. Technol.* **2011**, 3196–3201.

(54) Zhang, H.; Andrews, S. A. Catalysis of copper corrosion products on chlorine decay and HAA formation in simulated distribution systems. *Water Res.* **2012**, *46* (8), 2665–2673.

(55) Liu, S. G.; Zhu, Z. L.; Tan, X. C.; Feng, X. H.; Huang, Z. Y.; Qiu, Y. L.; Zhao, J. F. The influence of Cu(II) on the formation and distribution of disinfection by-products during the chlorination of drinking water. *Water. Air. Soil Pollut.* **2013**, *224* (4).

(56) Barnes, D.; FitzGerald, P. A.; Swan, H. B. Catalysed formation of chlorinated organic materials in waters. *Water Sci. Technol.* **1989**, *21* (2), 59–63.

CHAPTER TWO

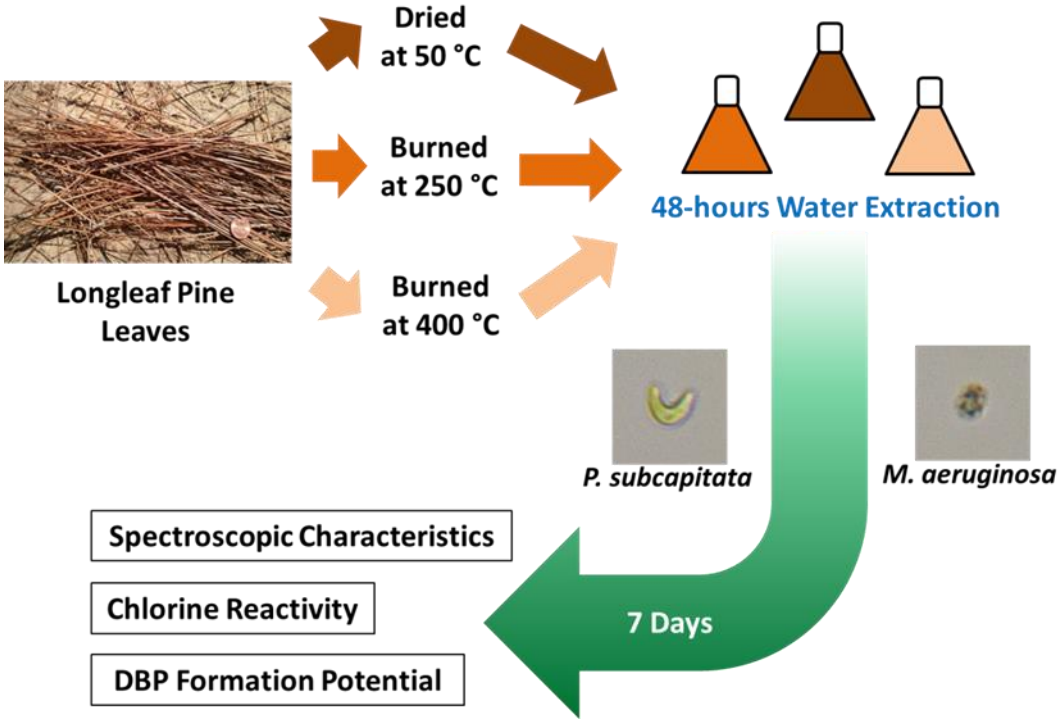
Growing Algae Alter Spectroscopic Characteristics and Chlorine Reactivity of Dissolved Organic Matter from Thermally-Altered Forest Litters

ABSTRACT

Previous studies demonstrated that wildfires alter spectroscopic characteristics of terrestrial dissolved organic matter (DOM) and increase specific disinfection byproduct formation potential (SDBP-FP). However, it is unclear whether characteristics of thermally-altered DOM (TA-DOM) are altered by biogeochemical processes (e.g., transformed by growing algae) before entering water treatment facilities. The freshwater green algae *Pseudokirchneriella subcapitata* and blue-green algae *Microcystis aeruginosa* were separately incubated in the mixture of cultural medium and pine (*Pinus palustris*) litter-derived TA-DOMs (50 °C, 250 °C, and 400 °C) over 7 days to demonstrate the effects of algal growth on alterations in SDBP-FP. TA-DOM optical characteristics and SDBP-FP were quantified by absorption and fluorescence spectroscopy and chlorination-based DBP-FP experiments. After the inoculation with *P. subcapitata*, TA-DOM aromaticity (indicated by $SUVA_{254}$) increased from 1.19 to 1.90 L/mg/m for 50 °C-extract but decreased from 4.95 to 3.75 L/mg/m for 400 °C-extract. The fraction of tyrosine-like components decreased from 25.9 to 9.3% for 50 °C-extract but increased from 0.9 to 1.3% for 400 °C-extract. Same patterns were also observed for *M. aeruginosa*. Growing algae generally increased chlorine reactivities and formations of trihalomethanes, haloacetonitriles, chloral hydrate, and haloketones. Our data suggest that

the biodegradable dissolved organic carbon in TA-DOM decreases as fire intensity (i.e., temperature) increases. Post-fire algal blooms can increase chlorine reactivity of fire-affected terrestrial DOM for DBP formation.

GRAPHICAL ABSTRACT



INTRODUCTION

Wildfire can affect stream water quality by elevating dissolved organic matter (DOM) concentrations released from burned materials and potentially increase treatment costs for drinking water supply.[1,2] DOMs are important precursors of hazardous disinfection byproducts (DBPs) formed during drinking water chlorination processes,[3] and DBP formations are highly related to DOM composition and chemical characteristics.[4] With respect to potential impacts of wildfire on drinking water quality, wildfire could increase chlorine reactivity of terrestrial DOM and thus DBP formations during water chlorination.[5,6] During transportation of fire-affected terrestrial DOMs from forested watersheds to water treatment plants, various biogeochemical processes (e.g., biotransformation) can further alter their chlorine reactivities. To better understand impacts of wildfire on drinking water quality, a comprehensive knowledge on physical, chemical, and biological processes that may occur during DOM transport is essential. Due to global warming and increasing human activities in forested watersheds such as forest fertilization practices in pine plantations, elevation of nutrients after wildfire events concomitant with occurrences of algal blooms in downstream waterbodies will likely happen more frequently.[7–9] In addition to utilizing inorganic nutrients and carbon dioxide, freshwater algae can also use DOM as carbon and nitrogen sources.[10,11] Accordingly, it is expected that growing algae will potentially alter chemistry of fire-affected DOM.

Wildfire can significantly change chemical structure of forest detritus with subsequent effects on the chemical reactivity of fire-affected terrestrial DOM.[6,12] Using optical indices (e.g., $SUVA_{254}$ and fluorescence index) to examine DOM molecular

weight (MW) and aromaticity, Wang et al.[6] found that DOMs from burned forest detritus exhibited lower MW and higher aromaticity compared to that from unburned detritus. Furthermore, temperature and oxygen levels during combustion regulate DOM chemistry and chlorine reactivity. Wang et al.[5] demonstrated that chlorine reactivity of thermally-altered DOM (TA-DOM) for the formation of trihalomethanes (THMs), chloral hydrate (CHD), and haloketones (HKs) decreased with increasing of combustion temperature from 50 to 400 °C. However, chlorine reactivity for the formation of haloacetonitriles (HANs) as well as formation of brominated DBPs was elevated as combustion temperature increased. Those data suggest that chemical reactivity of TA-DOM is temperature dependent.

Regarding biological reactivities of burned materials, it has been well studied that biochar degradability is related to charring temperature.[13–15] For example, Bruun et al.[13] showed that microbial mineralization and assimilation of charcoal decreased with increasing char production temperature. Thus, it is expected that TA-DOMs would also exhibit different biological reactivities (e.g., extent of degradation by growing algae), depending on fire temperature. To evaluate the fate of burned DOM in streams, Norwood et al.[16] incubated pyrogenic water soluble organic matter (Py-WSOM) from 250 °C-chars with unsterilized river water and found Py-WSOM and associated biomarkers significantly decreased within a month, suggesting that wildfire combustion may contribute labile terrestrial organic matters to aquatic systems. To determine degradability of DOM, biodegradable dissolved organic carbon (BDOC) has been used as an indicator of the fraction of labile compounds in DOM, where the loss of DOC after

incubations of DOM with microorganisms was regarded as BDOC.[17–19] Previous studies showed that extent and rate of DOM biodegradation were closely related to DOM fluorescence characteristics.[18–20] It is expected that biodegradation of TA-DOM would substantially alter DOM composition and characteristics and consequently affect chlorine reactivity in DBP formation.[21–24]

Planktonic algae are ubiquitous microorganisms playing important roles in DOM cycles in freshwaters.[11] Understanding the effects of growing algae on TA-DOMs will improve our knowledge on impacts of wildfire on drinking water quality. Noticeably, either bacteria or unspecified microorganisms from natural waters were usually used in culture-based experiments to examine biodegradation-induced changes of DOM characteristics. To date, there is still a lack of information regarding the alteration of TA-DOM characteristics by growing algae. Cyanobacteria (blue-green algae) and green algae revealed different capabilities to metabolize and transform DOMs.[10,11,25] Both species also have been proven to be able to remove nutrients from a wide variety of wastewaters.[26] Since bacterial C/N ratios are lower than those for phytoplankton, cyanobacteria may need more nitrogen per unit biomass than green algae.[27] In addition, it has been demonstrated that the addition of biochar water extractable substances or humic acids has a promoting effect on algal growth.[28,29] Hence, it is likely that some DOMs exported from fire-affected forest materials, including amino acids and aliphatic acids which have been identified as DBP precursors,[28,30,31] can be uptaken by growing algae for their growth. In addition to TA-DOM, organic matters produced from green algae and cyanobacteria reveal different chlorine reactivities for DBP formation.[32,33] Simultaneous

consumption and production of DOM by growing algae would lead to either more or less DBP precursors compared with original TA-DOM. The net effects appear to be related to the original DOM characteristics as well as algal species.[34] Among all regulated DBPs in the U.S., bromine-containing DBPs usually reveal more toxic effects on testing animals than chlorine-containing DBPs, and the toxicity is correlated with the number of bromine atoms.[35] To minimize formation of Br-DBPs in finished waters, it is important to understand the sources of Br-DBP precursors and their fate during water transportation.

The overall objective of this study is to understand how TA-DOMs are quantitatively and qualitatively altered by growing green algae *Pseudokirchneriella subcapitata* and cyanobacteria *Microcystis aeruginosa* and the subsequent chlorine reactivities for DBP formation. The specific objectives were to: (1) measure water quality of three TA-DOMs (50, 250, and 400 °C) inoculated with algae and respective algal growth over 7 days; (2) compare biodegradability of TA-DOMs by measuring spectroscopic characteristics; (3) compare chlorine reactivities of TA-DOMs before and after inoculations with algae and associated DBP (THMs, HANs, CHD, and HKs) formation potential; (4) assess alterations of Br-THMs by growing algae.

MATERIALS AND METHODS

Preparation of TA-DOM

The intact auburn-color longleaf pine (*Pinus palustris*) litters were collected from the top litter layer (0-2 cm) at Hobcaw Barony in Georgetown, South Carolina, on April 9th, 2015. All of the litters were partially-decayed. The samples were sealed in paper bags and

stored in a drying oven at 50 °C. To simulate burned leaf litters in wildfire, 10 grams of dried litter were put in an aluminum foil and placed in a pre-heated muffle furnace at 250 and 400 °C under oxic conditions for an hour. The selection of burning temperatures was based on the common range of historical fire temperature in coniferous forests and the distinctive characteristics of TA-DOM.[5,36] All dried and burned litters were ground ≤ 2 mm using a mortar and a pestle. To obtain litter extracts and minimize contaminations of dissolved organic carbon (DOC), 5 g of each type of litter was mixed with 150 ml Milli-Q water in a 250 ml Erlenmeyer flask covered with aluminum foil. The water-litter mixtures were shaken for 24 hours using an orbital shaker at 250 rpm and remained another 24 hours without shaking. Extracts were filtered using 0.45 μm polyethersulfone membrane filters (Supor-450, Pall Gelman Science) rinsed three times with 20 mL of Milli-Q water. Three types of extracts termed 50 °C-extract, 250 °C-extract, and 400 °C-extract were used for further algal bioassay.

Algal Culture and Bioassay

Pseudokirchneriella subcapitata UTEX 1648 and *Microcystis aeruginosa* UTEX 2385 (University of Texas at Austin, Austin, TX) were separately cultured in the medium. *P. subcapitata* and *M. aeruginosa* commonly occur in fresh waters and are capable of uptaking and transforming organic compounds.[11] The composition of culture medium was reported in Table S1. The algal culture conditions were followed by the previous study.[37] Algal cultures were maintained at a temperature of 24 ± 2˚C and a 12:12-hour light-dark photoperiod illuminated by cool white fluorescent lighting at an

intensity of 2100 lux. Algal growth was monitored every day throughout the entire experiment by measuring optical density at 680 nm (OD_{680}) using UV-VIS spectrophotometer (Shimadzu UV-1800). The litter extracts were prepared freshly prior to the algal bioassays to maintain original DOC characteristics. The algal bioassay conditions were the same as for algal cultures. Experimental chambers consisted of 250 mL of solution in Erlenmeyer flasks. Algal bioassays were conducted using three replicates of each type of extract inoculated with algae. DOC concentrations after forest fire events in the streams of fire-impacted forest watershed have been reported to be an order of magnitude higher than from unburned areas.[38,39] Because the theoretical DOC concentration of cultural medium was 0.6 mg/L, we adjusted initial TA-DOM concentrations in the algal bioassay solutions to approximate 10 mg-DOC/L by diluting raw litter extracts with the cultural medium. The initial OD_{680} values were adjusted to 0.05, where DOC concentrations contributed from algae and medium were less than 1 mg/L. Algal cells in late exponential growth phase usually have relatively high ability for removing and biodegrading organic compounds.[40,41] Based on the experimental conditions both algae reached late exponential growth phase in 7-8 days; therefore, a 7-days incubation time was used in this study. Samples including extracts before and after inoculations with algae were collected at the beginning (day 0) and end (day 7) of experiment and were filtered using 0.45 μm polyethersulfone membrane filters (Supor-450, Pall Gelman Science) for chemical analyses.

Chemical Analyses

Chemical analyses, including water chemistry (pH, DOC, total dissolved nitrogen), DOM optical characteristics ($SUVA_{254}$, HIX, E2/E3, FI, β/α, and EEM) and DBP (trihalomethanes, haloacetonitriles, chloral hydrate, and haloketones) formation, were published previously.[6] Detailed descriptions are presented in the Supporting Information (APPENDIX I). Statistically significant differences between the treatments were determined using one-way ANOVA with Tukey's test. Significance was considered as $P < 0.05$.

RESULTS AND DISCUSSION

Water Quality and Algal Growth

Detailed descriptions of algal growth in the cultural medium and associated water quality are presented in the Supporting Information (APPENDIX I). After 48-h water extractions, DOC concentration extracted from thermally-altered litters was 680.6 ± 18.6, 217.6 ± 4.3, and 20.0 ± 0.2 mg/L for 50, 250, and 400 °C-extract, respectively (Table 1). Before inoculations with algae, the respective DOC concentration was 14.3 ± 0.3, 10.4 ± 0.1, and 9.7 ± 0.0 mg/L (Figure 1B); under these bioassay conditions, DOC contributed from the cultural medium (0.7 mg/L, Table 1) was negligible. Except for the 250 °C-extract inoculated with *M. aeruginosa*, DOC concentrations decreased throughout the experiment; and the lowest extent of DOC loss was observed for 400 °C-extract. For example, the loss of DOC for 50, 250, and 400 °C-extract inoculated with *P. subcapitata* was 28% (from 14.3 ± 0.3 to 10.0 ± 0.5 mg/L), 30% (from 10.4 ± 0.1 to 7.4 ± 0.7 mg/L), and 20% (from 9.7 ± 0.0 to 8.0 ± 0.3 mg/L), respectively. Hur et al. observed that

inoculation of leaf litter extract with microorganisms resulted in a decrease in DOC.[23] In this study, decreases of DOC concentrations after inoculations with algae could be attributed to the utilization by growing algae and microbes in culture medium. Besides, the decreases of DOC could be also due to the fluorescent lighting.[42] The extent of decrease suggested that DOM in 50 and 250 °C-extracts may contain more labile and biodegradable compounds compared to that in 400 °C-extract. Comparing OD_{680} values for algae growing in the medium, the addition of 50 and 400 °C-extracts into the medium resulted in higher OD_{680} values at the end of experiment (Figure 1A), suggesting that DOM from fire-affected areas may promote algal growth.

DOM spectroscopic Characteristics and Biodegradability

As indicated by $SUVA_{254}$, DOM aromaticity statistically increased in the 50 and 250 °C-extracts but decreased in the 400 °C-extract after inoculations with algae (Figure 2A). Inoculation of extracts with *M. aeruginosa* resulted in higher $SUVA_{254}$ than with *P. subcapitata*. For example, $SUVA_{254}$ for the 50 °C-extract was increased from 1.19 ± 0.02 to 2.20 ± 0.10 and 1.90 ± 0.09 L/mg/m after inoculation with *M. aeruginosa* and *P. subcapitata*, respectively. Similarly, humification index (HIX) significantly increased in the 50 and 250 °C-extracts after inoculations with algae but decreased in the 400 °C-extract (Figure 2B), suggesting that growing algae can alter aromaticity and humic substance content of DOM. Regarding the increases in aromaticity and degree of humification in 50 and 250 °C-extracts after inoculations with algae, the findings were consistent with previous studies demonstrating that the inoculation of DOM with

microorganisms resulted in increases in $SUVA_{254}$ and HIX values.[23,43,44] Humification of DOMs involves a series of degradation reactions of original compounds. Several studies showed that an increase in $SUVA_{254}$ or HIX represented DOM biodegradation processes.[19,21,23] The increases in $SUVA_{254}$ and HIX in this study could be due to the uptake of non-aromatic DOM such as carbohydrates by growing algae,[11,45] and the biotic transformation of labile DOM to products containing more unsaturated carbon bonds such as polyunsaturated fatty acids.[46] Decreases in $SUVA_{254}$ and HIX observed in the 400 °C-extract (e.g., HIX decreased from 31.5 ± 1.2 to 17.6 ± 0.6 after the inoculation with *P. subcapitata*) suggest that DOM was less impacted by biodegradation processes and the degraded DOM was dominated by non-aromatic compounds instead of humic substances.[21] Similar results were reported by Hertkorn et al. showing that utilization of humic substances by autochthonous microorganisms resulted in a loss of aromaticity and an increase in aliphatic and carbohydrate-like structures.[22] According to the E2/E3 value, an indicator inversely correlated with DOM MW, inoculations of 50 and 250 °C-extracts with algae resulted in significantly higher MW (Figure 2C). But the MW in 400 °C-extract decreased after inoculations with algae. Previous studies also reported that humification of plant-derived DOM caused increases in average DOM molecular size.[47,48] Increases in MW in this study were in accordance with increases of humification for the 50 and 250 °C-extracts. Therefore, increases in MW may result from biodegradation of DOM containing simple structures with low MW and exhibiting relatively high percentage of biodegradable carbon by growing algae. In contrast, DOM in the 400 °C-extract may contain relatively low amounts of biodegradable carbon and

high amount of compounds with condensed aromatic structures, which would prevent biodegradation by growing algae. The freshness index (β/α) represents proportion of contribution of recently produced microbial DOM.[43] Regardless of the type of extract, the inoculations of extract with algae resulted in significant increases in freshness index (Figure 2D), indicating increases of algae-related organic matter during algal growth. The fluorescence index (FI) provides information on DOM source, which can be either from algae or from terrestrial plant.[43] The FI values significantly decreased after inoculation of 50 and 250 °C-extracts with *P. subcapitata* but increased with *M. aeruginosa* (Figure 2E), suggesting that the extent of utilization of TA-DOM by *P. subcapitata* was likely greater than that by *M. aeruginosa*, and the amount of organic matter produced by *M. aeruginosa* was greater than that by *P. subcapitata*. Under the experimental conditions of this study, alterations of TA-DOM characteristics could be also caused by fluorescent lighting and microbes in the cultural medium.[42]

The fluorescence EEM of DOM showed that after the inoculations of extracts with algae, the proportion of tyrosine-like components significantly decreased from 25.9 ± 0.0% to 9.3 ± 0.4% and 17.1 ± 0.3% for 50 °C-extract after inoculation with *P. subcapitata* and *M. aeruginosa*, respectively (Figures 3A-3C). In contrast, it significantly increased from 0.9 ± 0.0% to 1.3 ± 0.1% and 2.0 ± 0.4% for 400 °C-extract inoculated with *P. subcapitata* and *M. aeruginosa*, respectively (Figures 3G-3I). For the 250 °C-extract, the changes were algal species dependent. Effects of growing algae on the alteration of fulvic acid-like and humic acid-like components revealed opposite patterns compared to tyrosine-like component. For example, the proportion of fulvic acid-like

component significantly increased from 16.2 ± 0.0% to 18.1 ± 0.4% for 50 °C-extract inoculated with *P. subcapitata* (Figures 3A, 3B), but it significantly decreased from 44.5 ± 0.0% to 41.3 ± 0.6% for 400 °C-extract (Figures 3G, 3H). The proportion of soluble microbial byproduct-like components in 50, 250, and 400 °C-extracts inoculated with *P. subcapitata* was 30.4 ± 0.9%, 27.3 ± 1.1%, and 13.7 ± 0.5%, respectively (Figures 3B, 3E, 3H), which were significantly higher than before inoculations (25.5 ± 0.0%, 25.7 ± 0.0%, and 11.2 ± 0.0%) (Figures 3A, 3D, 3G). For the extracts after inoculations with *M. aeruginosa*, an increase was only observed for 400 °C-extract (from 11.2 ± 0.0% to 14.3 ± 0.5%) (Figures 3G, 3I).

The patterns regarding effects of growing algae (*P. subcapitata*) on the alterations of fulvic and humic-acid like components were consistent with alterations in aromaticity and degree of humification. Previous studies showed that microbial transformation of DOM led to loss of protein-like compounds and increases of fulvic and humic-like compounds of DOM,[18,23,49] which is in agreement with our observations for the DOMs from 50 and 250 °C-extracts but not with that from 400 °C-extract. The extent of biotransformation of DOM is positively related to the quantity of biodegradable dissolved organic carbon (BDOC).[20] In addition, Fellman et al.[17,49] reported that fraction of BDOC in the DOMs from stream waters was positively correlated with the proportion of protein-like components but negatively correlated with $SUVA_{254}$ and humic-like components. Accordingly, DOM in the 50 and 250 °C-extracts may be more biodegradable than that in the 400 °C-extract. Autochthonous natural organic matter (NOM) is usually more biodegradable than allochthonous NOM.[50] The proportion of autochthonous and

allochthonous DOM in the original litter extracts (β/α) (Figure 2E) also suggested that DOM in 400 °C-extracts contains less BDOC. Biotransformation of BDOM (e.g., humification) would lead to increases in humic substances, while utilization of amino acids or peptides by growing algae would result in the loss of tyrosine-like components.[51] Biodegradation processes might have less influence on the changes of protein-like components in DOM from 400 °C-litters compared to the DOMs from 50 and 250 °C-litters. Alternatively, the increase in tyrosine-like component was likely due to productions of protein-like compound from algae such as phycocyanin and phycoerythrin in cyanobacteria;[32,52,53] fluorescence reductions of fulvic and humic-like components might be due to the degradation of fluorescing material or quenching of humic substances caused by organic molecules formed during degradation.[52,54]

In additional to DOM chemical structure and optical characteristics, biodegradability of DOM was also related to DOM composition. In terms of relationships in the fraction of BDOC and composition of DOM, Qualls and Haines[55] found a highly positive correlation (R = 0.83) between the loss of DOC and initial content of hydrophilic neutral substances. Carbohydrate content in DOM also has been used as an indicator representing the extent of DOM biodegradation.[19,55–57] Quill et al.[12] found that increases in burning temperature decreased the terrestrial DOM carbohydrate composition and aliphatic carboxylate species in the DOM and consequently reduce the polarity and solubility of DOM. In this study relative low proportion of BDOC in 400 °C-extract might be associated with lacks of carbohydrate and hydrophilic substances.

DOM Chlorine Reactivity and DBP Formation Potential

After the inoculation of 50 °C-extract with algae, specific chlorine demand (SCD) significantly increased from 0.71 ± 0.08 to 1.27 ± 0.05 and 1.60 ± 0.13 mg-Cl_2/mg-DOC for *P. subcapitata* and *M. aeruginosa*, respectively (Figure 4A). The same patterns were also observed for 250 °C-extract. For those extracts, inoculation with *M. aeruginosa* resulted in significantly higher SCD than *P. subcapitata*. In contrast, decreases of SCD were observed for the 400 °C-extract, and there was no significant difference in the SCD after the inoculation with *P. subcapitata* and *M. aeruginosa*. Before the inoculations with algae, SCD of TA-DOM significantly increased with burning temperature. After the inoculations with *P. subcapitata* in 50 °C and 250 °C-extracts, SCD exhibited no significant difference (1.26 ± 0.05 and 1.18 ± 0.10 mg-Cl_2/mg-DOC for the 50 and 250 °C-extract). There were also no differences in SCD for all TA-DOMs after inoculations with *M. aeruginosa*. These results suggest that algae-induced alteration of SCD was associated with algal species and DOM characteristics, and the algae-induced alteration would diminish the differences of SCD among TA-DOMs.

The patterns of algae-induced changes in SCD (Figure 4A) were the same as that in DOM aromaticity and humic substance content (Figures 2A, 2B,), suggesting that chlorine reactivity of DOM was closely related to the proportion of aromatic compound or humic substance. Beggs and Summers[21] demonstrated that alterations in chlorine reactivity of three types of litter (tree, empty bed, and established bed) leachate after biodegradation was dependent on the characteristics of litter leachate. Biodegradation of the leachate from established bed, which had less biodegradable organic materials than

the other two types, decreased the chlorine reactivity. On the contrary, biodegradation of tree and empty bed litter leachates increased chlorine reactivity, indicative of an increase in humic substance fraction. The decreases in chlorine reactivity for 400 °C-extract may be caused by the biotransformation of chlorine oxidizable organic matter to non-chlorine oxidizable organic matter by growing algae.

In this study most of SDBP-FPs after inoculations with algae over 7 days were significantly changed (Figures 4B-E). Regardless of algal species, growing algae had a promoting effect on specific trihalomethane formation potential (STHM-FP) (Figure 4B). For example, STHM-FPs for the 50 °C-extract after inoculations of algae increased from 26.39 ± 1.48 to 55.12 ± 2.14 and 53.31 ± 4.11 µg-THMs/mg-DOC for *P. subcapitata* and *M. aeruginosa*, respectively. Substantial increases in STHM-FP after inoculations with algae for 50 and 250 °C-extracts suggested that non-THM precursors might be preferentially removed during biodegradation and additional THM precursors were produced by growing algae.[18] These results are in agreement with a previous study,[21] showing that biodegradation of tree and empty bed litter leachates increased STHM-FP. STHM-FP for 400 °C-extract, which had less fraction of biodegradable DOM, also significantly increased after the inoculation with algae (increased from 17.88 ± 3.99 to 32.86 ± 0.73 and 29.57 ± 0.31 µg-THMs/mg-DOC for *P. subcapitata* and *M. aeruginosa*, respectively). Although the aromaticity of DOM from 400 °C-litter decreased after inoculations with algae, non-aromatic DOM produced from algae may have higher chlorine reactivity than aromatic DOM for the THMs formation. Increases in protein-like compounds were observed for the 400 °C-extract with inoculations with algae. Since

moieties of proteins are known as highly reactive THM precursors,[58] the promotion of STHM-FP after inoculations of 400 °C-extract with algae was likely due to increases in proteinaceous THM precursors produced by algae. After inoculations with algae, the patterns of specific haloacetonitriles formation potential (SHAN-FP) were consistent with that in SCD, except for 400 °C-extract after inoculation with *M. aeruginosa* (Figures 4C, 4A). Many algae-related proteinaceous compounds such as amino acids and heterocyclic nitrogen in nucleic acid have been reported as HANs precursors,[58,59] and SHAN-FP for the organic matters produced from green algae and cyanobacteria are different. For example, Oliver[60] reported that the blue-green alga *Anabaena* Texas 1447 had a much higher organic nitrogen content and yielded more dihaloacetonitriles on chlorination than the green alga *Scenedesmus basiliensis*. Yang et al.[61] showed that the yield of dichloroacetonitrile after chlorination of algae-produced organic matter ranged from 0.03 to 0.15 μmol/mmol-C for *M. aeruginosa* and from 0.04 to 0.07 μmol/mmol-C for green alga *Chlorella vulgaris*. The observed higher SHAN-FP after inoculation with *M. aeruginosa* may result from higher amounts of DON (Table S2) and highly reactive proteinaceous compounds it produced. After the inoculations with algae, increases of specific chloral hydrate formation potential (SCHD-FP) were observed for 50 and 400 °C-extracts. But it significantly decreased from 8.32 ± 2.13 to 3.41 ± 0.18 and 3.66 ± 0.81 μg-CHD/mg-DOC after inoculations of 250 °C-extract with *P. subcapitata* and *M. aeruginosa*, respectively (Figure 4D). Before inoculations with algae, SCHD-FP for the 250 °C-extract was significantly higher than that for 50 °C-extract, while it did not reveal significant difference after inoculations with algae. Similarly to the formation of HANs,

amino acids and nitrogenous compounds are also potential precursors for CHD.[62] Freshwater algae are able to assimilate a variety of organic nitrogen sources including amino acids,[46] and extracellular oxidation and hydrolysis of amino acids and proteins have been reported as common pathways as these compounds were assimilated by algae.[63] The decreases of SCHD-FP for 250 °C-extract after inoculations with algae as well as the decrease of SHAN-FP for 400 °C-extract after inoculation with *P. subcapitata* were likely due to the uptake of amino acids exported from burned litters by growing algae. After the inoculations with algae, SHK-FP for 50 and 400 °C-extracts significantly increased (Figure 4E). The formations of haloketones may originate from reactions between chlorine and compounds containing carbonyl functional groups.[64] Increases in carbonyl groups during humification of fulvic acid[65] as well as oxidization of primary amino groups by chlorine[58] would lead to the increases in SHK-FP for 50 and 400 °C-extracts.

Growing algae also altered the amount of DBP formed during water chlorination processes (Figure S1). Except for the fraction of tryptophan-like components, highly correlated relationships ($P < 0.05$) in DOM characteristics and chlorine reactivities in forming HANs and CHD were observed after inoculations with algae (Tables S3, S4). In contrast, no significant correlations at all were observed in STHM-FP. After inoculation with *P. subcapitata*, significant correlations were observed in fractions of fluorescence compounds and SHK-FP. Relationships in DBP-FP and DOM properties have also been derived in previous studies.[18,21] For example, Hur et al. reported that either before or after biodegradation, the STHM-FP for six different DOMs was highly correlated with MW

information.[18] Beggs and summers found that aromaticity of pine leachates was a strong indicator of THM formation.[21] In this study, before inoculations with algae, STHM-FP and SHAN-FP were also significantly correlated with DOM MW; and after inoculations with algae, aromaticity of TA-DOM was significantly correlated with SHAN-FP and SCHD-FP.

Fractions of Brominated-THMs

The bromine incorporation factor (BIF) for trihalomethanes increased from 4.47 ± 0.02% to 5.25 ± 0.65% and 6.20 ± 1.02% for 400 °C-extract inoculated with *P. subcapitata* and *M. aeruginosa*, respectively (Figure 5). During water chlorination processes, studies demonstrated that bromine incorporation of DBPs was associated with DOM characteristics and bromide concentration in waters.[4,66,67] Decreases of $SUVA_{254}$ and MW were observed for the 400 °C-extract after inoculations with algae (Figures 2A, 2C). The increases of BIF for 400 °C-extract after inoculations with algae were in agreement with the studies showing that bromine was more effectively incorporated into low $SUVA_{254}$ and low MW of DOM.[4,66,67] Increases in algae-produced organic matter were observed for the 400 °C-extract (Figure 2E). Since production of bromine from algal cells has not been reported and more than 70% of algal organic matters are hydrophilic compounds,[53] the increases in BIF could also be explained by the findings that hydrophilic fractions of DOM were generally more reactive with bromine than chlorine.[4,66,67] Maes et al.[25] found that exposure of the green alga *Desmodesmus subspitacus* to xenoestrogen 17α-ethinylestradiol (EE2) in the bromine-containing

medium led to transformation of EE2 into two brominated analogues, suggesting that algae could brominate EE2 when bromide was available in the solution. In this study, increases in the BIF of THM may result from the biotransformation of no bromine-containing THM precursors into bromine-containing precursors by growing algae.

Environmental Significance and Implications

In order to accurately predict impacts of wildfire on downstream water quality as well as to take precautions against negative impacts on community drinking water supply, it is essential for responsible agencies to understand how the quality and quantity of DOM from fire impacted areas are changed during its transport from forested watersheds to water treatment facilities. This study presents evidence that growing algae alter terrestrial DOM composition and chlorine reactivity after wildfire. The fraction of biodegradable DOM exported from forested watersheds decreases with an increase in fire intensity (i.e., fire temperature), implying that DOMs exported from mildly burned (≤ 250 °C) areas are more likely subjected to biotic transformations in downstream compared to that from severely burned (≥ 400 °C) areas. Fire-affected DOMs can accelerate proliferations of phytoplankton in drinking water resources. Algal blooms may have an ephemeral effect on decreasing DBP precursors in fire-affected terrestrial DOM; but as algal populations increase, concomitant productions of algal organic matters will likely outweigh that loss, resulting in increases of DOM and chlorine reactivity for DBP formations as well as increases of the proportion of brominated THMs. Clearly, despite negative impacts of algal organic matters on drinking water quality such as taste-and-odor compounds

(geosmin and 2-methylisoborneol) and cyanotoxins (microcystins) produced by cyanobacteria (e.g., *Oscillatoria* sp. and *Microcystis* sp.), occurrence of wildfire followed by algal blooms in drinking water resources will create additional challenges for drinking water treatment facilities.

Despite findings that elevated DOM concentrations after wildfire could lead to algal blooms in downstream waters,[68–70] occurrences of wildfire and seasonal proliferation of algae as a result of global warming are more likely observed in spring and summer,[71–73] which makes proliferation of algae an important consideration in terms of assessing impacts of wildfire on drinking water quality. Algal bloom species can differ from site to site. Additionally, algal capabilities to uptake DOM as well as characteristics of algal organic matter are related to the species and growth phase.[40,41,53,74] Thus, it is challenging to generalize the impacts of wildfire and algal bloom on drinking water quality. Further studies are still needed to understand that after wildfire events how algal species alter water quality during their life cycle.

REFERENCES

(1) Bladon, K. D.; Emelko, M. B.; Silins, U.; Stone, M. Wildfire and the future of water supply. *Environ.Sci.Technol.* **2014**, *48* (16), 8936–8943.

(2) Spencer, C. N.; Gabel, K. O.; Hauer, F. R. Wildfire effects on stream food webs and nutrient dynamics in Glacier National Park, USA. *For. Ecol. Manage.* **2003**, *178* (1–2), 141–153.

(3) Kraus, T. E. C.; Anderson, C. a; Morgenstern, K.; Downing, B. D.; Pellerin, B. a; Bergamaschi, B. a. Determining sources of dissolved organic carbon and disinfection byproduct precursors to the McKenzie River, Oregon. *J. Environ. Qual.* **2010**, *39* (6), 2100–2112.

(4) Kitis, M.; Karanfil, T.; Wigton, A.; Kilduff, J. E. Probing reactivity of dissolved organic matter for disinfection by-product formation using XAD-8 resin adsorption and ultrafiltration fractionation. *Water Res.* **2002**, *36* (15), 3834–3848.

(5) Wang, J. J.; Dahlgren, R. A.; Chow, A. T. Controlled Burning of Forest Detritus Altering Spectroscopic Characteristics and Chlorine Reactivity of Dissolved Organic Matter: Effects of Temperature and Oxygen Availability. *Environ. Sci. Technol.* **2015**, *49* (24), 14019–14027.

(6) 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.

(7) Bisson, P.A., Ice, G.G., Perrin, C.J., Bilby, R. E. Effects of forest fertilization on water quality and aquatic resources in the Douglas-fir region. In *Forest Fertilization: Sustaining and Improving Nutrition and Growth of Western Forests. Institute of Forest Resources*; Chappel, H.N. Wettman, G.F. Miller, R. E., Ed.; College of Forest Resources, University of Washington, Seattle, WA, 1992; pp 179–193.

(8) Fox TR, Allen HL, Albaugh TJ, Rubilar R, C. C. Forest Fertilization and Water Quality in the United States. *Better Crop.* **2007**, No. 91, 7–9.

(9) Anderson, C. W. Ecological effects on streams from forest fertilization; literature review and conceptual framework for future study in the western Cascades. *Water-Resources Investig. Rep.* **2002**.

(10) Markou, G.; Vandamme, D.; Muylaert, K. Microalgal and cyanobacterial cultivation: The supply of nutrients. *Water Res.* **2014**, *65*, 186–202.

(11) Subashchandrabose, S. R.; Ramakrishnan, B.; Megharaj, M.; Venkateswarlu, K.; Naidu, R. Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. *Environ. Int.* **2013**, *51*, 59–72.

(12) Quill, E. S.; Angove, M. J.; Morton, D. W.; Johnson, B. B. Characterisation of dissolved organic matter in water extracts of thermally altered plant species found in boxironbark forests. *Aust. J. Soil Res.* **2010**, *48* (8), 693–704.

(13) Bruun, S.; Jensen, E. S.; Jensen, L. S. Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration. *Org. Geochem.* **2008**, *39* (7), 839–845.

(14) Hilscher, A.; Knicker, H. Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28month microcosm experiment. *Org. Geochem.* **2011**, *42* (1), 42–54.

(15) Harvey, O. R.; Kuo, L. J.; Zimmerman, A. R.; Louchouarn, P.; Amonette, J. E.; Herbert, B. E. An index-based approach to assessing recalcitrance and soil carbon sequestration potential of engineered black carbons (biochars). *Environ. Sci. Technol.* **2012**, *46* (3), 1415–1421.

(16) Norwood, M. J.; Louchouarn, P.; Kuo, L. J.; Harvey, O. R. Characterization and biodegradation of water-soluble biomarkers and organic carbon extracted from low temperature chars. *Org. Geochem.* **2013**, *56*, 111–119.

(17) Fellman, J. B.; D'Amore, D. V.; Hood, E.; Boone, R. D. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. *Biogeochemistry* **2008**, *88* (2), 169–184.

(18) Hur, J.; Lee, M. H.; Song, H.; Schlatman, M. A. Microbial transformation of dissolved organic matter from different sources and its influence on disinfection byproduct formation potentials. *Environ. Sci. Pollut. Res.* **2013**, *20* (6), 4176–4187.

(19) Kalbitz, K.; Schmerwitz, J.; Schwesig, D.; Matzner, E. Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma* **2003**, *113* (3–4), 273–291.

(20) Lim, M. H.; Snyder, S. A.; Sedlak, D. L. Use of biodegradable dissolved organic carbon (BDOC) to assess the potential for transformation of wastewater-derived contaminants in surface waters. *Water Res.* **2008**, *42* (12), 2943–2952.

(21) Beggs, K. M. H.; Summers, R. S. Character and chlorine reactivity of dissolved organic matter from a mountain pine beetle impacted watershed. *Environ. Sci. Technol.* **2011**, *45* (13), 5717–5724.

(22) Hertkorn, N.; Claus, H.; Schmitt-Kopplin, P.; Perdue, E. M.; Filip, Z. Utilization and transformation of aquatic humic substances by autochthonous microorganisms. *Environ. Sci. Technol.* **2002**, *36* (20), 4334–4345.

(23) Hur, J.; Park, M.-H.; Schlautman, M. A. Microbial transformation of dissolved leaf litter organic matter and its effects on selected organic matter operational descriptors. *Environ. Sci. Technol.* **2009**, *43* (7), 2315–2321.

(24) Pellerin, B. a; Hernes, P. J.; Saraceno, J.; Spencer, R. G. M.; Bergamaschi, B. a. Microbial degradation of plant leachate alters lignin phenols and trihalomethane precursors. *J. Environ. Qual.* **2010**, *39* (3), 946–954.

(25) Maes, H. M.; Maletz, S. X.; Ratte, H. T.; Hollender, J.; Schaeffer, A. Uptake, elimination, and biotransformation of 17α-ethinylestradiol by the freshwater alga Desmodesmus subspicatus. *Environ. Sci. Technol.* **2014**, *48* (20), 12354–12361.

(26) Cai, T.; Park, S. Y.; Li, Y. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renew. Sustain. Energy Rev.* **2013**, *19*, 360–369.

(27) Granéli, E.; Carlsson, P.; Legrand, C. The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. *Aquat. Ecol.* **1999**, *33* (1), 17–27.

(28) Bährs, H.; Steinberg, C. E. W. Impact of two different humic substances on selected coccal green algae and cyanobacteria-changes in growth and photosynthetic performance. *Environ. Sci. Pollut. Res.* **2012**, *19* (2), 335–346.

(29) Smith, C. R.; Buzan, E. M.; Lee, J. W. Potential impact of biochar water-extractable substances on environmental sustainability. *ACS Sustain. Chem. Eng.* **2013**, *1* (1), 118–126.

(30) Kirso, U.; Irha, N. Role of algae in fate of carcinogenic polycyclic aromatic hydrocarbons in the aquatic environment. *Ecotoxicol. Environ. Saf.* **1998**, *41* (41), 83–89.

(31) Singer, P. C. Humic Substances as Precursors for Potentially Harmful Disinfectant by-products. *Water Sci. Technol. Water Supply* **1999**, *40* (9), 25–30.

(32) Nguyen, M.; Westerhoff, P.; Baker, L.; Hu, Q.; Esparza-Soto, M.; Sommerfeld, M. Characteristics and Reactivity of Algae-Produced Dissolved Organic Carbon. *J. Environ. Eng.* **2005**, *131*, 1574–1582.

(33) Wert, E. C.; Rosario-Ortiz, F. L. Intracellular organic matter from cyanobacteria as a precursor for carbonaceous and nitrogenous disinfection byproducts. *Environ. Sci. Technol.* **2013**, *47* (12), 6332–6340.

(34) Guillemette, F.; del Giorgio, P. A. Simultaneous consumption and production of fluorescent dissolved organic matter by lake bacterioplankton. *Environ. Microbiol.* **2012**, *14* (6), 1432–1443.

(35) Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; DeMarini, D. M.

Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutat. Res. - Rev. Mutat. Res.* **2007**, *636* (1–3), 178–242.

(36) Bodí, M. B.; Martin, D. A.; Balfour, V. N.; Santín, C.; Doerr, S. H.; Pereira, P.; Cerdà, A.; Mataix-Solera, J. Wildland fire ash: Production, composition and eco-hydro-geomorphic effects. *Earth-Science Rev.* **2014**, *130*, 103–127.

(37) Tsai, K.-P. Effects of two copper compounds on Microcystis aeruginosa cell density, membrane integrity, and microcystin release. *Ecotoxicol. Environ. Saf.* **2015**, *120*, 428–435.

(38) McEachern, P.; Prepas, E. E.; Gibson, J. J.; Dinsmore, W. P. Forest fire induced impacts on phosphorus, nitrogen, and chlorophyll a concentrations in boreal subarctic lakes of northern Alberta. *Can. J. Fish. Aquat. Sci.* **2000**, *57* (S2), 73–81.

(39) Hohner, A. K.; Cawley, K. M.; Rosario-ortiz, F. L. *Impact of the High Park wildfire on water quality*; Department of Civil, Environmental and Architectural Engineering, University of Colorado, 2014.

(40) Mailhot, H. Prediction of algal bioaccumulation and uptake rate of nine organic compounds by ten physicochemical properties. *Environ. Sci. Technol.* **1987**, *21* (10), 1009–1013.

(41) Woertz, I.; Feffer, a.; Lundquist, T.; Nelson, Y. Algae Grown on Dairy and Municipal Wastewater for Simultaneous Nutrient Removal and Lipid Production for Biofuel Feedstock. *J. Environ. Eng.* **2009**, *135* (11), 1115–1122.

(42) Lu, Y.; Bauer, J. E.; Canuel, E. A.; Yamashita, Y.; Chambers, R. M.; Jaffé, R. Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use. *J. Geophys. Res. Biogeosciences* **2013**, *118* (2), 566–580.

(43) 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.

(44) Hur, J. Microbial changes in selected operational descriptors of dissolved organic matters from various sources in a watershed. *Water. Air. Soil Pollut.* **2011**, *215* (1–4), 465–476.

(45) Kobayashi, H.; Rittmann, B. E. Microbial removal of hazardous organic compounds. *Environ. Sci. Technol.* **1982**, *16* (3), 170A–183A.

(46) Perez-Garcia, O.; Escalante, F. M. E.; de-Bashan, L. E.; Bashan, Y. Heterotrophic cultures of microalgae: Metabolism and potential products. *Water Res.* **2011**, *45* (1), 11–36.

(47) Merritt, K. A.; Erich, M. S. Influence of organic matter decomposition on soluble carbon and its copper-binding capacity. *J. Environ. Qual.* **2003**, *32* (6), 2122–2131.

(48) Leloup, M.; Pallier, V.; Nicolau, R.; Feuillade-Cathalifaud, G. Assessing Transformations of Algal Organic Matter in the Long-Term: Impacts of Humification-Like Processes. *Int. J. Mol. Sci.* **2015**, *16* (8), 18096–18110.

(49) Fellman, J. B.; Hood, E.; Edwards, R. T.; D'Amore, D. V. Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. *J. Geophys. Res. Biogeosciences* **2009**, *114* (1), 1–14.

(50) Leenheer, J. J. a.; Croué, J. J.-P. Peer reviewed: characterizing aquatic dissolved organic matter. *Environ. Sci. Technol.* **2003**, *37* (1), 18A–26A.

(51) Znachor, P.; Nedoma, J. Importance of dissolved organic carbon for phytoplankton nutrition in a eutrophic reservoir. *J. Plankton Res.* **2010**, *32* (3), 367–376.

(52) Korak, J. A.; Wert, E. C.; Rosario-Ortiz, F. L. Evaluating fluorescence spectroscopy as a tool to characterize cyanobacteria intracellular organic matter upon simulated release and oxidation in natural water. *Water Res.* **2015**, *68*, 432–443.

(53) Henderson, R. K.; Baker, A.; Parsons, S. A.; Jefferson, B. Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. *Water Res.* **2008**, *42* (13), 3435–3445.

(54) Saadi, I.; Borisover, M.; Armon, R.; Laor, Y. Monitoring of effluent DOM biodegradation using fluorescence, UV and DOC measurements. *Chemosphere* **2006**, *63* (3), 530–539.

(55) Qualls, R. G.; Haines, B. L. Biodegradability of Dissolved Organic Matter in Forest Throughfall, Soil Solution, and Stream Water. *Soil Sci. Soc. Am. J* **1992**, *586*, 578–586.

(56) Dowell, W. H. . Kinetics and mechanisms of dissolved organic carbon retention in a headwater stream. *Biogeochemistry* **1985**, *1* (4), 329–352.

(57) Leenheer, J. A. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environ. Sci. Technol.* **1981**, *15* (5), 578–587.

(58) Scully, F. E.; Howell, G. D.; Kravitz, R.; Jewell, J. T.; Hahn, V.; Speed, M. Proteins in natural waters and their relation to the formation of chlorinated organics during water disinfection. *Environ. Sci. Technol.* **1988**, *22* (5), 537–542.

(59) Lee, W.; Westerhoff, P.; Croué, J.-P. Dissolved Organic Nitrogen as a Precursor for Chloroform, Dichloroacetonitrile, N -Nitrosodimethylamine, and Trichloronitromethane. *Environ. Sci. Technol.* **2007**, *41* (15), 5485–5490.

(60) Oliver, B. Dihaloacetonitriles in Drinking Water: Algae and Fulvic Acids as Precursors. *Environ. Sci. Technol.* **1983**, *17* (2), 80–83.

(61) Yang, X.; Guo, W.; Shen, Q. Formation of disinfection byproducts from chlor(am)ination of algal organic matter. *J. Hazard. Mater.* **2011**, *197*, 378–388.

(62) Trehy, M. L.; Yost, R. A.; Miles, C. J. Chlorination byproducts of amino acids in natural waters. *Environ. Sci. Technol.* **1986**, *20* (11), 1117–1122.

(63) Burkholder, J. M.; Glibert, P. M.; Skelton, H. M. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* **2008**, *8* (1), 77–93.

(64) Deborde, M.; von Gunten, U. Reactions of chlorine with inorganic and organic compounds during water treatment-Kinetics and mechanisms: A critical review. *Water Res.* **2008**, *42* (1–2), 13–51.

(65) Bolan, N. S.; Adriano, D. C.; Kunhikrishnan, A.; James, T.; McDowell, R.; Senesi, N. *Dissolved Organic Matter. Biogeochemistry, Dynamics, and Environmental Significance in Soils.*, 1st ed.; Elsevier Inc., 2011; Vol. 110.

(66) Liang, L.; Singer, P. C. Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water. *Environ. Sci. Technol.* **2003**, *37* (12), 2920–2928.

(67) Chowdhury, S.; Champagne, P.; James McLellan, P. Investigating effects of bromide ions on trihalomethanes and developing model for predicting bromodichloromethane in drinking water. *Water Res.* **2010**, *44* (7), 2349–2359.

(68) Cooper, S. D.; Page, H. M.; Wiseman, S. W.; Klose, K.; Bennett, D.; Even, T.; Sadro, S.; Nelson, C. E.; Dudley, T. L. Physicochemical and biological responses of streams to wildfire severity in riparian zones. *Freshw. Biol.* **2015**, *60* (12), 2600–2619.

(69) Silins, U.; Bladon, K. D.; Kelly, E. N.; Esch, E.; Spence, J. R.; Stone, M.; Emelko, M. B.; Boon, S.; Wagner, M. J.; Williams, C. H. S.; et al. Five-year legacy of wildfire and salvage logging impacts on nutrient runoff and aquatic plant, invertebrate, and fish productivity. *Ecohydrology* **2014**, *7* (6), 1508–1523.

(70) Smith, H. G.; Sheridan, G. J.; Lane, P. N. J.; Nyman, P.; Haydon, S. Wildfire effects on water quality in forest catchments: A review with implications for water supply. *J. Hydrol.* **2011**, *396* (1–2), 170–192.

(71) Running, S. W. Is Global Warming Causing More, Large Wildfires. *Science (80-.).* **2006**, *313* (August), 927–928.

(72) Westerling, A. L.; Hidalgo, H. G.; Cayan, D. R.; Swetnam, T. W. Warming and Earlier Spring Increase Western U.S. Forest Wildfire Activity. *Science (80-.).* **2006**, *313* (5789), 940–943.

(73) Michalak, A. M.; Anderson, E. J.; Beletsky, D.; Boland, S.; Bosch, N. S.; Bridgeman, T. B.; Chaffin, J. D.; Cho, K.; Confesor, R.; Daloglu, I.; et al. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (16), 6448–6452.

(74) Leloup, M.; Nicolau, R.; Pallier, V.; Yéprémian, C.; Feuillade-Cathalifaud, G. Organic matter produced by algae and cyanobacteria: Quantitative and qualitative characterization. *J. Environ. Sci.* **2013**, *25* (6), 1089–1097.

Table 1. Characteristics of the algal culture medium and raw litter extracts after 48-h water extractions (Average ± Standard Deviation, n = 3).

	Culture medium	50 °C-extracts	250 °C-extracts	400 °C-extracts
pH	9.52 ± 0.00	4.34 ± 0.00	5.57 ± 0.00	9.28 ± 0.00
Conductivity (μS/cm)	260 ± 0	170 ± 0	128 ± 0	740 ± 0
DOC (mg/L)	0.7 ± 0.0	680.6 ± 18.6	217.6 ± 4.3	20.0 ± 0.2
TDN (mg/L)	11.8 ± 0.2	8.5 ± 0.4	1.4 ± 0.1	3.1 ± 0.1
NH_4^+-N (mg/L)	0.6 ± 0.2	1.1 ± 0.0	0.3 ± 0.0	0.7 ± 0.2
NO_x^--N (mg/L)	13.7 ± 1.0	1.1 ± 0.1	0.03 ± 0.01	0.50 ± 0.03
DON (mg/L)	ND	6.3 ± 0.5	1.1 ± 0.1	1.9 ± 0.2
DOC/DON (mg/mg)	ND	107.9 ± 5.4	197.5 ± 21.0	10.7 ± 1.0
$SUVA_{254}$ (L/mg/m)	NA	1.19 ± 0.02	0.91 ± 0.00	4.95 ± 0.00
HIX	NA	1.02 ± 0.04	0.86 ± 0.14	31.53 ± 1.18
E2/E3	NA	4.86 ± 0.00	5.22 ± 0.00	6.00 ± 0.00
β/α	NA	0.50 ± 0.00	0.59 ± 0.03	0.36 ± 0.00
FI	NA	1.81 ± 0.04	2.06 ± 0.04	2.50 ± 0.06

ND: Not detectable
NA: Not available

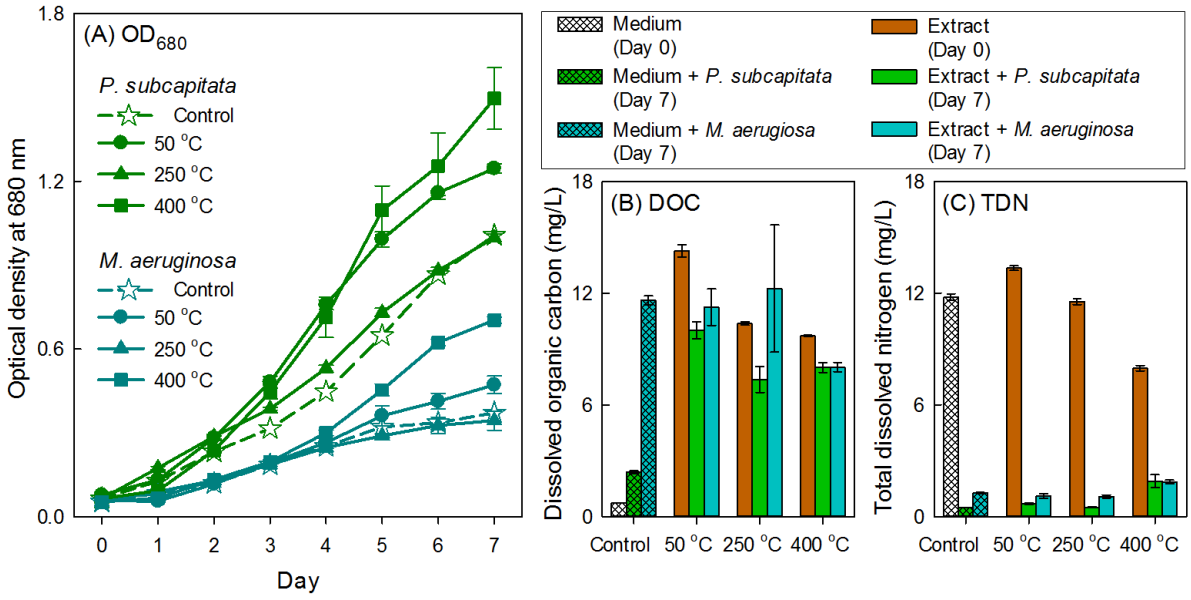


Figure 1. Time-course changes of optical density at 680 nm (OD_{680}) for *P. subcapitata* and *M. aeruginosa* cultured in the medium (control) and in the medium mixed with thermally-altered extracts (A). Associated concentrations of dissolved organic carbon (DOC) (B) and total dissolved nitrogen (TDN) (C) on day 0 and day 7. Error bars represent the standard deviation.

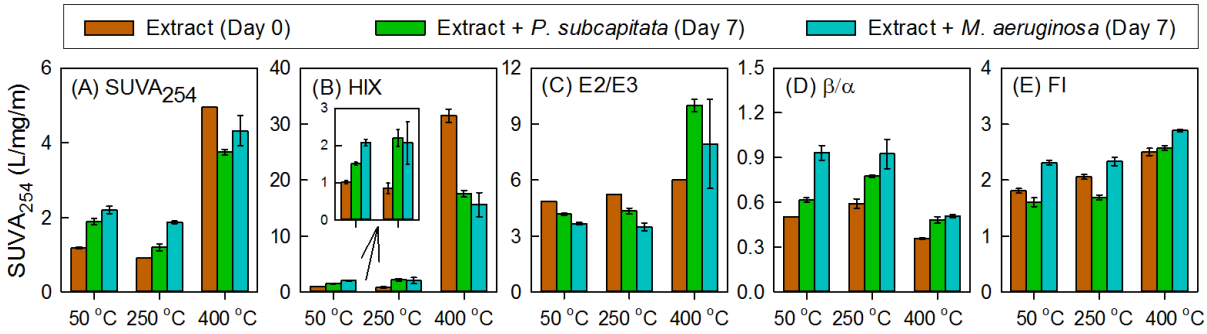


Figure 2. Optical characteristics of thermally-altered extracts before (Day 0) and after (Day 7) inoculations with *P. subcapitata* and *M. aeruginosa*. Error bars represent the standard deviation. $SUVA_{254}$, specific ultraviolet absorbance at 254 nm; HIX, humification index; E2/E3, UVA at 254 nm divided by UVA 365 nm; β/α, freshness index; FI, fluorescence index.

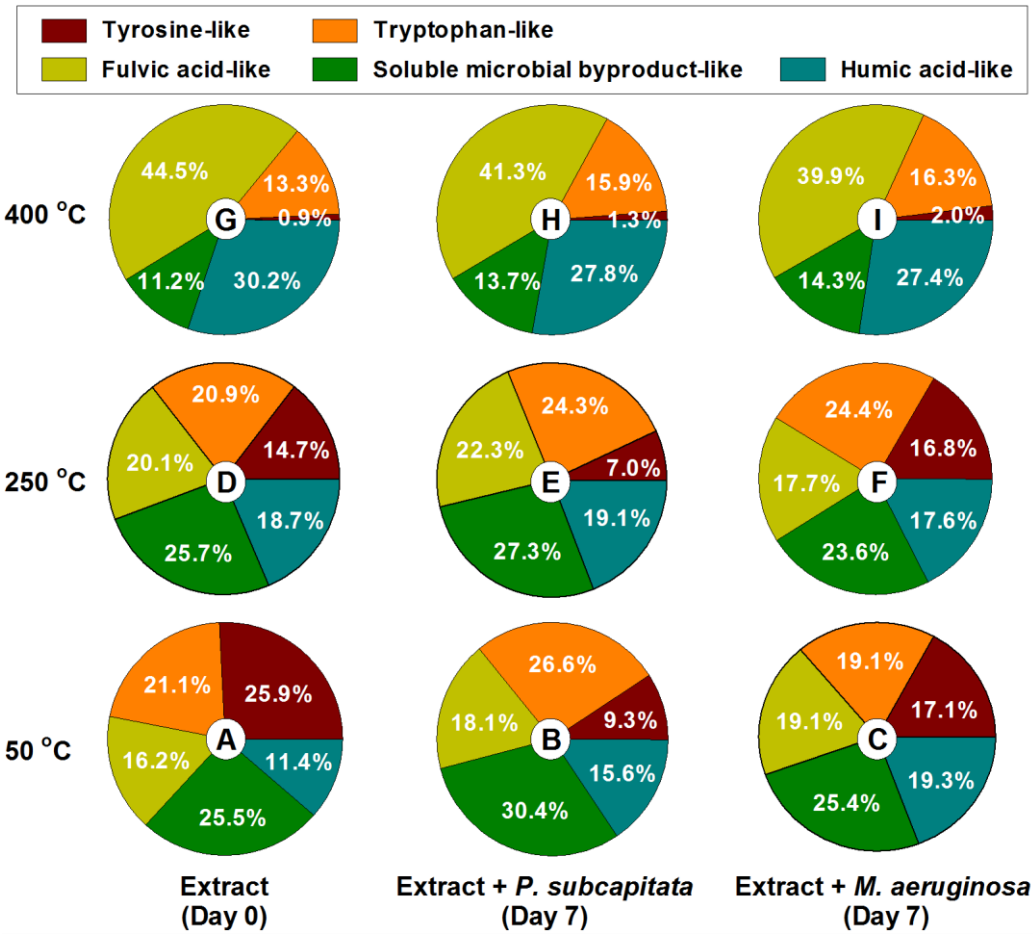


Figure 3. Percent fluorescence responses of five EEM regions for thermally-altered extracts before (Day 0) and after (Day 7) inoculations with *P. subcapitata* and *M. aeruginosa*.

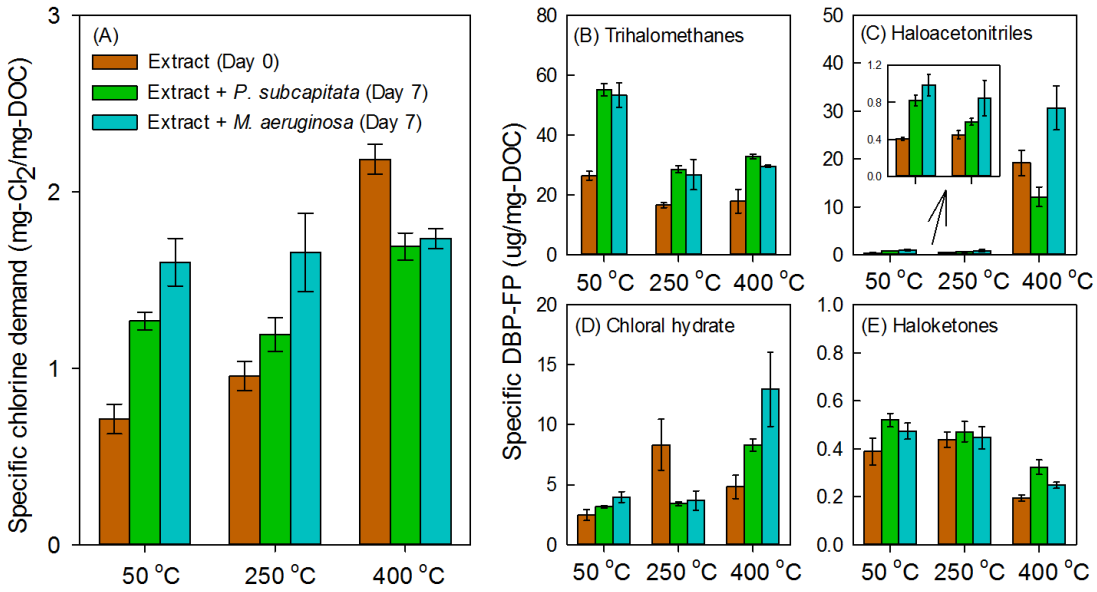


Figure 4. Specific chlorine demand (A) and specific disinfection byproduct formation potential (DBP-FP) (B-E) of thermally-altered extracts before (Day 0) and after (Day 7) inoculations with *P. subcapitata* and *M. aeruginosa*. Error bars represent the standard deviation.

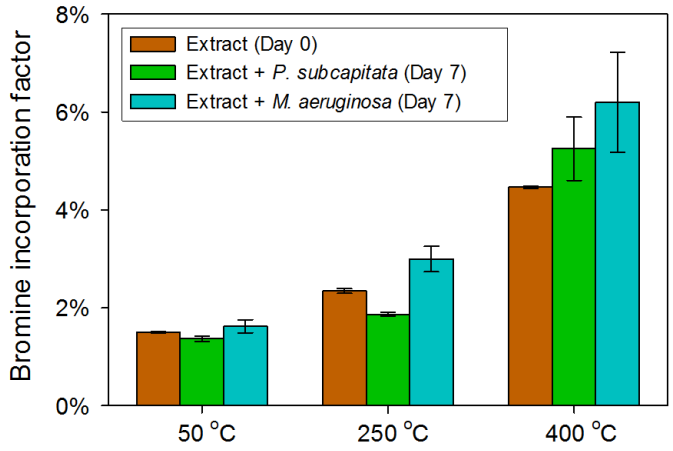


Figure 5. Bromine incorporation factor of trihalomethanes formed from thermally-altered extracts before (Day 0) and after (Day 7) inoculations with *P. subcapitata* and *M. aeruginosa*. Error bars represent the standard deviation.

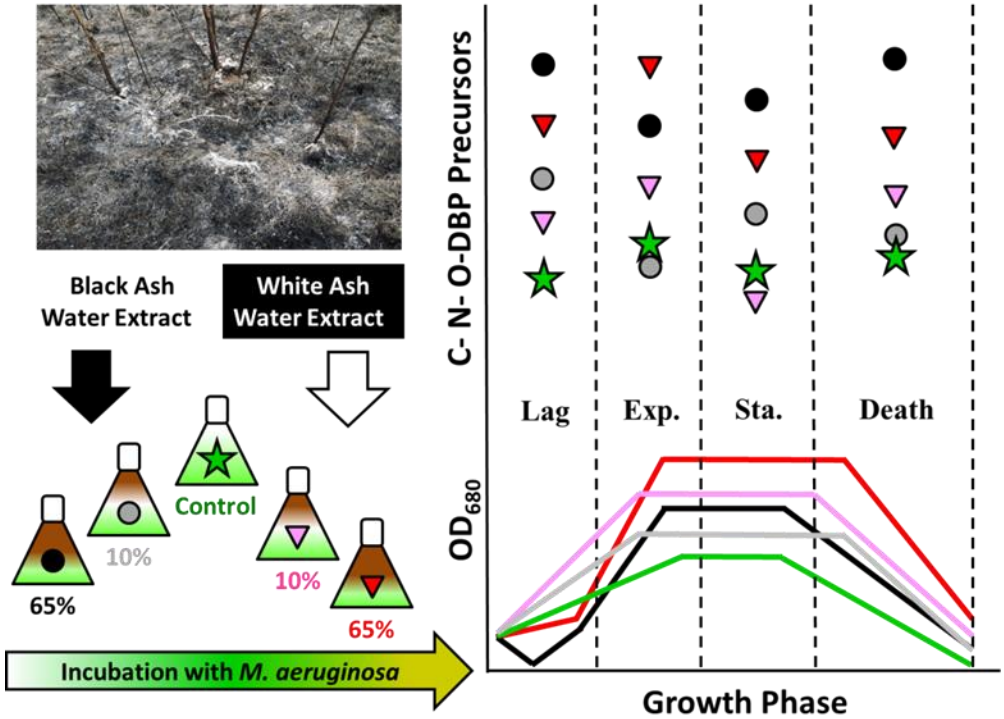
CHAPTER THREE

Dynamic Changes of Disinfection Byproduct Precursors following Exposures of Microcystis aeruginosa to Wildfire Ash Solutions

ABSTRACT

Wildfires can elevate dissolved organic matter (DOM) levels due to ash input and algal growth in source waters, and consequently impacting disinfection byproduct formation in finished water; however, it remains unclear how quality and quantity of overall allochthonous and autochthonous DOM as well as associated DBP formation are changed during an entire algal life cycle. *Microcystis aeruginosa* was cultured in the medium containing low and high concentrations [10% and 65% (v/v)] of black and white ash water extracts (BE and WE) to study dynamic changes of carbonaceous, nitrogenous, and oxygenated DBP precursors during algal growth. DOM was characterized by absorption and fluorescence spectroscopy and chlorination/chloramination-based DBP formation experiments. In the treatment with 10% BE, the amount of C-DBP precursors decreased from 6.8 to 3.0 mmol/mol-C at lag-exponential phase then increased to 4.2 mmol/mol-C at death phase. The same trend was observed for O-DBP precursors. However, these dynamic changes of C- and O-DBP precursors exhibited opposite patterns in 65% extracts. Similar patterns were also observed in the WE treatments. On the other hand, N-DBP precursors continuously declined in all treatments. These results indicate that postfire ash loading and algal bloom stage may significantly affect DBP formation in source water.

GRAPHICAL ABSTRACT



INTRODUCTION

Wildfires convert forest biomass and detritus into ash. After wildfires high load of ash in stream waters and elevated concentration of dissolved organic matter (DOM) have been reported in downstream source waters.[1–3] In addition, increases in nutrient concentrations (e.g., nitrogen and phosphorus) concomitant with proliferation of algae also have been observed.[4–7] Algae are prevailing producers of autochthonous DOM in freshwaters,[8] and the level of DOM released from algae is usually proportional to their population.[9] Thus, increases of DOM in fire-impacted source water can be attributed to both allochthonous inputs from ash and autochthonous productions from algae. Regardless of wildfires, successions of harmful algal bloom, especially *Microcystis aeruginosa*, often occur in reservoirs causing serious threat to drinking water quality due to DOM and toxins released from algal cells;[10] and excess inputs of nutrient and organic matter in waters are important factors for stimulating its population.[11,12] DOMs released from both ash and *M. aeruginosa* are precursors of potentially carcinogenic disinfection byproducts (DBPs) [e.g., trihalomethanes (THMs) and haloacetic acids (HAAs)] formed during water treatment oxidation/disinfection processes such as chlorination and chloramination. Importantly, DBP formation potential (DBP-FP) is correlated with DOM quality and quantity.[13,14] To understand how postfire elevated DOM affects DBP-FP in drinking water supply, it is essential to obtain comprehensive knowledge on the changes in concentration and composition of DOM during algal blooms in the absence and presence of ash-contaminated solutions.

The amounts of ash-derived DOM and nutrient entering downstream source water could be changed by environmental variables such as postfire precipitation, snowmelt, and hydrologic conditions.[15,16] For example, Wang et al.[15] reported that increasing cumulative precipitation in the field resulted in a significant decrease in dissolved organic carbon (DOC) released from ash. Compositions of ash-derived DOM are related to ash characteristics. Black and white ashes are commonly observed in burned forest watersheds. Generally, black ash is produced at relatively lower burning temperature and contains higher amount of organic compounds.[1,17] Regarding quality and quantity of DOMs released from black and white ashes, Wang et al.[18] demonstrated that concentrations of DOC in black and white ash solutions were not statistically different, but black ash solution contained significantly less aromatic carbon content than white ash solution. Black ash solution revealed lower pH and amounts of NO_x^-- and NH_4^+-N. Quill et al.[19] also found an increase of aromatic structure and a decrease of aliphatic carbohydrate compounds and polysaccharides in the DOM derived from ash formed at higher temperature.

Variations in water quality caused by wildfires would lead to changes in algal population.[20] Ecotoxicological studies found that exposures of freshwater algae to DOM released from wildfire ash or biochar elicited different degrees of impact on its population, depending on the DOM concentration and composition.[21–23] For example, using optical density at 680 nm (OD_{680}) to monitor daily growth of *Synechococcus elongatus* following 7-days exposures to DOC extracted from biochar, Smith et al.[23] found that growth curve in the presence of 25 mg/L of DOC was similar to the control

but OD_{680} value in the treatment with 25 mg/L of DOC was approximately three times greater than with 150 mg/L of DOC on day 7. They also observed that exposure to biochar-derived DOM containing organic acids and phenols remarkably inhibited algal population growth. Furthermore, it is well documented that quality and quantity of algae-produced organic matter (AOM) can be substantially altered by growth conditions.[24–27] Previous studies showed that growing algae with an ample supply of nutrients excrete higher amount of DOM to the medium.[28,29] Using optical indices (e.g., $SUVA_{254}$, HIX, and FI) to examine AOM characteristics, Huang et al.[24] observed that changes in nitrogen and phosphorus concentrations in the cultural medium altered *M. aeruginosa* AOM aromaticity, molecular weight, protein and chlorophyll concentrations. Moreover, AOM quality and quantity vary at different algal growth stages.[29–32] Henderson et al.[31] reported that the AOM released by *M. aeruginosa* at exponential phase contained greater amount of aromatic compounds than at stationary phase. These studies suggest that concentration and composition of ash solution can play crucial roles in affecting *M. aeruginosa* population and associated AOM quality and quantity during its life cycle (i.e., from lag to death phase), which would consequently lead to substantial changes in DBP precursor in a DOM pool.

Recently, we demonstrated that exponentially growing *M. aeruginosa* altered DBP precursors in a bulk DOM pool containing thermally-altered DOM.[33] However, it is unclear whether and to what extent overall DBP precursors are affected by ash-derived DOM concentrations during the initiation and end of algal blooms. Overall, the aim of this study was to assess the effects of wildfire ash solutions on *M. aeruginosa* population

and to evaluate dynamic changes of DOM properties and DBP precursors at lag, exponential, stationary, and death phases. The specific objectives were to: (1) compare *M. aeruginosa* population and growth rate in the absence and presence of black and white ash water extracts with low and high concentrations; (2) evaluate alterations of DOM spectroscopic characteristics; (3) assess DOM reactivities and DBP-FP at each growth phase; and (4) identify correlations among specific DBP-FP and DOM optical indices.

MATERIALS AND METHODS

Sampling Site and Ash Collection

Ash samples were collected on October 2nd from the 2013 Rim Fire, which started on August 17th and is recorded as the third largest wildfire in California history covering more than 100,000 ha in watersheds. The sampling site was located approximately 2 km south west of the Cherry Lake in Tuolumne River Watershed within the Stanislaus National Forest (Figure S1, Supporting Information, APPENDIX I), where the dominate vegetation type was ponderosa pine. Based on the visual color of ash, postfire ash samples including black ash and white ashes were collected using a 7.6 cm diameter × 5.0 cm depth metal coring device. Each type of ash samples consisted of three subsamples collected within a 10 m radius.

Preparation of Black and White Ash Extracts

Black and white ash samples were air-dried at room temperature (22 ± 1 °C) and passed through a 2 mm screen. To obtain black and white ash extracts, 50 g of each type

of ash was mixed with 200 ml Milli-Q water in a 250 ml Erlenmeyer flask. The water-ash mixtures were shaken for 72 hours using an orbital shaker at 250 rpm. Extracts were filtered using Millipore 0.45 µm filters rinsed three times with 20 mL of Milli-Q water. Black ash water extract (BE) and white ash water extract (WE) were used for further algal bioassay.

Algal Culture and Bioassay

The blue-green alga *Microcystis aeruginosa* UTEX 2385 (University of Texas at Austin, Austin, TX) was cultured non-axenically in the medium. The medium composition is provided in Table S1. Algal cultures were maintained at a temperature of 24 ± 2˚C and a 12:12-hour light-dark photoperiod illuminated by cool white fluorescent lighting at an intensity of 2100 lux.[33] The algal bioassay conditions were the same as for algal cultures, where algal population was monitored every day throughout the entire experiment by measuring optical density at 680 nm (OD_{680}) using UV-VIS spectrophotometer (Shimadzu UV-1800).

Concentrations of dissolved organic carbon (DOC) in lakes across the U.S. range from 2 to 10 mg/L.[34] Regarding wildfire impacts on stream water DOC concentration, Hohner et al.[35] have reported that postfire DOC concentration in the fire-impacted Upper Cache la Poudre River was 18.2 mg/L, significantly greater than annual average of 3.7 mg/L in the reference site. To simulate algal blooms in downstream receiving water in the absence and presence of low and high concentrations of wildfire ash solution, the initial OD_{680} values for algal bioassays including control and treatments were adjusted to

0.07 by adding 333 mL of algal stock solution into 1L volumetric flasks without (control) and with amendment of 10% and 65% of BE and WE (treatments). The flasks were then filled up to a final volume of 1 L by adding cultural medium and were mixed thoroughly. For example, 10% BE consisted of 100 mL of raw BE, 333 mL of algal stock solution, and 567 mL of cultural medium. Afterwards, 200 mL of mixtures were distributed separately to 250 mL acid washed Erlenmeyer flasks. Three replicate flasks were included for the control and treatments. Subsamples from the control and treatments were collected on day 0, 7, 28, and 35, which was operationally defined as lag, exponential, stationary, and death phase, respectively. Growth rate during cultivation day *t1* and day *t2* was calculated as follows:

$$\text{Growth rate (day}^{-1}) = \frac{\ln \text{OD}_{680,t2} - \ln \text{OD}_{680,t1}}{t2 - t1}$$

where $\text{OD}_{680,t2}$ and $\text{OD}_{680,t1}$ refer to OD_{680} values on the cultivation day t2 and day t1.

Chemical and Statistical Analyses

Subsamples collected in the algal bioassay were filtered using pre-washed Millipore 0.45 μm filters for chemical analyses, which contained extracellular AOM and ash-derived DOM. Methods of all analyses were published previously.[18] Detailed descriptions are presented in the Supporting Information (APPENSIX II). Spectroscopic characteristics of dissolved organic matter were analyzed, including specific UV absorbance at 254 nm (SUVA_{254}), humification index (HIX), E2/E3, and fluorescence index (FI). SUVA_{254} was calculated by normalizing UV absorbance at 254 nm to DOC

concentration. Fluorescence excitation-emission matrices (EEMs) from spectrofluorometry were analyzed by fluorescence regional integration (FRI).[18]

Carbonaceous, nitrogenous, and oxygenated disinfection byproducts (C-, N-, O-DBPs) were analyzed. C-DBPs included trihalomethanes (THMs) and haloacetic acids (HAAs); N-DBPs consisted of haloacetonitriles (HANs) and N-nitrosodimethylamine (NDMA); O-DBPs were chloral hydrate (CHD) and haloketones (HKs). DBPs were formed during DOM chlorination, except that NDMA was formed from the reaction of DOM and chloramine. The DOM reactivities for DBP formation were expressed as specific DBP formation potential (specific DBP-FP), which was calculated as the DBP concentration divided by the DOC concentration (mmol/mol-C).

Statistically significant differences between the control and treatments were determined using one-way ANOVA with Tukey's test. Significance was considered as $P < 0.05$. Correlations between specific DBP-FPs and optical indices were analyzed using principal component analysis (PCA).

RESULTS AND DISCUSSION

Water chemistry and *M. aeruginosa* growth following exposures to ash extracts

Characteristics of the raw black and white ash extracts (BE and WE) were reported in Table S2. Selected water quality parameters in the control (no ash extracts) and treatments (with 10% and 65% BE or WE) varied at the beginning of experiments, and they are provided in Table 1. For example, DOC concentration in the 65% BE (23.1 ± 0.4 mg/L) was significantly higher ($P < 0.05$) than the 65% WE (17.7 ± 0.4 mg/L) and

control (2.1 ± 0.2 mg/L). Ammonium (NH_4^+-N) concentration in the 65% BE (3.9 ± 0.2 mg/L) was significantly higher than the control (0.1 ± 0.04 mg/L) and other treatments. Approximately, 1 mg/L of DOC in the 10% BE and WE (3.8 – 2.1 and 3.3 – 2.1 mg/L) as well as 20 and 15 mg/L of DOC in the 65% BE (23.1 – 2.1 mg/L) and WE (17.7 – 2.1 mg/L) originated from raw ash extracts. Some metals that have been reported as constituents of wildfire ash, including As, Cd, Cr, and Pb[1,3] were not detectable in all treatments. These results imply that the property and amount of ash exported from wildfire-impacted areas into waters are key factors affecting downstream water quality.

OD_{680} decreased from 0.11 to 0.06 after 1-day exposure of 65% BE and then started to increase to 0.07 and 0.17 on day 3 and 4 (Figure 1A). On the contrary, it continuously increased from 0.15 to 0.23 in the 65% WE. The highest OD_{680} values during exponential (day 7) and stationary phase (day 28) were observed in the 65% WE (0.36 ± 0.01) and 65% BE (1.15 ± 0.04). After day 30 OD_{680} in the control and treatments started to decline. *M. aeruginosa* cultured in the 65% WE revealed the highest growth rate (0.22 ± 0.00 day^{-1}) and decline rate (0.23 ± 0.01 day^{-1}) during lag-exponential and stationary-death phase, respectively (Figure 1B). These results suggest that wildfire ash solution can cause different degrees of stimulation or ephemeral inhibition effects on *M. aeruginosa* population, depending on the exposure time as well as property and amount of ash solution.

Several compounds released from ash such as polycyclic aromatic hydrocarbons (PAHs) can potentially elicit adverse effects on algal growth.[36,37] Moreover, although ammonium and ammonia appear to be an ideal nitrogen source for algal growth, algal

growth can be inhibited following exposures to excessive amounts of ammonium and ammonia.[38] The temporary inhibition of *M. aeruginosa* growth following 2-day exposure to 65% BE was likely due to short-term transient physiological responses to relatively high concentration of ammonium (3.9 mg/L);[38] and the observed recovery of growth on day 4 and highest OD_{680} at stationary phase may be attributed to the growth stimulation from relatively high phosphate concentration (6.4 mg/L) masking negative impacts caused by the toxicants.[39] The highest specific growth rate observed in the 65% WE at lag-exponential phase could be ascribed to high nutrient (14.48 mg/L as NO_x-N) along with high electrical conductivity (623 µS/cm).[40,41] These findings may be applied to explain why proliferation of algae in streams or ponds is sometimes but not always observed after wildfires.[7,42]

DOC concentrations in the control and treatments increased over time throughout the experiment (Figure 1C). There was no significant difference in the DOC concentrations for 10% BE and WE (3.8 ± 0.1 and 3.3 ± 0.1 mg/L) at lag phase, but DOC concentrations in the 10% BE were significantly greater than that in the 10% WE at exponential and stationary phases. During lag-exponential phase, significant increases in DOC concentrations in 10% ash solutions were observed, but it was not observed in 65% ash solutions until exponential-stationary phase. At death phase, there was no significant difference in DOC concentrations in the control, 10% BE and WE. In contrast, DOC concentrations in the 65% ash solutions remained higher than the control and 10% ash solutions regardless of growth phase. These results suggest that DOC concentration generally increases over time during algal growth irrespective of presence of wildfire ash

derived solution; and effect of wildfire ash solution on the increment of DOC is dependent on the algal growth phase as well as characteristics and amount of ash solution.

Dynamic changes of DOM optical characteristics

DOM optical characteristics in the control and treatments varied during *M. aeruginosa* growth (Figure 2). $SUVA_{254}$, an indicator of DOM aromatic carbon content, in the control increased from 0.8 ± 0.0 to 1.3 ± 0.1 L/mg/m during lag-stationary phase and slightly decreased to 1.0 L/mg/m at death phase (Figure 2A). During lag-exponential phase, $SUVA_{254}$ significantly decreased in the 10% BE (from 3.6 ± 0.0 to 1.8 ± 0.1 L/mg/m) and WE but slightly increased in the 65% BE and WE. During exponential-death phase, $SUVA_{254}$ remained nearly constant in the 10% BE and WE but gradually decreased in the 65% BE and WE.

Humification index (HIX), an indicator of DOM humification extent or humic substance content, in the control significantly decreased from 9.7 ± 0.0 to 1.0 ± 0.1 during lag-stationary phase and then increased to 1.5 ± 0.1 at death phase (Figure 2B). Similar trends were also observed in all treatments. Regardless of characteristics and amount of ash extract, $SUVA_{254}$ and HIX in the control were lower than treatments, suggesting that presence of ash solution during an ongoing *M. aeruginosa* bloom results in higher DOM aromatic carbon content and humification extent. Our findings were in agreement with the study by Leloup et al.[43] showing that $SUVA_{254}$ of DOM released from *M. aeruginosa* was less than that from surface waters regardless of its growth phase.

The patterns showing that $SUVA_{254}$ and HIX decreased over time in the treatments suggest that exposure of *M. aeruginosa* to ash solution can transform humic substances to less aromatic compounds with aliphatic and carbohydrate-like structures,[43,44] and the degree of transformation increases along with growth phase.

E2/E3 ratio, an index inversely correlated with DOM molecular weight (MW),[45] in the control decreased from 5.7 to 3.4 during lag-exponential phase and then increased to 3.7 ± 0.1 at death phase (Figure 2C). E2/E3 ratio in the treatments, except for the 65% WE, also showed the same pattern as the control, indicating that MW of DOM increased during lag-exponential phase and started to decrease until death phase. MW of DOM in the 65% WE was higher than the control and other treatment at lag phase. Noticeably, during lag-exponential phase *M. aeruginosa* cultured in the 65% WE also showed the highest growth rate compared to control and treatments (Figure 1B). These results are in accordance with previous studies[46,47] showing that growth rate of algae was positively correlated with the MW of DOM in the cultural medium. Declines in MW of DOM from exponential to death phase in this study were also observed in Tai Lake, China, as a result of DOM transformation during summer and fall season.[48]

An increase in FI during algal growth was observed in all treatments. For example, FI in the 65% WE was 1.8 and 2.0 at lag and death phase, respectively. Since FI values provide information on the relative contribution of microbial and terrestrial sources to the DOM pool (higher values represent more microbial origins), these results suggest that DOM released from *M. aeruginosa* increased throughout the experiment. DOC in the control significantly increased from 4.7 ± 0.3 to 11.1 ± 0.3 mg/L during exponential-

stationary phase (Figure 1C). However, FI decreased during that period (from 2.5 ± 0.0 to 2.1 ± 0.0). Nevertheless, an increase in FI may be indicative of released cyanobacterial DOM, changes of FI may not proportionally represent the changes of DOM mass due to competing effects of peak emission wavelength and spectrum curvature.[49,50]

When *M. aeruginosa* was cultured in the presence of 10% BE at exponential phase, proportions of tyrosine- and tryptophan-like compounds started to decrease, and proportions of fulvic acid- and humic acid-like compounds started to increase (Figure 2F). It was also observed in the 10% WE and 65% WE at stationary phase (Figures 2H, I). For example, in the 10% WE during stationary-death phase, tyrosine-like compounds significantly decreased from 9.3 ± 0.3 to 6.0 ± 0.1%, and fulvic acid-like compounds increased from 23.9 ± 0.1 to 30.0 ± 0.1%. Since a decline in protein-like compounds and increases in humic substances of DOM are indicative of microbial transformation of DOM,[51,52] these results suggest that onset of transformation of black ash-DOM by growing *M. aeruginosa* may occur earlier than that of white ash-DOM. These observations are supported by Tsai and Chow[33] who demonstrated that DOMs released from 50 and 250 °C burned litter extracts are more biodegradable than that from 400 °C-extracts. Increases in burning temperature can decrease the terrestrial DOM carbohydrate composition and aliphatic carboxylate species resulting in more recalcitrant and hydrophobic compounds in DOM.[19] In addition, Liu et al.[53] found hydrophobic dissolved organic nitrogen (DON) had little or no effect on algal growth. Thus, it is likely that acclimation time for *M. aeruginosa* to uptake less labile or hydrophilic DOM released from white ash was longer compared to the DOM released from black ash, which was

produced at lower burning temperature. Dynamic change of tyrosine-like compound in the 65% BE was less pronounced than 10% BE, which was possibly due to higher concentrations of PAHs or low molecular weight organic acids and phenols[23,54] reducing biotransformation activities.

Dynamic changes of DOM reactivity and DBP concentration

Different patterns in dynamic changes of carbonaceous, nitrogenous, and oxygenated specific disinfection byproduct formation potential (C-, N-, O-SDBP-FP) were observed when *M. aeruginosa* was cultured in the absence and presence of ash solutions (Figure 3). Dynamic changes of individual SDBP-FP for each DBP group are depicted in Figure S2. Concentration of each DBP species will be discussed later. In control, the specific C-DBP-FP revealed little changes during lag-stationary phase (range from 2.4 to 2.8 mmol/mol-C) and significantly increased ($P < 0.05$) from 2.5 ± 0.2 to 3.1 ± 0.1 mmol/mol-C during stationary-death phase (Figure 3A). Specific N-DBP-FP significantly increased from 0.3 to 0.6 mmol/mol-C during lag-exponential phase and then continuously decreased until death phase; similar trend was also observed for specific O-DBP-FP. In the previous study by Huang et al,[14] variations in specific THM- and HAA-FPs during *M. aeruginosa* growth from lag to death phase were also not obvious. In contrast to the control, specific C-DBP-FP significantly decreased during lag-exponential phase in the 10% BE (from 6.8 ± 0.0 to 3.0 ± 0.2 mmol/mol-C) and increased to 4.2 ± 0.2 mmol/mol-C at death phase, and it also exhibited similar trends for O-DBP-FP (Figure 3B). Interestingly, dynamic changes of specific C- and O-DBP-FPs in the 65% BE and

WE (Figures 3C, E) exhibited opposite trends to the 10% BE and WE (Figures 3B, D). For example, specific C-DBP-FP slightly increased from 6.0 ± 0.0 to 7.0 ± 1.1 mmol/mol-C during lag-exponential phase and decreased to 5.1 ± 0.8 mmol/mol-C at death phase in the 65% BE (Figure 3C). In addition, similar trends of dynamic changes in all DBP precursors were observed in 10% BE and WE, as well as in 65% BE and WE. Specific N-DBP-FP continuously decreased in all treatments, regardless of concentration or composition of ash solution. These results imply that concentration of ash solutions has greater influence on the dynamic changes in C- and O-DBP precursors compared to composition of ash solution which can be influenced by burning temperature.

Exposures of algae to different concentrations of chemicals can alter cells' internal constituents and physiological functions (e.g., membrane integrity and barrier function of plasma membrane) to different degrees.[55,56] For example, *M. aeruginosa* cell membrane permeability was dependent on cupric ion concentration; exposure to higher copper concentration could lyse cell membrane causing release of higher amount of intracellular microcystins.[57] Healthy algal cells release small amounts of their intracellular materials as a normal growth process; while cells that are compromised, during nutrient depletion or death phase may lose cellular soluble contents into surrounding environments, and cells can also be degraded or solubilized.[29] In the 10% BE and WE, decreases in specific C- and O-DBP-FP during lag-exponential phase might be due to increases in membrane permeability allowing release of small molecular weight non-DBP precursors into water solution. Bacteria-produced polysaccharides have been reported as C-DBP precursors;[58,59] after exponential growth phase, increases in C- and O-DBP precursors

were likely attributed to elevated levels of polysaccharide such as β-1,3-glucan when nutrient concentrations started to decline.[60] In the 65% BE and WE solutions, increases in specific C- and O-DBP-FP during lag-exponential phase could be due to cell lysis following release of those C- and O-DBP precursors. Gough et al.[32] found that AOM became more hydrophilic and less aromatic during cell lysis, which may explain the decline of C- and O-DBP precursors during exponential-death phase.[61] Elevation of AOM was found related to increases in the propensity of DOM pool to form THMs and HAAs in San Luis Reservoir, California.[62] Also, Jack et al.[63] showed that algal production and senescence in outdoor mesocosms increased THM-FP. These studies are in agreement with our findings that ongoing *M. aeruginosa* blooms increase C-DBP precursors during different growth phases, regardless of concentration of ash solution.

DONs constitute the pool for N-DBP precursors; some of them in fire-affected DOM (e.g., urea and amino acids) might be uptaken by *M. aeruginosa*.[64–66] Moreover, depletion of nutrient[60] could cause reduction in cellular nitrogenous component; and presence of fire-affected DOM may enhance uptake of those DBP precursors by *M. aeruginosa*,[67] leading to continuous declines in the amount of N-DBP precursors in all treatments. Carbohydrate is the major compound in extracellular AOM,[29] and stagnation in algal growth would cause the accumulation of dissolved carbohydrates. Therefore, it is also possible that the decline in N-DBP precursors was due to increases in carbohydrate-associated non-N-DBP precursors. Pivokonsky et al.[68] showed that aging of algal culture was accompanied by an increase in protein-related AOM. Some N-DBP precursors are proteins released from algae.[69] In this study an increase in N-DBP precursors in the

control was only observed during lag-exponential phase but not in all treatments, suggesting that characteristics of protein-related AOM was likely altered following exposure to ash solution.

All DBP-FP in the control increased over time throughout the experiment, and it was significantly greater in death phase than lag phase (Figure 4). Patterns in dynamic changes of DBP-FP were associated with the concentration of ash solution in all treatments. For example, THM-FP in the 10% WE during lag-death phase approximately increased by 200% (from 193.4 ± 0.0 to 409.0 ± 23.1 μg/L), but it remained relatively unchanged in the 65% WE (from 1171.2 ± 0.0 to 1259.4 ± 68.9 μg/L) (Figure 4A). In contrast, HAN-FP in the 65% WE significantly decreased ($P < 0.05$) from 129.9 ± 0.0 to 83.5 ± 4.3 μg/L, but it remained nearly constant in the 10% WE (from 36.2 ± 0.0 to 31.8 ± 1.6 μg/L) (Figure 4C). DBP-FP in the 65% ash solutions, with the exception of NDMA-FP (Figure 4D), was significantly higher than the control at each growth phase; however, during some growth phases DBP-FP in the 10% ash solutions was lower than the control. For example, HAN-FP in the 10% WE at lag phase (36.2 ± 0.0 μg/L) was significantly higher than the control (8.0 ± 0.0 μg/L), but it showed lower or no difference with the control during exponential-death phase (Figure 4C). Similar situations were also observed for HAA-, CHD-, and HK-FPs (Figures 4B, E, and F).

DOM quality and quantity can significantly affect DBP species and concentration. All DBP concentrations in the control increased with an increase in DOC concentration throughout all growth phases despite of fluctuation in DOM reactivity (Figure 3A), suggesting that pre-fire AOM quantity has greater influence on DBP concentration than

its quality. Although postfire DOC amounts also increased over time in all treatments, influences of most DOM reactivity outweighed DOC quantity, leading to some DBP concentrations declined or no significantly changed during growth phases. In this regard, as algae grew with a low concentration of ash solution, overall DBP concentrations could be lower than or similar to the fire-affected DOM-free scenario. Figures 3 and 4 provide evidences that concentration of ash solution can not only affect postfire DBP concentration but can also alter amount of DBP precursor during an ongoing algal bloom.

Correlations among specific DBP-FP and DOM optical properties

Principal component analysis (PCA) was run on spectroscopic characteristics and specific DBP-FP for all samples (Figure 5). Principal component 1 (PC 1) represented aromatic carbon content, which explained 54.74% of the total variance; and principal component 2 (PC 2) reflected DOM origins, which explained 14.58% of the total variance (Table S3). PC 1 showed high positive loadings for $SUVA_{254}$, HIX, E2/E3, III, V, STHM-FP, and SHAA-FP; and PC 2 showed high positive loadings for SCHK-FP, SHAN-FP, and SCHD-FP. Based on the loadings of variance, THM and HAA precursors were related to aromatic carbon content, humic substances and DOM MW; and NDMA precursor was associated with microbial related compounds (IV). The PCA results suggest that DOM reactivity in forming THMs, HAAs, HANs, and CHD were higher following exposures of *M. aeruginosa* to high concentration of ash solutions during lag-stationary phase than death phase; while NDMA was likely formed after exposures to low concentration of ash solutions during exponential-death phase.

Implications for Water Resources Management

In an effort to predict impact of wildfire on DBP-FP in drinking water, effects of wildfires on terrestrial DOM quality and quantity have been extensively studied.[15,18,70,71] In fact, DOM pool in source water contains both allochthonous and autochthonous DOMs. Wildfires and harmful algal blooms (HABs) in source water will likely happen more frequently as a result of global warming and eutrophication.[72–74] In this study, we demonstrated dynamic changes of DBP precursors by incorporating different quality and quantity of DOMs released from ash and noxious algae *M. aeruginosa* during its growth. Dynamic changes in DOM reactivities and associated DBP concentrations would provide useful information for water resource managers, regarding the timing for treating waters impaired by ongoing HAB and wildfire. The conceptual model in Figure 3 shows that the pattern of specific DBP-FP during bloom process is more influenced by the concentration than composition of ash solution. Forest and water resource managers shall put efforts to reduce postfire ash load in forested watersheds or amount of ash-derived DOM in stream waters to minimize DBP precursors in source water. Also, authorities need to be particularly cautious about postfire precipitation or snowmelt that would carry significant amount of ash-derived DOM into source water during ongoing HABs.[16]

Furthermore, this study suggests that postfire DBP precursors can be dynamically changed during an entire algal life cycle, and the changes are different in C-, N-, and O-DBP species. NDMA-FP was found to increase throughout this experiment but remained below 20 ng/L. Previous studies have found that algal bloom-impacted waters were less prone to NDMA formation compared to other types of water.[75–77] However, those studies

did not incorporate algal growth phase. Importantly, in addition to nutrient levels in water (i.e., N and P), organic and metallic compounds can affect algal population as well as associated AOM quality and quantity.[78–80] Linkages between responses of algae to environmental contaminants and subsequent DBP precursors are essential knowledge for source water quality management and need to be further investigated.

REFERENCES

(1) Bodí, M. B.; Martin, D. A.; Balfour, V. N.; Santín, C.; Doerr, S. H.; Pereira, P.; Cerdà, A.; Mataix-Solera, J. Wildland fire ash: Production, composition and eco-hydro-geomorphic effects. *Earth-Science Rev.* **2014**, *130*, 103–127.

(2) Audry, S.; Akerman, A.; Riotte, J.; Oliva, P.; Maréchal, J. C.; Fraysse, F.; Pokrovsky, O. S.; Braun, J. J. Contribution of forest fire ash and plant litter decay on stream dissolved composition in a sub-humid tropical watershed (Mule Hole, Southern India). *Chem. Geol.* **2014**, *372*, 144–161.

(3) Smith, H. G.; Sheridan, G. J.; Lane, P. N. J.; Nyman, P.; Haydon, S. Wildfire effects on water quality in forest catchments: A review with implications for water supply. *J. Hydrol.* **2011**, *396* (1–2), 170–192.

(4) Cook, P. L. M.; Holland, D. P. Long term nutrient loads and chlorophyll dynamics in a large temperate Australian lagoon system affected by recurring blooms of cyanobacteria. *Biogeochemistry* **2012**, *107* (1–3), 261–274.

(5) Charette, T.; Prepas, E. E. Wildfire impacts on phytoplankton communities of three small lakes on the Boreal Plain, Alberta, Canada: a paleolimnological study. *Can. J. Fish. Aquat. Sci.* **2003**, *60* (5), 584–593.

(6) Beganyi, S. R.; Batzer, D. P. Wildfire induced changes in aquatic invertebrate communities and mercury bioaccumulation in the Okefenokee Swamp. *Hydrobiologia* **2011**, *669* (1), 237–247.

(7) Spencer, C. N.; Gabel, K. O.; Hauer, F. R. Wildfire effects on stream food webs and nutrient dynamics in Glacier National Park, USA. *For. Ecol. Manage.* **2003**, *178* (1–2), 141–153.

(8) Leenheer, J. J. a.; Croué, J. J.-P. Peer reviewed: characterizing aquatic dissolved organic matter. *Environ. Sci. Technol.* **2003**, *37* (1), 18A–26A.

(9) Hoehn, R. C.; Barnes, D. B.; Thompson, B. C.; Randall, C. W.; Grizzard, T. J.; Shaffer, P. T. B. Algae as sources of trihalomethane precursors. *J. Am. Water Work. Assoc.* **1980**, *72* (6), 344–350.

(10) Paerl, H. W.; Paul, V. J. Climate change: Links to global expansion of harmful cyanobacteria. *Water Res.* **2012**, *46* (5), 1349–1363.

(11) Burkholder, J. M.; Glibert, P. M.; Skelton, H. M. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* **2008**, *8* (1), 77–93.

(12) Paerl, H. W.; Scott, J. T.; McCarthy, M. J.; Newell, S. E.; Gardner, W. S.; Havens, K. E.; Hoffman, D. K.; Wilhelm, S. W.; Wurtsbaugh, W. A. It Takes Two to

Tango: When and Where Dual Nutrient (N & P) Reductions Are Needed to Protect Lakes and Downstream Ecosystems. *Environ. Sci. Technol.* **2016**, *50* (20), 10805–10813.

(13) Hua, G.; Reckhow, D. A.; Abusallout, I. Correlation between SUVA and DBP formation during chlorination and chloramination of NOM fractions from different sources. *Chemosphere* **2015**, *130*, 82–89.

(14) 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.

(15) Wang, J. J.; Dahlgren, R. A.; Erşan, M. S.; Karanfil, T.; Chow, A. T. Temporal variations of disinfection byproduct precursors in wildfire detritus. *Water Res.* **2016**, *99*, 66–73.

(16) Writer, J. H.; Hohner, A.; Oropeza, J.; Schmidt, A.; Cawley, K.; Rosario-Ortiz, F. L. Water treatment implications after the High Park Wildfire in Colorado. *J. Am. Water Works Assoc.* **2014**, *106* (4), 85–86.

(17) Pereira, P.; Úbeda, X.; Martin, D. A. Fire severity effects on ash chemical composition and water-extractable elements. *Geoderma* **2012**, *191*, 105–114.

(18) 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.

(19) Quill, E. S.; Angove, M. J.; Morton, D. W.; Johnson, B. B. Characterisation of dissolved organic matter in water extracts of thermally altered plant species found in boxironbark forests. *Aust. J. Soil Res.* **2010**, *48* (8), 693–704.

(20) Neary, D. G. .; Ryan, K. C. .; DeBano, L. F. Wildland Fire in Ecosystems, effects of fire on soil and water. *USDA-FS Gen. Tech. Rep.* **2005**, *4* (September), 250.

(21) Silva, V.; Pereira, J. L.; Campos, I.; Keizer, J. J.; Gonçalves, F.; Abrantes, N. Toxicity assessment of aqueous extracts of ash from forest fires. *Catena* **2015**, *135*, 401–408.

(22) Smith, C. R.; Buzan, E. M.; Lee, J. W. Potential impact of biochar water-extractable substances on environmental sustainability. *ACS Sustain. Chem. Eng.* **2013**, *1* (1), 118–126.

(23) Smith, C. R.; Hatcher, P. G.; Kumar, S.; Lee, J. W. Investigation into the Sources of Biochar Water-Soluble Organic Compounds and Their Potential Toxicity on Aquatic Microorganisms. *ACS Sustain. Chem. Eng.* **2016**, *4* (5), 2550–2558.

(24) 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.

(25) Obernosterer, I.; Herndl, G. J. Phytoplankton extracellular release and bacterial growth: Dependence on the inorganic N:P ratio. *Mar. Ecol. Prog. Ser.* **1995**, *116* (1–3), 247–258.

(26) Gupta, N.; Bhaskar, A. S. B.; Lakshmana Rao, P. V. Growth characteristics and toxin production in batch cultures of Anabaena flos-aquae: Effects of culture media and duration. *World J. Microbiol. Biotechnol.* **2002**, *18* (1), 29–35.

(27) Piorreck, M.; Baasch, K. H.; Pohl, P. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry* **1984**, *23* (2), 207–216.

(28) Myklestad, S.; Holm-hansen, O.; Vårum, K. M.; Volcani, B. E. Rate of release of extracellular amino acids and carbohydrates from the marine diatom Chaetoceros affinis. *J. Plankton Res.* **1989**, *11* (4), 763–773.

(29) Myklestad, S. M. Dissolved Organic Carbon from Phytoplankton. *Mar. Chem.* **2000**, *5*, 112–144.

(30) Leloup, M.; Nicolau, R.; Pallier, V.; Yéprémian, C.; Feuillade-Cathalifaud, G. Organic matter produced by algae and cyanobacteria: Quantitative and qualitative characterization. *J. Environ. Sci.* **2013**, *25* (6), 1089–1097.

(31) Henderson, R. K.; Baker, A.; Parsons, S. A.; Jefferson, B. Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. *Water Res.* **2008**, *42* (13), 3435–3445.

(32) Gough, R.; Holliman, P. J.; Cooke, G. M.; Freeman, C. Characterisation of algogenic organic matter during an algal bloom and its implications for trihalomethane formation. *Sustain. Water Qual. Ecol.* **2015**, *6*, 11–19.

(33) Tsai, K.-P.; Chow, A. T. Growing Algae Alter Spectroscopic Characteristics and Chlorine Reactivity of Dissolved Organic Matter from Thermally-Altered Forest Litters. *Environ. Sci. Technol.* **2016**, *50* (15), 7991–8000.

(34) Wetzel, R. G. *Limnology: Lake and River Ecosystems*; 2001; Vol. 37.

(35) Hohner, A. K.; Cawley, K.; Oropeza, J.; Summers, R. S.; Rosario-Ortiz, F. L. Drinking water treatment response following a Colorado wildfire. *Water Res.* **2016**, *105* (2016), 187–198.

(36) Ekelund, N. G. A.; Andreas Aronsson, K. Changes in chlorophyll a fluorescence in Euglena gracilis and Chlamydomonas reinhardtii after exposure to wood-ash. *Environ. Exp. Bot.* **2007**, *59* (1), 92–98.

(37) Campos, I.; Abrantes, N.; Vidal, T.; Bastos, A. C.; Gonçalves, F.; Keizer, J. J. Assessment of the toxicity of ash-loaded runoff from a recently burnt eucalypt plantation. *Eur. J. For. Res.* **2012**, *131* (6), 1889–1903.

(38) Collos, Y.; Harrison, P. J. Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Mar. Pollut. Bull.* **2014**, *80* (1–2), 8–23.

(39) Hund, K. Algal growth inhibition test - Feasibility and limitations for soil assessment. *Chemosphere* **1997**, *35* (5), 1069–1082.

(40) Moisander, P. H.; McClinton, E.; Paerl, H. W. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. *Microb. Ecol.* **2002**, *43* (4), 432–442.

(41) Blackburn, S. I.; McCausland, M. a.; Bolch, C. J. S.; Newman, S. J.; Jones, G. J. Effect of salinity on growth and toxin production in cultures of the bloom-forming cyanobacterium Nodularia spumigena from Australian waters. *Phycologia* **1996**, *35* (6), 511–522.

(42) Earl, S. R.; Blinn, D. W. Effects of wildfire ash on water chemistry and biota in south-western U.S.A. streams. *Freshw. Biol.* **2003**, *48* (6), 1015–1030.

(43) Leloup, M.; Pallier, V.; Nicolau, R.; Feuillade-Cathalifaud, G. Assessing Transformations of Algal Organic Matter in the Long-Term: Impacts of Humification-Like Processes. *Int. J. Mol. Sci.* **2015**, *16* (8), 18096–18110.

(44) Hertkorn, N.; Claus, H.; Schmitt-Kopplin, P.; Perdue, E. M.; Filip, Z. Utilization and transformation of aquatic humic substances by autochthonous microorganisms. *Environ. Sci. Technol.* **2002**, *36* (20), 4334–4345.

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

(46) Fagerberg, T.; Jephson, T.; Carlsson, P. Molecular size of riverine dissolved organic matter influences coastal phytoplankton communities. *Mar. Ecol. Prog. Ser.* **2010**, *409* (1996), 17–25.

(47) Tulonen, T.; Salonen, K.; Arvola, L. Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. *Hydrobiologia* **1992**, *229* (1), 239–252.

(48) Zhang, F.; Harir, M.; Moritz, F.; Zhang, J.; Witting, M.; Wu, Y.; Schmitt-Kopplin, P.; Fekete, A.; Gaspar, A.; Hertkorn, N. Molecular and structural characterization of dissolved organic matter during and post cyanobacterial bloom in Taihu by combination of NMR spectroscopy and FTICR mass spectrometry. *Water Res.* **2014**, *57*, 280–294.

(49) Korak, J. A.; Wert, E. C.; Rosario-Ortiz, F. L. Evaluating fluorescence spectroscopy as a tool to characterize cyanobacteria intracellular organic matter upon simulated release and oxidation in natural water. *Water Res.* **2015**, *68*, 432–443.

(50) Korak, J. A.; Dotson, A. D.; Summers, R. S.; Rosario-Ortiz, F. L. Critical analysis of commonly used fluorescence metrics to characterize dissolved organic matter. *Water Res.* **2014**, *49*, 327–338.

(51) Fellman, J. B.; Hood, E.; Edwards, R. T.; D'Amore, D. V. Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. *J. Geophys. Res. Biogeosciences* **2009**, *114* (1), 1–14.

(52) Hur, J.; Park, M.-H.; Schlautman, M. A. Microbial transformation of dissolved leaf litter organic matter and its effects on selected organic matter operational descriptors. *Environ. Sci. Technol.* **2009**, *43* (7), 2315–2321.

(53) Liu, H.; Jeong, J.; Gray, H.; Smith, S.; Sedlak, D. L. Algal uptake of hydrophobic and hydrophilic dissolved organic nitrogen in effluent from biological nutrient removal municipal wastewater treatment systems. *Environ. Sci. Technol.* **2012**, *46* (2), 713–721.

(54) Buss, W.; Mašek, O.; Graham, M.; Wüst, D. Inherent organic compounds in biochar–Their content, composition and potential toxic effects. *J. Environ. Manage.* **2015**, *156*, 150–157.

(55) Beauvais-Flück, R.; Slaveykova, V. I.; Cosio, C. Transcriptomic and Physiological Responses of the Green Microalga Chlamydomonas reinhardtii during Short-Term Exposure to Subnanomolar Methylmercury Concentrations. *Environ. Sci. Technol.* **2016**, *50* (13), 7126–7134.

(56) Rioboo, C.; González, O.; Herrero, C.; Cid, A. Physiological response of freshwater microalga (Chlorella vulgaris) to triazine and phenylurea herbicides. *Aquat. Toxicol.* **2002**, *59* (3–4), 225–235.

(57) Tsai, K.-P. Effects of two copper compounds on Microcystis aeruginosa cell density, membrane integrity, and microcystin release. *Ecotoxicol. Environ. Saf.* **2015**, *120*, 428–435.

(58) Lee, W.; Westerhoff, P.; Croué, J.-P. Dissolved Organic Nitrogen as a Precursor for Chloroform, Dichloroacetonitrile, N -Nitrosodimethylamine, and Trichloronitromethane. *Environ. Sci. Technol.* **2007**, *41* (15), 5485–5490.

(59) Wang, Z.; Kim, J.; Seo, Y. Influence of bacterial extracellular polymeric substances on the formation of carbonaceous and nitrogenous disinfection byproducts. *Environ. Sci. Technol.* **2012**, *46* (20), 11361–11369.

(60) Granum, E.; Myklestad, S. M. Cellular and extracellular production of carbohydrates and amino acids by the marine diatom. *Mar. Ecol. Prog. Ser.* **2002**, *242* (Werner 1977), 83–94.

(61) Chang, E. E.; Chiang, P. C.; Ko, Y. W.; Lan, W. H. Characteristics of organic precursors and their relationship with disinfection by-products. *Chemosphere* **2001**, *44* (5), 1231–1236.

(62) Kraus, T. E. C.; Bergamaschi, B. a.; Hernes, P. J.; Doctor, D.; Kendall, C.; Downing, B. D.; Losee, R. F. How reservoirs alter drinking water quality: Organic matter sources, sinks, and transformations. *Lake Reserv. Manag.* **2011**, *27* (3), 205–219.

(63) Jack, J.; Sellers, T.; Bukaveckas, P. A. Algal production and trihalomethane formation potential: an experimental assessment and inter-river comparison. *Can. J. Fish. Aquat. Sci.* **2002**, *59*, 1482–1491.

(64) Bronk, D. A.; See, J. H.; Bradley, P.; Killberg, L. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences Discuss.* **2006**, *3* (4), 1247–1277.

(65) Berman, T.; Chava, S. Algal growth on organic compounds as nitrogen sources. *J. Plankton Res.* **1999**, *21* (8), 1423–1437.

(66) Lian, L.; Yue, E.; Li, J.; Blatchley, E. R. Volatile disinfection byproducts resulting from chlorination of uric acid: Implications for swimming pools. *Environ. Sci. Technol.* **2014**, *48* (6), 3210–3217.

(67) Khan, M.; Yoshida, N. Effect of l-glutamic acid on the growth and ammonium removal from ammonium solution and natural wastewater by Chlorella vulgaris NTM06. *Bioresour. Technol.* **2008**, *99* (3), 575–582.

(68) Pivokonsky, M.; Kloucek, O.; Pivokonska, L. Evaluation of the production, composition and aluminum and iron complexation of algogenic organic matter. *Water Res.* **2006**, *40* (16), 3045–3052.

(69) Bond, T.; Huang, J.; Templeton, M. R.; Graham, N. Occurrence and control of nitrogenous disinfection by-products in drinking water - A review. *Water Res.* **2011**, *45* (15), 4341–4354.

(70) Cawley, K. M.; Hohner, A. K.; Podgorski, D. C.; Cooper, W. T.; Korak, J. A.; Rosario-Ortiz, F. L. Molecular and Spectroscopic Characterization of Water Extractable Organic Matter from Thermally Altered Soils Reveal Insight into Disinfection Byproduct Precursors. *Environ. Sci. Technol.* **2016**, acs.est.6b05126.

(71) Wang, J. J.; Dahlgren, R. A.; Chow, A. T. Controlled Burning of Forest Detritus Altering Spectroscopic Characteristics and Chlorine Reactivity of Dissolved Organic Matter: Effects of Temperature and Oxygen Availability. *Environ. Sci.*

Technol. **2015**, *49* (24), 14019–14027.

(72) Odhiambo, M.; Routh, J. Does Black Carbon Contribute to Eutrophication in Large Lakes? *Curr. Pollut. Reports* **2016**, *2* (4), 213–223.

(73) Paerl, H. W.; Huisman, J. Blooms like it hot. *Science* **2008**, *320* (5872), 57–58.

(74) Westerling, A. L.; Hidalgo, H. G.; Cayan, D. R.; Swetnam, T. W. Warming and Earlier Spring Increase Western U.S. Forest Wildfire Activity. *Science (80-.).* **2006**, *313* (5789), 940–943.

(75) Dai, N.; Mitch, W. A. Relative iImportance of N-Nitrosodimethylamine Compared to Total N-Nitrosamines in Drinking Waters. *Environ. Sci. Technol.* **2013**, *47* (8), 3648–3656.

(76) Zeng, T.; Glover, C. M.; Marti, E.; Woods, G.; Karanfil, T.; Mitch, W. A.; Dickenson, E. R. V. Relative Importance of Different Water Categories as Sources of N-Nitrosamine Precursors. *Environ. Sci. Technol.* **2016**, acs.est.6b04650.

(77) Bond, T.; Templeton, M. R.; Graham, N. Precursors of nitrogenous disinfection by-products in drinking water--A critical review and analysis. *J. Hazard. Mater.* **2012**, *235–236*, 1–16.

(78) Lin, J. H.; Kao, W. C.; Tsai, K. P.; Chen, C. Y. A novel algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants. *Water Res.* **2005**, *39*, 1869–1877.

(79) Tsai, K.-P.; Chen, C.-Y. An algal toxicity database of organic toxicants derived by a closed-system technique. *Environ. Toxicol. Chem.* **2007**, *26* (9), 1931–1939.

(80) Vogs, C.; Altenburger, R. Time-Dependent Effects in Algae for Chemicals with Different Adverse Outcome Pathways: A Novel Approach. *Environ. Sci. Technol.* **2016**, *50* (14), 7770–7780.

Table 1. Characteristics of the control and mixtures containing 10% and 65% (v/v) of black ash extract (BE) and white ash extract (WE) in culture medium. (average ± standard deviation, n =3). Both raw ash water extracts were prepared by mixing 50 g of individual black ash and white ash with 200 ml Milli-Q water and shaking for 72 hours.

Parameter	Control	10% BE	65% BE	10% WE	65% WE
pH	**9.5 ± 0.1**^{a}	9.4 ± 0.0^{a}	8.9 ± 0.1^{b}	9.4 ± 0.1^{a}	8.8 ± 0.0^{b}
OD_{680}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Conductivity (µS/cm)	342 ± 5^{a}	381 ± 2^{b}	569 ± 8^{c}	390 ± 5^{b}	**623 ± 1**^{d}
DOC (mg/L)	2.1 ± 0.2^{a}	3.8 ± 0.1^{b}	**23.1 ± 0.4**^{c}	3.3 ± 0.1^{b}	17.7 ± 0.4^{d}
TDN (mg/L)	15.6 ± 0.5^{a}	18.7 ± 0.4^{a}	**25.0 ± 0.4**^{b}	17.0 ± 0.1^{a}	18.4 ± 0.1^{a}
NH_4^+-N (mg/L)	0.1 ± 0.0^{a}	0.8 ± 0.1^{b}	**3.9 ± 0.2**^{c}	0.6 ± 0.1^{b}	0.9 ± 0.0^{d}
NO_x^--N (mg/L)	12.8 ± 0.6^{a}	14.3 ± 0.1^{b}	14.2 ± 0.3^{b}	13.7 ± 0.1^{a}	**14.5 ± 0.4**^{b}
DON (mg/L)	2.2 ± 0.6^{a}	3.6 ± 0.2^{a}	**7.0 ± 0.3**^{b}	2.7 ± 0.1^{a}	3.0 ± 0.3^{a}
PO_4^{3-} (mg/L)	3.4 ± 0.0^{a}	4.1 ± 0.0^{b}	**6.4 ± 0.0**^{c}	3.8 ± 0.0^{a}	4.3 ± 0.0^{b}
Zn (mg/L)	**0.02 ± 0.00**	< 0.01	< 0.01	< 0.01	< 0.01
Cu(mg/L)	**0.02 ± 0.00**	< 0.01	< 0.01	< 0.01	< 0.01
As (mg/L)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Cd (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cr (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pb (mg/L)	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03

Bold numbers indicate the highest number among groups.
Lowercase letters refer to the significantly different groups ($P < 0.05$).

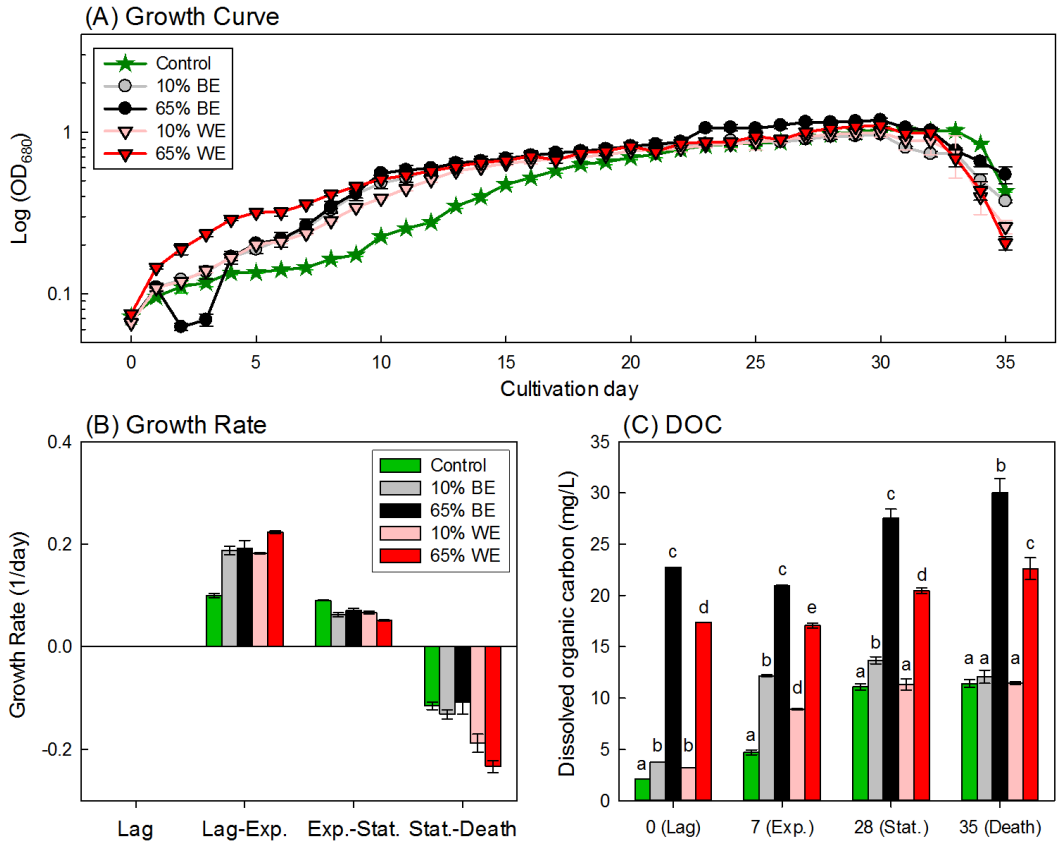


Figure 1. (A) Time-course changes of optical density at 680 nm for *M. aeruginosa* cultured in the absence (control), and presence of 10% and 65% of black and white ash extracts (10% and 65% BE and WE). (B) Growth rate of *M. aeruginosa* in the control and treatments at different growth phases. (C) Corresponding concentrations of dissolved organic carbon (DOC) in the solution on day 0 (lag phase), 7 [exponential phase (exp.)], 28 [stationary phase (stat.)], and 35 (death phase). Lowercase letters indicate statistically different groups ($P < 0.05$). Error bars represent the standard deviation (n = 3).

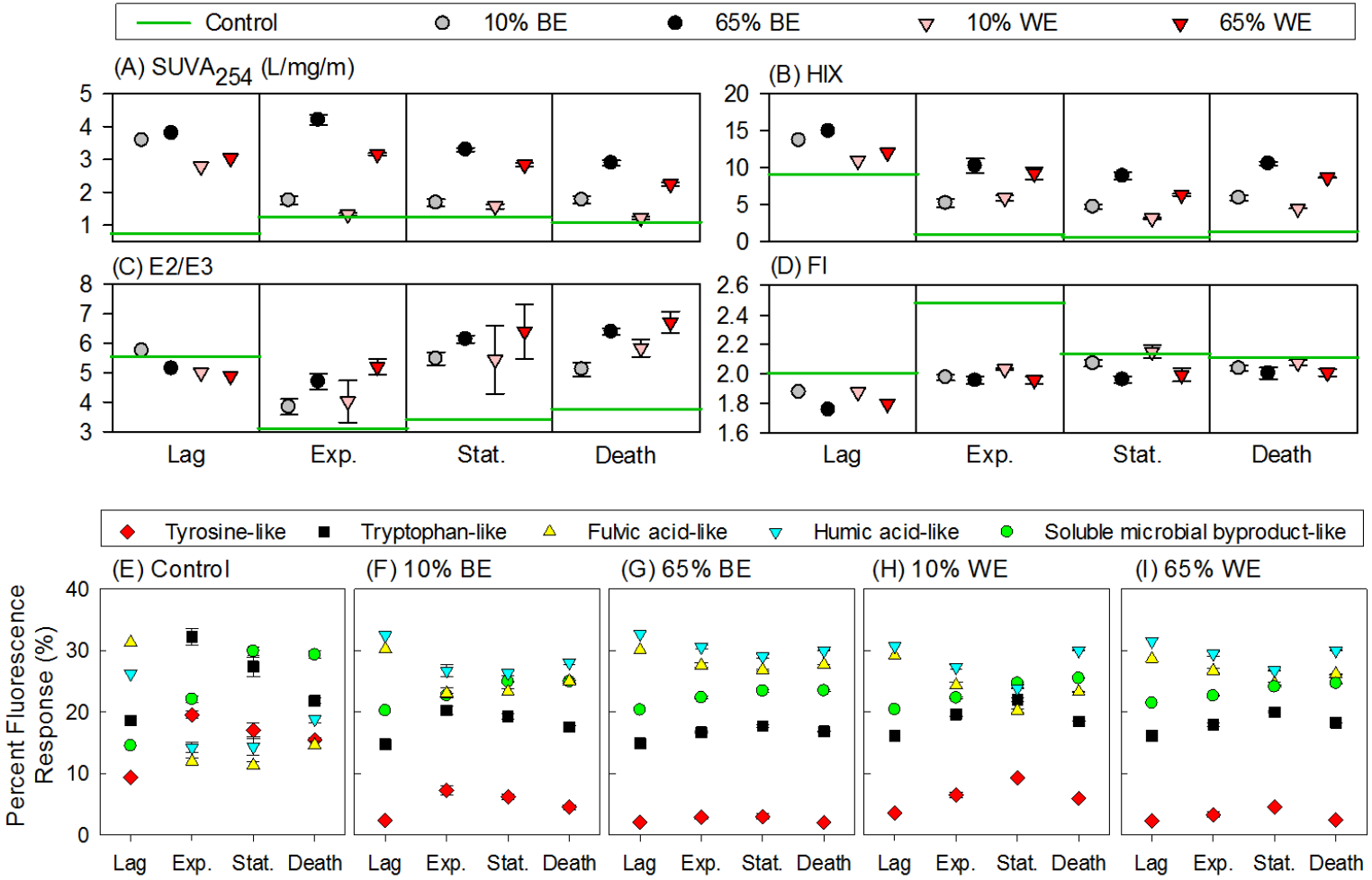


Figure 2. (A-D) Spectroscopic characteristics of DOM in the absence (control) and presence of black and white ash extracts (10% and 65% BE and WE) at different algal growth phases. $SUVA_{254}$, specific ultraviolet absorbance at 254 nm; HIX, humification index; E2/E3 ratio, UVA at 254 nm divided by UVA at 365 nm; FI, fluorescence index. (E-I) Percent DOM fluorescence responses of five EEM regions. Error bars represent the standard deviation (n = 3).

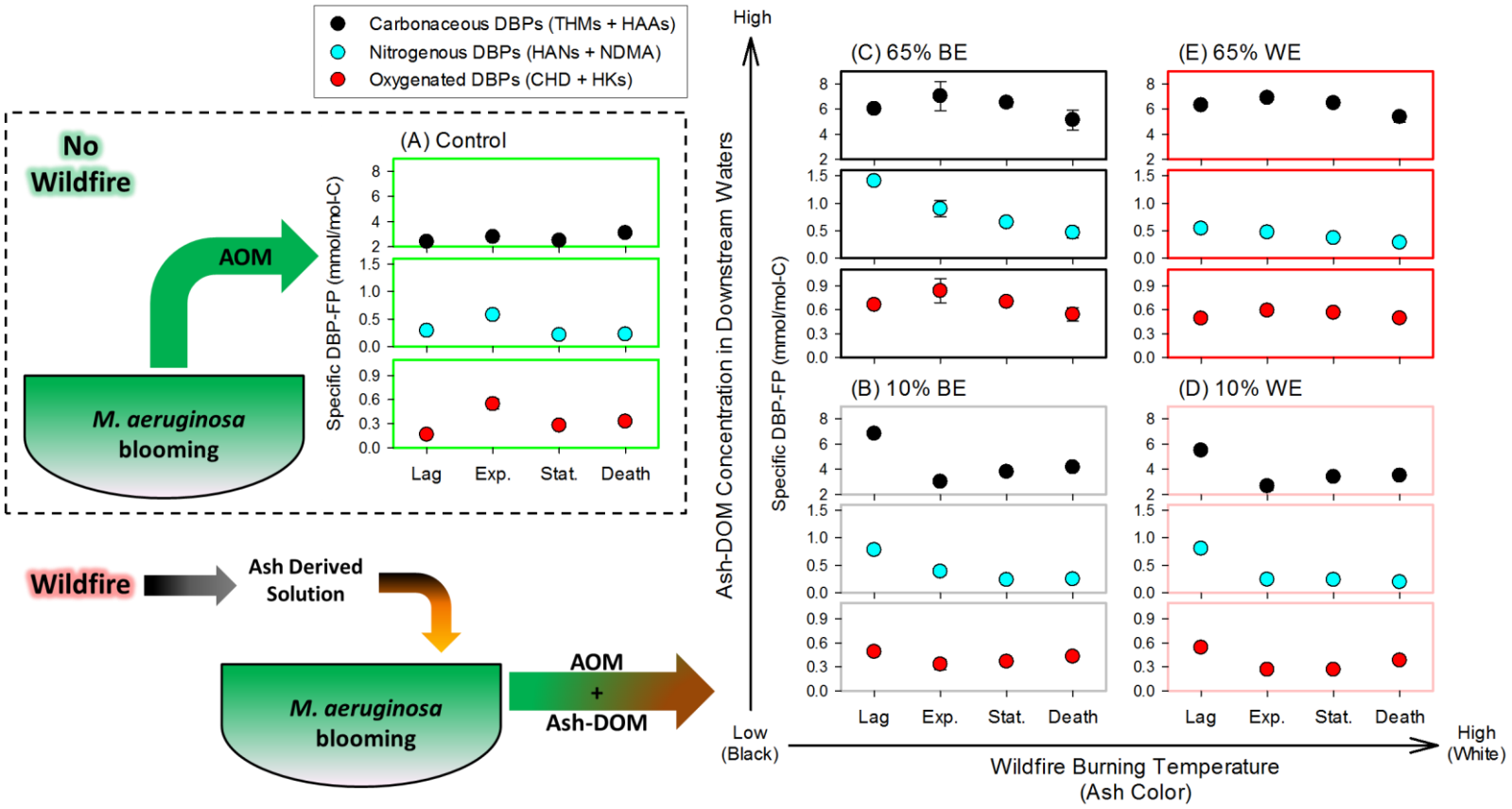


Figure 3. (A) Results of specific disinfection byproduct formation potential (SDBP-FP) in the absence (control) and (B to E) presence of black and white ash extracts (10% and 65% BE and WE) at different algal growth phases as a conceptual model of dynamic changes of DBP precursors during algal blooms without (only algal organic matter (AOM)) and with inflow of wildfire ash-derived solution (AOM + Ash-DOM). Error bars represent the standard deviation (n = 3).

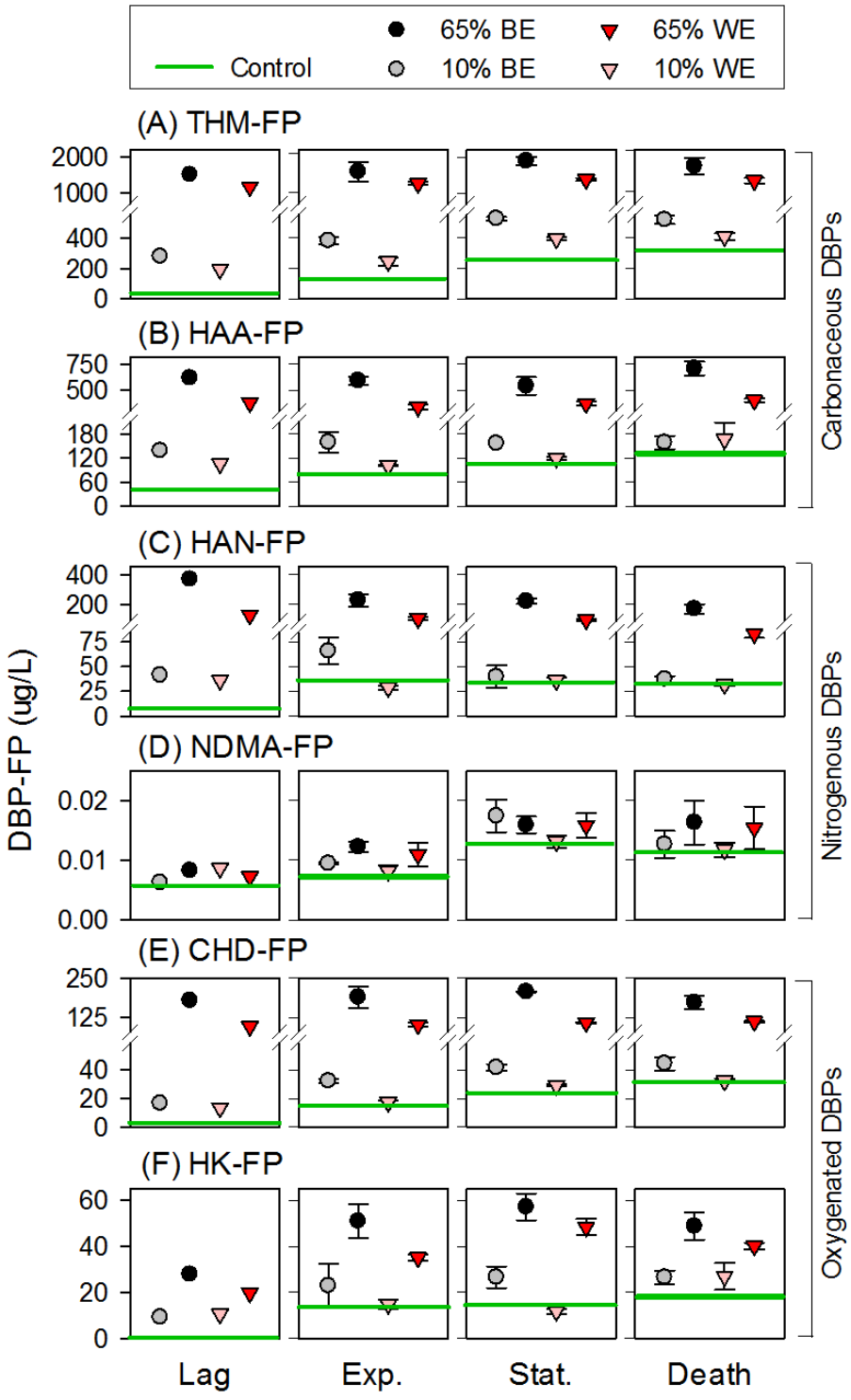


Figure 4. Disinfection byproduct formation potential (DBP-FP) in the absence (control) and presence of black and white ash extracts (10% and 65% BE and WE) at different algal growth phases. THM, trihalomethane; HAA, haloacetic acid;

HAN, haloacetonitrile; NDMA, N-nitrosodimethylamine; CHD, chloral hydrate; HK, haloketone. Error bars represent the standard deviation (n = 3).

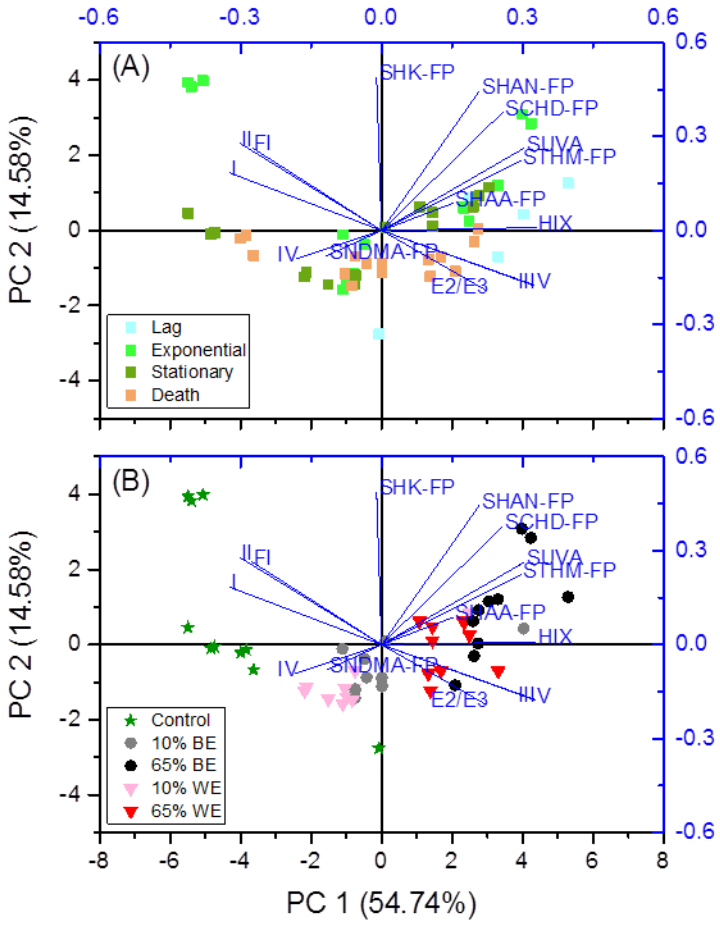


Figure 5. Principle component analysis of spectroscopic characteristics and chlorine/chloramine reactivity of DOM in the absence (control) and presence of black and white ash extracts (10% and 65% BE and WE) at different algal growth phases. Component scores of different algal growth phases and growth conditions are shown in the panel (A) and (B), respectively. Roman numerals I, II, III, IV, and V represent proportion of tyrosine-like, tryptophan-like, fulvic acid-like, soluble microbial byproduct-like, and humic acid-like components analyzed by fluorescence EEM, respectively.

CHAPTER FOUR

Control Wildfire-Induced Microcystis aeruginosa blooms by Copper Sulfate: Trade-Offs between Reducing Algal Organic Matter and Promoting Disinfection Byproduct Formation

ABSTRACT

Elevated levels of nutrient due to wildfire ash input into stream waters will likely cause algal blooms. When resource water is impeded by algae and require immediate restoration, copper algaecides are usually applied to control their growth. Previous studies suggest that Cu^{2+} can promote reactivity of dissolved organic matter (DOM) in forming disinfection byproducts (DBPs). However, it is unclear that how DBP formation is changed after treatment of fire-induced algal bloom by copper algaecide. *Microcystis aeruginosa* was cultured in the medium containing black/white ash extracts (BE and WE) to study DBP concentrations before and after 4-days exposures to 0.5 and 1.0 mg-Cu/L. Algal population was indicated by optical density at 680 nm (OD_{680}). DOM was characterized by absorption and fluorescence spectroscopy and chlorination/chloramination-based DBP formation experiments. In the end of experiment, OD_{680} and DOM in the treatments were lower than control. N-nitrosodimethylamine concentrations in both treatments were 4-6 times higher than that in control, but haloacetonitrile concentrations in the treatments and control revealed no significant difference. The results may serve to support risk evaluations of algal population and DBP concentration when wildfire-induced *M. aeruginosa* bloom is left untreated and when it is treated by copper algaecide.

GRAPHICAL ABSTRACT

INTRODUCTION

Elevated levels of nutrient (e.g., phosphorous, nitrogen, carbon) and dissolved organic matter (DOM) due to inputs of wildfire ash in stream waters will likely cause excessive growth of algae and create great challenges to downstream drinking water treatments.[1,2] DOMs released from wildfire ash and algae are precursors of potentially carcinogenic disinfection byproducts (DBPs) [e.g., trihalomethanes (THMs) and haloacetic acids (HAAs)] formed during water treatment disinfection processes such as chlorination and chloramination.[3,4] In addition, toxic algal blooms can exacerbate postfire drinking water quality. For example, blue-green alga *Microcystis aeruginosa* commonly occurs in eutrophic reservoirs and often produces microcystins, which are potent hepatotoxins and tumor promoters, causing serious problems to public health.[5] In order to control abundance of noxious algae in drinking water resources, copper-based algaecides are extensively applied.[6,7] The amount of algae-produced organic matter (AOM) usually increases with increasing algal population. Following exposure of algae to copper algaecides, concentration of AOM and associated DBP precursors may not increase owing to inhibition of algal growth.[8]

Application of copper sulfate ($CuSO_4$) for controlling noxious algae has been practiced for decades.[9] Noticeably, previous studies have showed that soluble copper may enhance DOM chlorine/chloramine reactivity in DBP formation potential (DBP-FP) by complexing with DOM and catalyzing haloform formation.[10–13] Thus, persistence of copper in the water column following application of copper sulfate may lead to increase in DBP-FP during water disinfection. In order to ensure drinking water safety after wildfires, minimizing AOM level and associated DBP precursors is particularly

important in situations where algal bloom is ongoing. However, it is unclear whether controlling wildfire-induced algal blooms by copper sulfate would face the trade-offs between reduction of AOM associated DBP precursors versus promotion of DBP formation. Therefore, it is needed to investigate alterations of DOM and DBP concentration following exposures of algae to copper sulfate during postfire algal blooms.

DBP formation potential is correlated with DOM quality and quantity.[14,15] Importantly, during wildfire-induced algal blooms, bulk DOM chemistry in a reservoir can be influenced by algal population and characteristics of DOM released from ash and algae. Black and white ashes are commonly observed in burned forested watersheds, and black ash is usually produced at relatively lower burning temperature and contains higher amount of organic compounds.[16,17] In addition, compared to DOM released from white ash, black ash derived DOM usually contains less aromatic carbon and higher amounts of aliphatic carbohydrate and polysaccharide compounds.[4,18] After wildfire events, exposures of algae to black and white ash solutions may elicit different responses in terms of algal populations as well as quantity and quality of AOM. Both autochthonous and allochthonous DOMs contain several metal-binding functional groups (e.g., carboxyl, carbonyl, phenolic, and alcohol groups) with various binding capacity.[19] Importantly, binding capacity of DOM and copper ions relates to DOM functional groups and copper concentration, which is critical for copper to act as a catalyst for DBP formation.[20,21] The amount of copper required for controlling problematic algae varies, and the maximum label recommended concentration of copper sulfate as an algaecide is 2 mg-Cu/L.[22] Also, a maximum contaminant level goal of 1.3 mg/L has been set for copper in drinking water supplies in the U.S.[23]

Regarding catalytic effect of copper concentration on DBP-FP, Zhang and Andrews[12] found that during DOM chloramination, as Cu^{2+} concentrations increased from 0 to 1 mg/L, N-nitrosodimethylamine (NDMA) concentrations proportionally increased from 31 to 104 ng/L. In addition, previous studies showed that effects of Cu^{2+} on DOM chlorine/chloramine reactivities in DBP formation were related to DOM properties. For example, Blatchley et al.[10] found that addition of 1 mg-Cu/L as $CuSO_4$ in the citric acid solution resulted in a significant increase in chloroform formation; however this was not observed when using 2,6-dihydroxybenzoic acid instead. Liu et al.[21] also found that during Cu-catalytic chlorination reactions, the THMs and HAAs formations from carboxylic acids was more enhanced than carbohydrates. During chloramination of a series of concentrations of Suwannee River DOM (SR-DOM) spiked with 1 mg-Cu/L as $CuSO_4$, Zhang and Andrews[12] found that NDMA-FP significantly decreased as SR-DOM concentration increased. On the contrary, Barnes et al.[24] reported that concentrations of chloroform formed during chlorination were positively correlated with concentrations of humic acid. Moreover, it was documented that influence of Cu^{2+} on promoting THMs formation was more remarkable than HAAs during the chlorination of humic acid.[21] It suggests that catalytic effect of copper on DBP-FP is also DBP species dependent. Exposures of algae to copper can significantly impact algal populations as well as AOM quantity and quality,[8] which would consequently affect Cu-AOM complexation. Hence, it is expected that controlling wildfire-induced noxious algal blooms by copper sulfate affect DBP formation during subsequent water disinfection process.

Overall, in this study we investigated changes of DOM quantity and quality as well as DBP formation following 4-days exposures of *M. aeruginosa* to different amounts of

copper sulfate. To simulate fire-induced noxious algal blooms in source water contaminated by different types of ash solution, *M. aeruginosa* was separately cultured in the medium containing black and white ash water extracts. The specific objectives were to: (1) measure DOM concentration and population of *M. aeruginosa* following 4-day exposures of 0, 0.5, and 1 mg-Cu/L as copper sulfate; (2) demonstrate changes of DOM spectroscopic characteristics in the absence and presence of copper sulfate; and (3) identify differences in DOM reactivity for DBP formation without and with exposures to copper sulfate.

MATERIALS AND METHODS

Ash Collection and Ash Water Extract Preparation

Black and white ash samples were collected on October 2nd from the 2013 Rim Fire in California. Detailed descriptions in sampling location and collection approach were published previously[4] and are presented in the Supporting Information (APPENDIX III). All ash samples were air-dried at room temperature (22 ± 1 °C) and passed through a 2 mm screen. To obtain black and white ash extracts, 50 g of each type of ash was mixed with 200 ml Milli-Q water in a 250 ml Erlenmeyer flask. The water-ash mixtures were shaken for 24 hours using an orbital shaker at 250 rpm. Extracts were filtered using Millipore 0.45 µm filters rinsed three times with 20 mL of Milli-Q water. Black ash water extract (BE) and white ash water extract (WE) were used for further algal bioassay.

Algal Culture and Bioassay

The blue-green alga *Microcystis aeruginosa* UTEX 2385 (University of Texas at Austin, Austin, TX) was cultured non-axenically in the medium. The medium composition is provided in Table S1. Algal cultures were maintained at a temperature of 24 ± 2˚C and a 12:12-hour light-dark photoperiod illuminated by cool white fluorescent lighting at an intensity of 2100 lux.[25] The algal bioassay conditions were the same as for algal cultures, where algal population was monitored at the beginning and end of experiment (day 0 and 4) by measuring optical density at 680 nm (OD_{680}) using UV-VIS spectrophotometer (Shimadzu UV-1800). Four-days exposure time was chosen because significant decrease in *M. aeruginosa* population after exposure to copper algaecide was often observed within 4 days in laboratory studies.[8,22]

To simulate postfire algal bloom in downstream receiving water, the initial OD_{680} values for algal bioassays were adjusted to 0.09 by adding algal stock solution into 1L volumetric flasks containing culture medium and BE or WE. According to the range of DOM concentrations across U.S. lakes as well as pre- and post-fire DOM concentrations published in previous studies,[26,27] dissolved organic carbon (DOC) concentrations derived from BE and WE in the algal bioassays were adjusted to 5 mg/L. A stock solution (1000 mg-Cu/L) of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, Sigma Chemical Co., St. Louis, MO) was prepared within one hour prior to experiment initiation using Milli-Q water. To study effects of controlling *M. aeruginosa* blooms by copper sulfate on the changes of DOM and DBP formation, algal bioassays were conducted using three replicates of treatment of 0.5 and 1.0 mg-Cu/L as nominal concentrations and an untreated control. Experimental chambers consisted of 250 mL of treated and untreated *M. aeruginosa* in the mixtures of culture medium and wildfire ash solutions (BE and WE) in 1000 mL acid

washed Erlenmeyer flasks. Subsamples from the control and treatments were collected at the beginning and end of experiment (day 0 and 4) for further chemical analysis.

Chemical and Statistical Analyses

Subsamples collected in the algal bioassay were filtered using pre-washed Millipore 0.45 μm filters for chemical analyses, which contained extracellular AOM and ash-derived DOM. Methods of all analyses including water chemistry and DBP-FP were published previously.[4,28] Detailed descriptions are presented in the Supporting Information (APPENDIX III). Spectroscopic characteristics of dissolved organic matter were analyzed, including specific UV absorbance at 254 nm ($SUVA_{254}$), humification index (HIX), E2/E3, and fluorescence index (FI). $SUVA_{254}$ was calculated by normalizing UV absorbance at 254 nm to DOC concentration. Fluorescence excitation-emission matrices (EEMs) from spectrofluorometry were analyzed by fluorescence regional integration (FRI).[4]

Six DBP species were analyzed, including trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), N-nitrosodimethylamine (NDMA), chloral hydrate (CHD), and haloketones (HKs). Most DBP formation potentials were examined under DOM chlorination condition, except that for HANs and NDMA where chloramination was used instead. Yu and Reckhow[29] showed that hypochlorite can react with HANs via nucleophilic attack on the nitrile carbon, and the products might be transformed or degraded to other DBPs or intermediate forms. It has been reported that chloramines could enhance HAN formation.[29,30] Therefore, we tested HAN formation potential under chloramination condition. The DOM reactivities for DBP formation were expressed as

specific DBP formation potential (specific DBP-FP), which was calculated as the DBP concentration divided by the DOC concentration (mmol/mol-C). Statistically significant differences between the control and treatments were determined using one-way ANOVA with Tukey's test. Significance was considered as $P < 0.05$.

RESULTS AND DISCUSSION

Changes of *M. aeruginosa* Population and DOM following Exposures to Copper Sulfate

Characteristics for *M. aeruginosa* growing in the presence of black and white ash extracts (BE and WE) as well as measured soluble copper concentration (Cu^{2+}) in the control and treatments are reported in Table 1. Throughout the experiment, OD_{680} value for *M. aeruginosa* growing in the presence of BE significantly increased from 0.09 ± 0.00 to 0.18 ± 0.03 ($p < 0.05$) in the control (Figure 1A1). On the contrary, after 4-days exposures of 0.5 and 1.0 mg-Cu/L, OD_{680} values (0.03 ± 0.00 and 0.03 ± 0.00) were significantly lower than the value at the beginning of experiment (Figure 1A1). Besides, there was no significant difference in OD_{680} values between the two treatments. Regarding changes of dissolved organic carbon (DOC) concentration, DOC significantly increased from 6.2 ± 0.3 to 8.9 ± 0.4 in the control (Figure 1A2); increases in DOC were also observed for both treatments (7.5 ± 0.6 and 6.9 ± 0.1 mg/L for the treatments of 0.5 and 1.0 mg-Cu/L), but the increments for the treatments (17% and 11%) were less than the control (30%). Similar patterns of the changes in OD_{680} values and DOC concentration were also observed for the treatment with WE (Figures 1B1 and 1B2). These results suggest that postfire *M. aeruginosa* population and DOM concentration in

resource water may increase over time if no algaecide is applied for controlling its growth. In contrast, *M. aeruginosa* population can be significantly decreased along with a minimal increase in DOM concentration as copper sulfate is applied. Previous studies also suggest that as *M. aeruginosa* bloom is initiated, total microcystin concentration will increase along with cell density; and applying copper algaecide can not only inhibit algal population but also decrease cellular and aqueous microcystin.[8,31]

DOM Spectroscopic Properties following Exposures to Copper Sulfate

$SUVA_{254}$, a proxy for DOM aromaticity, was 2.6 ± 0.1, 4.0 ± 0.3, and 5.2 ± 0.1 L/mg/m for *M. aeruginosa* growing in the BE following 4-days exposures to 0, 0.5, and 1.0 mg-Cu/L, respectively (Figure 2A1). $SUVA_{254}$ for *M. aeruginosa* growing in the presence of WE also proportionally increased with increasing copper exposure concentration in the end of experiment (Figure 2B1). DOM aromaticity has been reported to be highly related to copper binding affinity.[32,33] For example, Kikuchi et al.[32] found a positive correlation between $SUVA_{254}$ and copper-to-DOC ratio in the Sagami River Basin, Japan, which supported our findings that $SUVA_{254}$ increased with increases in Cu^{2+}/DOC (Table S2). Previous studies also indicate that copper is preferentially complexed by DOM acidic and aromatic moieties (e.g., carboxylic, phenolic, and aminopolycarboxylate ligands) with high $SUVA_{254}$.[33,34]

Humification index (HIX) describes the degree of aromatic structure polycondensation.[35] HIX was significantly higher in the control (8.2 ± 0.0) than treatments of 0.5 and 1.0 mg-Cu/L (6.0 ± 0.3 and 3.4 ± 0.1) for *M. aeruginosa* growing in the presence of BE (Figure 2A2). For the *M. aeruginosa* growing in the presence of WE,

changes of HIX revealed similar pattern to that with BE, but there was no significant difference ($p \geq 0.05$) in the control (4.6 ± 2.0) and treatment of 0.5 mg-Cu/L (3.9 ± 0.8) (Figure 2B2). E2/E3 ratio, an index inversely correlated with DOM molecular weight (MW),[36] was 4.3 ± 0.2 and 4.3 ± 0.0 in the treatment of 0.5 and 1.0 mg-Cu/L for the *M. aeruginosa* growing in the presence of BE, slightly higher than that in the control (4.09 ± 0.08) (Figure 2A3). Similar pattern for the changes in E2/E3 were also observed for *M. aeruginosa* growing in the presence of WE (Figure 2B3). Fluorescence index (FI) provides information on relative contribution of autochthonous and allochthonous DOM sources (i.e., higher values represent more autochthonous origins). Regarding changes of FI values in the control and treatments, there was no significant difference for the *M. aeruginosa* growing in the presence of BE (Figure 2A4); but FI values in both treatments (2.1 ± 0.0 and 2.2 ± 0.0) were significantly higher than the control (1.9 ± 0.0) for the *M. aeruginosa* growing in the presence of WE (Figure 2B4). Based on changes of DOM optical indices in the control and treatments, these results suggest that exposures of postfire *M. aeruginosa* bloom to copper sulfate may result in reducing humification extent and MW of bulk DOM, which is likely attributed to the presence of low MW compounds released from algal cells into surrounding water.[8,37,38] HIX values also represent characteristics of DOM source. HIX < 4 indicates that biological or aquatic bacterial origin is the dominant source; while HIX > 6 implies that terrestrial DOM can be the major source.[37] In this study, HIX values after exposures of *M. aeruginosa* to 1.0 mg-Cu/L were lower compared to the control and 0.5 mg-Cu/L treatment, suggesting that amount of intracellular compounds released from algal cells following exposures to copper sulfate was related to copper concentration.[8] Less DOM humification extent

observed in the treatment of 1.0 mg-Cu/L may also indicate presence of relative smaller amount of phenol groups but larger contribution of oxygen-containing functional groups (e.g., carboxylic and carbonylic groups) to the bulk DOM.[38]

Changes of DOM composition in the control and treatments after 4-days exposures of *M. aeruginosa* to copper sulfate were observed through 3D excitation emission matrices (Figures 3A1-A4 and 3B1-B4). The proportions of tyrosine- and tryptophan-like compounds (regions I and II) in the treatment of 1.0 mg-Cu/L were significantly higher ($p < 0.05$) than that in the control and treatment of 0.5 mg-Cu/L, regardless of type of ash extract in the culture medium (Figures 3A5 and 3B5). On the contrary, proportions of fulvic acid- and humic acid-like compounds (regions III and V) in the treatment of 1.0 mg-Cu/L were significantly lower than that in the control and treatment of 0.5 mg-Cu/L. For example, the proportion of tyrosine-like compound for the *M. aeruginosa* growing in the presence of BE was 10.3 ± 0.1, 5.0 ± 0.2, and 4.1 ± 0.1% in the treatments of 1.0 and 0.5 mg-Cu/L and control (Figure 3A5, region I), where the proportion of fulvic acid-like compound was 21.1 ± 0.2, 26.3 ± 0.3, and 27.3 ± 0.1%, respectively (Figure 3A5, region III). These results suggest that controlling wildfire-induced *M. aeruginosa* bloom by means of copper sulfate may lead to increases in proteinaceous substances, as well as decreases in humic substance with different degrees in bulk DOM, depending on the copper concentration applied.

Toxicity of copper sulfate to algae may result from production of reactive oxygen species through the redox cycling of copper ions in the cells, which would cause suppression of mitosis as well as loss of cell membrane integrity.[39–41] Generally, protein-like compounds are major constituent in algal cells, followed by carbohydrate, lipid, and

relatively low amount of humic substance.[42–44] During cell lysis, intracellular materials are released into surrounding water. A positive correlation between extracellular microcystin and soluble copper concentration has been found.[8,31] In addition, applying minimum amount of copper required for controlling *M. aeruginosa* bloom can not only reduce cell density but also may not compromise cell membranes, resulting in minimal release of microcystin.[8,31] These studies support our findings that controlling *M. aeruginosa* by applying low concentration of copper (0.5 mg-Cu/L) was as effective as high amount of copper (1.0 mg-Cu/L) (Figures 1A1 and 1B1), where treatment of 1.0 mg-Cu/L led to higher proportions of protein-like compounds in the solution compared to that in the treatment of 0.5 mg-Cu/L.

DOM Reactivity for DBP Formation following Exposures to Copper Sulfate

Specific disinfection byproduct formation potential (SDBP-FP) (grid and non-grid bars in Figure 4) and DBP formation (triangle and square symbols in Figure 4) for *M. aeruginosa* growing in the presence of BE or WE following 4-days exposures of copper sulfate varied with different extents in the untreated control and treatments. In terms of DOM reactivity for DBP formation, most SDBP-FPs in the treatments were higher than the control, except SHAN-FP for *M. aeruginosa* growing in the presence of BE. For example, for *M. aeruginosa* growing in the presence of BE, STHM-FP in the control and treatment of 0.5 and 1.0 mg-Cu/L was 4.4 ± 0.2, 7.3 ± 0.6 and 7.3 ± 0.5 mmol/mol-C (Figure 4A1); and SHAN-FP was 0.1 ± 0.0, 0.1 ± 0.0, and 0.2 ± 0.1 mmol/mol-C, respectively (Figure 4A3). It was noticed that SCHD-FP proportionally increased with increasing copper concentration, regardless of type of ash extract. For example, for *M.*

aeruginosa growing in the presence of BE, it was 0.4 ± 0.1, 0.8 ± 0.1, and 1.3 ± 0.1 mmol/mol-C for the control and treatments of 0.5 and 1.0 mg-Cu/L, respectively (Figure 4A5). In terms of DBP formation, CHD concentration also increased with increasing copper concentration (Figure 4A5). In addition to CHD concentration, formations of THMs, HAAs, NDMA, and HKs in the treatments were also higher than the control for *M. aeruginosa* growing in the presence of BE, and it revealed no significant difference for the treatment of 0.5 and 1.0 mg-Cu/L. For example, NDMA concentrations in the treatments with 0.5 and 1 mg-Cu/L were 326.1 ± 16.6 and 342.2 ± 15.1 ng/L, approximately 6 times higher than that in the control (50.8 ± 13.5 ng/L) (Figure 4A4). However, for *M. aeruginosa* growing in the presence of WE, there was no significant difference for the formations of THMs, HAAs, HANs, and CHD in the treatment of 0.5 mg-Cu/L and control. Concentrations of THMs, HAAs, and HKs in the treatment of 1.0 mg-Cu/L were lower than the control. These results suggest that controlling wildfire-induced *M. aeruginosa* bloom by copper sulfate may promote subsequent DOM chlorine/chloramine reactivities for DBP formation. The amount of copper applied and type of ash solution (black or white ash solutions) in fire-impacted source water are key factors affecting changes of DBP formation.

After exposures of *M. aeruginosa* to different amounts of copper, effect of copper on the alteration of DBP formation is likely related to the changes of AOM quantity and quality, which can alter Cu-AOM chelating compounds and associated chlorine/chloramine reactivity. Blue-green algae have been found to be able to excrete copper chelators as a defense mechanism to detoxify elevated cupric ion concentration,[45–47] and a positive relation in concentrations of copper and copper complexing ligands

released by blue-green algae has also been reported.[48] As copper concentration increased from 0.5 to 1.0 mg/L in this study, increases in tyrosine- and tryptophan-like compounds and decreases in fulvic acid-like compound were observed (Figures 3A5 and 3B5). Both protein-like compounds have been found to exhibit high copper binding capacity during algal blooms.[49–51] Therefore, compared with the untreated control, increased DBP formation in the treatments of 0.5 and 1.0 mg-Cu/L could be due to higher amounts of DBP precursors and copper complexing ligands (e.g., glutathione, N-acetylglucosamine, hydroxamic acids) released from *M. aeruginosa*.[45,46,52] During the development of algal bloom in Taihu Lake, China, Li et al.[49] found that nitrogenous functional groups in fulvic acid revealed strong binding reactivity with cupric ions. For the *M. aeruginosa* growing in the presence of WE, as copper concentration increased from 0.5 to 1.0 mg/L, decreases of THM and HAA concentrations were likely caused by decreases of nitrogenous compounds in fulvic acid.[53,54]

Importantly, the cupric ion binding capacity to DOM is limited; once the functional group or binding sites of DOM are fully occupied, catalytic effect of copper on DBP formation may not be significant.[20] In this study, while *M. aeruginosa* grew in the presence of BE, concentrations of DBP in the treatments of 1.0 mg-Cu/L were not higher than that in the 0.5 mg-Cu/L treatment (except CHD), which was likely attributed to saturated copper-DOM complexation by 0.5 mg-Cu/L. Furthermore, functional group species play a critical role for cupric ions acting as a catalyst for promoting DBP formation. Blatchley et al.[10] found that cupric ions promoted THM formation during chlorination of citric acid, possibly through complexing with carboxylic and hydroxyl groups, enhancing the oxidative decarboxylation of citric acid. Based on Fourier

transform infrared (FTIR) spectra, Fu et al.[11] confirmed that carboxylate and hydroxyl groups in humic acid were active sites for cupric ion complexation; Liu et al.[55] also found that catalytic effect of cupric ion on HAA formation was related to the generation of hydroxyl radical caused by the presence of cupric ion. Proposed reaction mechanisms for copper-catalyzed THM and HAA formations during chlorination and chloramination of humic acid were also illustrated in their studies.[11,55] In addition, effect of cupric ion on DBP formation can be influenced by the preference of complexation with DBP precursors. Zhang and Andrews[20] noticed that catalytic effect of cupric ion on HAAs formation was only observed for dichloroacetic acid (DCAA) but not for trichloroacetic acid (TCAA), suggesting that copper would preferentially complex with DCAA precursors and increase their reactivity with chlorine. In this study, no significant differences for HAN concentrations were found in the control and treatments (Figures 4A3 and 4B3), which may imply that cupric ions may prefer complexing with non-HAN precursors.

Interestingly, as *M. aeruginosa* grew with BE, we noticed that promoting effect of cupric ion on DBP formation was observed for THMs, HAAs, NDMA, CHD, and HKs (Figures 4A1-A6); however, as *M. aeruginosa* grew with WE, it was only observed for NDMA and CHD (Figures 4B1-B6). These results suggest that following exposure of fire-induced *M. aeruginosa* bloom to copper sulfate, type of ash solution (black or white ash extract) would affect overall DOM reactivity for DBP formation. As *M. aeruginosa* grows in the presence of black ash solution, the promoting effect of copper on DBP formation is more commonly observed. Using FTIR spectroscopy to examine chemical functionality of thermally-altered DOM, Quill et al.[18] found that DOM derived from plant

materials burnt at a lower temperature (e.g., 150 °C, black ash solution) contained more aliphatic carboxylate groups. In addition, Zhao et al.[13] found that catalytic effect of cupric ions on aliphatic DBP precursors was more significant than that on aromatic precursors. These studies imply that as *M. aeruginosa* grows in the presence of BE, bulk DOM may exhibit more carboxylic functional groups compared to growth in the presence of WE, leading to more pronounced promotion effect of copper sulfate on DBP formation.

Implications for Water Resources Management

As a result of global warming and eutrophication in source water, occurrence of wildfire events concomitant with harmful algal blooms (HABs) will likely become inevitable.[56–58] HABs often pose serious challenges to drinking water treatment and public health. Options for controlling HABs include mechanical, chemical, physical, and biological tactics. Among these approaches, application of copper-based algaecide is usually used to manage problematic algal population because of its effectiveness.[7,59] Public concerns regarding the potential risks created by applying copper algaecide, such as inducing excess toxin release into source water and impacts on aquatic ecosystem, have been extensively studied.[8,22,60] However, information about its influence on DBP formation during drinking water disinfection is not available. Understanding influences of copper algaecide application on DBP formation is important for making risk-based decisions regarding management of drinking water resource affected by wildfire. In this study, we comparatively investigate changes of DBP formation as well as algal population following exposures of toxin-producing *M. aeruginosa* to copper sulfate, when *M. aeruginosa* was cultured with black and white ash extracts to simulate growing

in ash-contaminated waters with scenarios of low and high burning temperature. We found that low copper concentration (0.5 mg-Cu/L) was as effective as high concentration (1.0 mg-Cu/L) in controlling *M. aeruginosa* while minimizing promotion of DBP formation. Although DBP concentrations in the control were generally lower than or similar to the treatments, OD_{680} value and DOM concentration were higher. The results may serve to support risk evaluations of algal population and DBP concentration when wildfire-induced *M. aeruginosa* bloom is left untreated and when it is treated by copper sulfate.

In addition to copper salt (e.g., copper sulfate), several chelated copper compounds (e.g., copper-ethanolamine, copper-citrate, copper-gluconate) also have been used as formulations for copper-based algaecides.[61,62] Following a copper sulfate treatment for controlling blue-green algae in channel catfish ponds, Liu et al.[63] found that over 99% of copper applied was transferred to the bottom sediment within approximately 2 days. Chelated copper algaecides have been reported to be able to provide a higher concentration of copper in the water column for a longer period of time and loss to the sediment is slower than for copper sulfate.[64] Formulation of copper algaecide used for controlling HABs can differ from site to site and also likely affect Cu-DOM concentration. Regarding impact of copper algaecide treatment on DBP formation, future studies are still needed to understand time-course changes of DBP concentration following chelated copper algaecide application.

REFERENCES

(1) Smith, H. G.; Sheridan, G. J.; Lane, P. N. J.; Nyman, P.; Haydon, S. Wildfire effects on water quality in forest catchments: A review with implications for water supply. *J. Hydrol.* **2011**, *396* (1–2), 170–192.

(2) Bladon, K. D.; Emelko, M. B.; Silins, U.; Stone, M. Wildfire and the future of water supply. *Environ.Sci.Technol.* **2014**, *48* (16), 8936–8943.

(3) Goslan, E. H.; Seigle, C.; Purcell, D.; Henderson, R.; Parsons, S. A.; Jefferson, B.; Judd, S. J. Carbonaceous and nitrogenous disinfection by-product formation from algal organic matter. *Chemosphere* **2017**, *170*, 1–9.

(4) 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.

(5) Otten, T. G.; Paerl, H. W. Health Effects of Toxic Cyanobacteria in U.S. Drinking and Recreational Waters: Our Current Understanding and Proposed Direction. *Curr. Environ. Heal. reports* **2015**, *2* (1), 75–84.

(6) Hoko, Z.; Makado, P. K. Optimization of algal removal process at Morton Jaffray water works, Harare, Zimbabwe. *Phys. Chem. Earth* **2011**, *36* (14–15), 1141–1150.

(7) McKnight, D. M.; Chisholm, S. W.; Harleman, D. R. F. CuSO4 treatment of nuisance algal blooms in drinking water reservoirs. *Environ. Manage.* **1983**, *7* (4), 311–320.

(8) Tsai, K.-P. Effects of two copper compounds on Microcystis aeruginosa cell density, membrane integrity, and microcystin release. *Ecotoxicol. Environ. Saf.* **2015**, *120*, 428–435.

(9) Haughey, M. A.; Anderson, M. A.; Whitney, R. D.; Taylor, W. D.; Losee, R. F. Forms and fate of Cu in a source drinking water reservoir following CuSO4 treatment. *Water Res.* **2000**, *34* (13), 3440–3452.

(10) Blatchley, E. R.; Margetas, D.; Duggirala, R. Copper catalysis in chloroform formation during water chlorination. *Water Res.* **2003**, *37* (18), 4385–4394.

(11) Fu, J.; Qu, J.; Liu, R.; Qiang, Z.; Liu, H.; Zhao, X. Cu(II)-catalyzed THM formation during water chlorination and monochloramination: A comparison study. *J. Hazard. Mater.* **2009**, *170* (1), 58–65.

(12) Zhang, H.; Andrews, S. A. Factors affecting catalysis of copper corrosion products in NDMA formation from DMA in simulated premise plumbing. *Chemosphere* **2013**, *93* (11), 2683–2689.

(13) Zhao, Y.; Yang, H.; Liu, S.; Tang, S.; Wang, X.; Xie, Y. F. Effects of metal ions on disinfection byproduct formation during chlorination of natural organic matter and surrogates. *Chemosphere* **2016**, *144*, 1074–1082.

(14) Hua, G.; Reckhow, D. A. Characterization of Disinfection Byproduct Precursors Based on Hydrophobicity and Molecular Size Characterization of Disinfection Byproduct Precursors Based on Hydrophobicity and Molecular Size. *Environ. Sci. Technol.* **2007**, 3309–3315.

(15) Hua, G.; Reckhow, D. A.; Abusallout, I. Correlation between SUVA and DBP formation during chlorination and chloramination of NOM fractions from different sources. *Chemosphere* **2015**, *130*, 82–89.

(16) Pereira, P.; Úbeda, X.; Martin, D. A. Fire severity effects on ash chemical composition and water-extractable elements. *Geoderma* **2012**, *191*, 105–114.

(17) Bodí, M. B.; Martin, D. A.; Balfour, V. N.; Santín, C.; Doerr, S. H.; Pereira, P.; Cerdà, A.; Mataix-Solera, J. Wildland fire ash: Production, composition and eco-hydro-geomorphic effects. *Earth-Science Rev.* **2014**, *130*, 103–127.

(18) Quill, E. S.; Angove, M. J.; Morton, D. W.; Johnson, B. B. Characterisation of dissolved organic matter in water extracts of thermally altered plant species found in boxironbark forests. *Aust. J. Soil Res.* **2010**, *48* (8), 693–704.

(19) Aiken, G. R.; Hsu-kim, H.; Ryan, J. N. Influence of Dissolved Organic Matter on the Environmental Fate of Metals, Nanoparticles, and Colloids. *Environ. Sci. Technol.* **2011**, 3196–3201.

(20) Zhang, H.; Andrews, S. A. Catalysis of copper corrosion products on chlorine decay and HAA formation in simulated distribution systems. *Water Res.* **2012**, *46* (8), 2665–2673.

(21) Liu, S. G.; Zhu, Z. L.; Tan, X. C.; Feng, X. H.; Huang, Z. Y.; Qiu, Y. L.; Zhao, J. F. The influence of Cu(II) on the formation and distribution of disinfection by-products during the chlorination of drinking water. *Water. Air. Soil Pollut.* **2013**, *224* (4).

(22) Tsai, K.-P. Management of Target Algae by Using Copper-Based Algaecides: Effects of Algal Cell Density and Sensitivity to Copper. *Water, Air, Soil Pollut.* **2016**, *227* (7), 238.

(23) Drinking Water Contaminants - Standards and Regulations https://www.epa.gov/dwstandardsregulations#List (accessed Mar 11, 2017).

(24) Barnes, D.; FitzGerald, P. A.; Swan, H. B. Catalysed formation of chlorinated organic materials in waters. *Water Sci. Technol.* **1989**, *21* (2), 59–63.

(25) Tsai, K.-P.; Chow, A. T. Growing Algae Alter Spectroscopic Characteristics and

Chlorine Reactivity of Dissolved Organic Matter from Thermally-Altered Forest Litters. *Environ. Sci. Technol.* **2016**, *50* (15), 7991–8000.

(26) Wetzel, R. G. *Limnology: Lake and River Ecosystems*; 2001; Vol. 37.

(27) Hohner, A. K.; Cawley, K.; Oropeza, J.; Summers, R. S.; Rosario-Ortiz, F. L. Drinking water treatment response following a Colorado wildfire. *Water Res.* **2016**, *105* (2016), 187–198.

(28) Murray-Gulde, C. L.; Bearr, J.; Rodgers, J. H. Evaluation of a constructed wetland treatment system specifically designed to decrease bioavailable copper in a wastestream. *Ecotoxicol. Environ. Saf.* **2005**, *61* (1), 60–73.

(29) Yu, Y.; Reckhow, D. A. Kinetic Analysis of Haloacetonitrile Stability in Drinking Waters. *Environ. Sci. Technol.* **2015**, *49* (18), 11028–11036.

(30) Bond, T.; Huang, J.; Templeton, M. R.; Graham, N. Occurrence and control of nitrogenous disinfection by-products in drinking water - A review. *Water Res.* **2011**, *45* (15), 4341–4354.

(31) Iwinski, K. J.; Calomeni, A. J.; Geer, T. D.; Rodgers, J. H. Cellular and aqueous microcystin-LR following laboratory exposures of Microcystis aeruginosa to copper algaecides. *Chemosphere* **2016**, *147*, 74–81.

(32) Kikuchi, T.; Fujii, M.; Terao, K.; Jiwei, R.; Lee, Y. P.; Yoshimura, C. Correlations between aromaticity of dissolved organic matter and trace metal concentrations in natural and effluent waters: A case study in the Sagami River Basin, Japan. *Sci. Total Environ.* **2017**, *576*, 36–45.

(33) Baken, S.; Degryse, F.; Verheyen, L.; Merckx, R.; Smolders, E. Metal complexation properties of freshwater dissolved organic matter are explained by its aromaticity and by anthropogenic ligands. *Environ. Sci. Technol.* **2011**, *45* (7), 2584–2590.

(34) Lu, Y.; Allen, H. E. Characterization of copper complexation with natural dissolved organic matter (DOM) - Link to acidic moieties of DOM and competition by Ca and Mg. *Water Res.* **2002**, *36* (20), 5083–5101.

(35) Kalbitz, K. Properties of organic matter in soil solution in a German fen area as dependent on land use and depth. *Geoderma* **2001**, *104* (3–4), 203–214.

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

(37) Huguet, A.; Vacher, L.; Relexans, S.; Saubusse, S.; Froidefond, J. M.; Parlanti, E. Properties of fluorescent dissolved organic matter in the Gironde Estuary. *Org. Geochem.* **2009**, *40* (6), 706–719.

(38) Fuentes, M.; Baigorri, R.; González-Gaitano, G.; García-Mina, J. M. The

complementary use of 1H NMR, 13C NMR, FTIR and size exclusion chromatography to investigate the principal structural changes associated with composting of organic materials with diverse origin. *Org. Geochem.* **2007**, *38* (12), 2012–2023.

(39) Qian, H.; Yu, S.; Sun, Z.; Xie, X.; Liu, W.; Fu, Z. Effects of copper sulfate, hydrogen peroxide and N-phenyl-2-naphthylamine on oxidative stress and the expression of genes involved photosynthesis and microcystin disposition in Microcystis aeruginosa. *Aquat. Toxicol.* **2010**, *99* (3), 405–412.

(40) Stauber, J. M.; Florence, T. M. Mechanism of toxicity of ionic copper and copper complexes to algae. *Mar. Biol.* **1987**, *94*, 511–519.

(41) Stevenson, J. Barwinska-Sendra, A., Tarrant, E., Waldron, K. W. Mechanism of action and applications of the antimicrobial properties of copper. *Formatex* **2013**, *1*, 468–479.

(42) Myklestad, S. M. Dissolved Organic Carbon from Phytoplankton. *Mar. Chem.* **2000**, *5*, 112–144.

(43) Pivokonsky, M.; Safarikova, J.; Baresova, M.; Pivokonska, L.; Kopecka, I. A comparison of the character of algal extracellular versus cellular organic matter produced by cyanobacterium, diatom and green alga. *Water Res.* **2014**, *51*, 37–46.

(44) 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.

(45) Gouvêa, S. P.; Vieira, A. A. H.; Lombardi, A. T. Copper and cadmium complexation by high molecular weight materials of dominant microalgae and of water from a eutrophic reservoir. *Chemosphere* **2005**, *60* (9), 1332–1339.

(46) Mcknight, D. M.; Morel, F. M. M. Release of weak and strong copper-complexing agents by algae. *Limnol. Oceanogr.* **1979**, *24* (5), 823–837.

(47) Tonietto, A. E.; Lombardi, A. T.; Henriques Vieira, A. A.; Parrish, C. C.; Choueri, R. B. Cylindrospermopsis raciborskii (Cyanobacteria) exudates: Chemical characterization and complexation capacity for Cu, Zn, Cd and Pb. *Water Res.* **2014**, *49*, 381–390.

(48) Jardim, W. F.; December, R. A study of the copper-complexing compounds released by some species of cyanobacteria. *Water Res.* **1984**, *18* (8), 985–989.

(49) Li, W.; Zhang, F.; Ye, Q.; Wu, D.; Wang, L.; Yu, Y.; Deng, B.; Du, J. Composition and copper binding properties of aquatic fulvic acids in eutrophic Taihu Lake, China. *Chemosphere* **2017**, *172*, 496–504.

(50) Xu, H.; Yan, Z.; Cai, H.; Yu, G.; Yang, L.; Jiang, H. Heterogeneity in metal

binding by individual fluorescent components in a eutrophic algae-rich lake. *Ecotoxicol. Environ. Saf.* **2013**, *98*, 266–272.

(51) Lorenzo, J. I.; Nieto-Cid, M.; Álvarez-Salgado, X. A.; Pérez, P.; Beiras, R. Contrasting complexing capacity of dissolved organic matter produced during the onset, development and decay of a simulated bloom of the marine diatom Skeletonema costatum. *Mar. Chem.* **2007**, *103* (1–2), 61–75.

(52) Hsu-Kim, H. Stability of metal-glutathione complexes during oxidation by hydrogen peroxide and Cu(II)-catalysis. *Environ. Sci. Technol.* **2007**, *41* (7), 2338–2342.

(53) Chang, H.; Chen, C.; Wang, G. Identification of potential nitrogenous organic precursors for C-, N-DBPs and characterization of their DBPs formation. *Water Res.* **2011**, *45* (12), 3753–3764.

(54) Yang, X.; Shang, C. Chlorination byproduct formation in the presence of humic acid, model nitrogenous organic compounds, ammonia, and bromide. *Environ. Sci. Technol.* **2004**, *38* (19), 4995–5001.

(55) Liu, X.; Chen, Z.; Wang, L.; Shen, J. Effects of metal ions on THMs and HAAs formation during tannic acid chlorination. *Chem. Eng. J.* **2012**, *211–212*, 179–185.

(56) Visser, P. M.; Verspagen, J. M. H.; Sandrini, G.; Stal, L. J.; Matthijs, H. C. P.; Davis, T. W.; Paerl, H. W.; Huisman, J. How rising CO2 and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae* **2016**, *54*, 145–159.

(57) Paerl, H. W.; Huisman, J. Blooms like it hot. *Science* **2008**, *320* (5872), 57–58.

(58) Odhiambo, M.; Routh, J. Does Black Carbon Contribute to Eutrophication in Large Lakes? *Curr. Pollut. Reports* **2016**, *2* (4), 213–223.

(59) Le Jeune, A. H.; Charpin, M.; Deluchat, V.; Briand, J. F.; Lenain, J. F.; Baudu, M.; Amblard, C. Effect of copper sulphate treatment on natural phytoplanktonic communities. *Aquat. Toxicol.* **2006**, *80* (3), 267–280.

(60) Willis, B. E.; Bishop, W. M. Understanding Fate and Effects of Copper Pesticides in Aquatic Systems. *J. Geosci. Environ. Prot.* **2016**, *4* (5), 37–42.

(61) Calomeni, A. J.; Iwinski, K. J.; Kinley, C. M.; McQueen, A.; Rodgers, J. H. Responses of Lyngbya wollei to algaecide exposures and a risk characterization associated with their use. *Ecotoxicol. Environ. Saf.* **2015**, *116*, 90–98.

(62) Bishop, W. M.; Rodgers, J. H. Responses of Lyngbya magnifica Gardner to an algaecide exposure in the laboratory and field. *Ecotoxicol. Environ. Saf.* **2011**, *74* (7), 1832–1838.

(63) Liu, R.; Zhao, D.; Barnett, M. O. Fate and transport of copper applied in channel catfish ponds. *Water, Air, Soil Pollut.* **2006**, *176* (1–4), 139–162.

(64) Murray-Gulde, C. L.; Heatley, J. E.; Schwartzman, A. L.; Rodgers, J. H. Algicidal effectiveness of Clearigate, Cutrine-Plus, and copper sulfate and margins of safety associated with their use. *Arch. Environ. Contam. Toxicol.* **2002**, *43* (1), 19–27.

Table 1. (A) Characteristics of solutions for *M. aeruginosa* growing in the presence of black ash extract (BE) and white ash extract (WE), and (B) nominal and measured copper concentration on day 0 and day 4. (average ± standard deviation, n =3).

(A) Selected water quality parameters and optical indices

Parameter	BE	WE
pH	10.0 ± 0.0	9.7± 0.0
OD_{680}	0.1 ± 0.0	0.1 ± 0.0
Conductivity (µS/cm)	252 ± 0	259 ± 0
DOC (mg/L)	6.2 ± 0.3	5.3 ± 0.7
TDN (mg/L)	9.1 ± 0.1	8.1 ± 0.3
NH_4^+-N (mg/L)	0.1 ± 0.0	0.0 ± 0.0
NO_x^--N (mg/L)	2.1 ± 0.5	2.0 ± 0.1
DON (mg/L)	6.9 ± 0.6	6.0 ± 0.2
$SUVA_{254}$ (L/mg/m)	3.5 ± 0.4	2.7 ± 0.2
HIX	9.4 ± 0.0	8.0 ± 0.2
E2/E3	5.3 ± 0.4	4.8 ± 1.5
FI	1.7 ± 0.0	1.8 ± 0.0

(B) Nominal and measured soluble copper concentrations

	BE			WE		
	Control	Treatment		Control	Treatment	
Nominal Cu^{2+} on day 0 (mg/L)	0	0.5	1.0	0	0.5	1.0
Measured Cu^{2+} on day 0 (mg/L)	0.1 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
Measured Cu^{2+} on day 4 (mg/L)	0.0 ± 0.0	0.5 ± 0.1	0.9 ± 0.0	0.0 ± 0.0	0.6 ± 0.0	0.8 ± 0.0

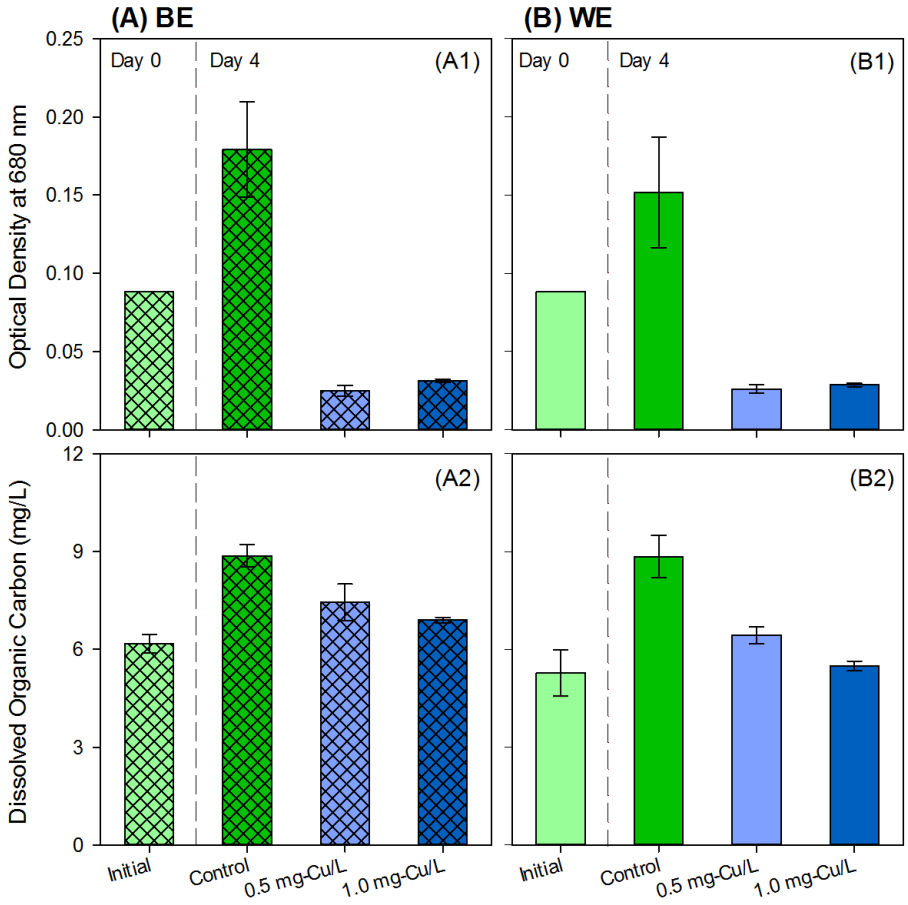


Figure 1. Optical density at 680 nm (OD_{680}) and dissolved organic carbon (DOC) for *M. aeruginosa* growing in the presence of (A) black ash extract (BE, A1 and A2) and (B) white ash extract (WE, B1 and B2) before (initial) and after 4-days exposures to copper sulfate (0.5 and 1.0 mg-Cu/L as nominal concentrations). Error bars represent the standard deviation (n = 3).

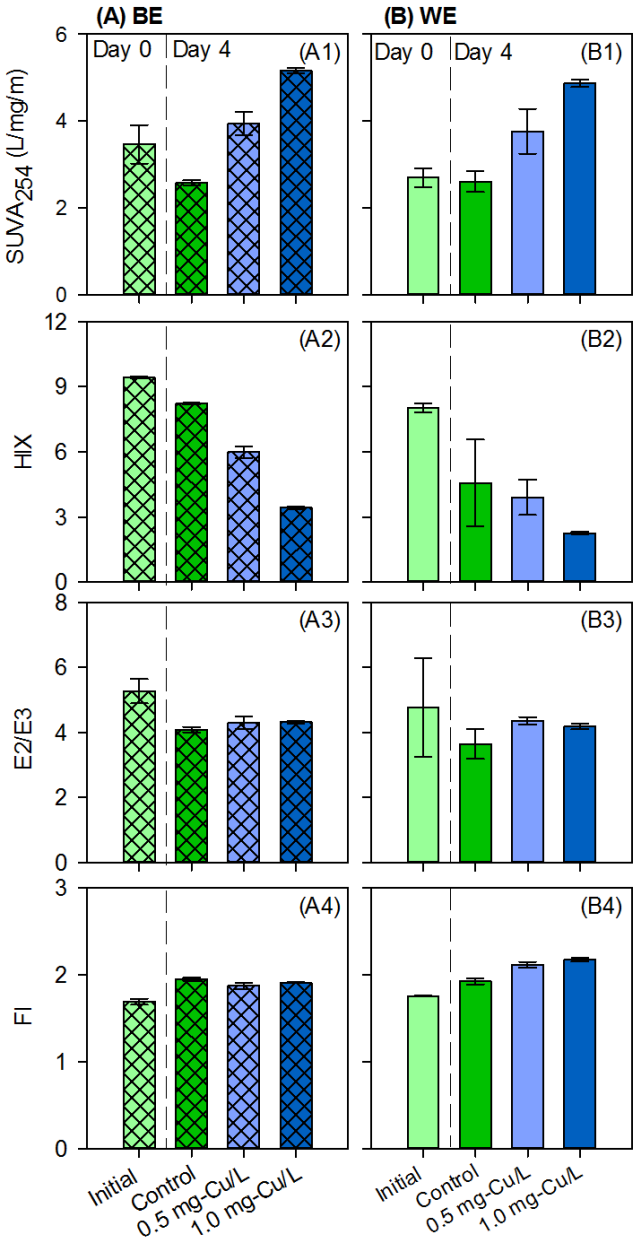


Figure 2. Spectroscopic characteristics of DOM for *M. aeruginosa* growing in the presence of (A) black ash extract (BE, A1-A4) and (B) white ash extract (WE, B1-B4) before (initial) and after 4-days exposures to copper sulfate (0.5 and 1.0 mg-Cu/L as nominal concentrations). Error bars represent the standard deviation (n = 3).

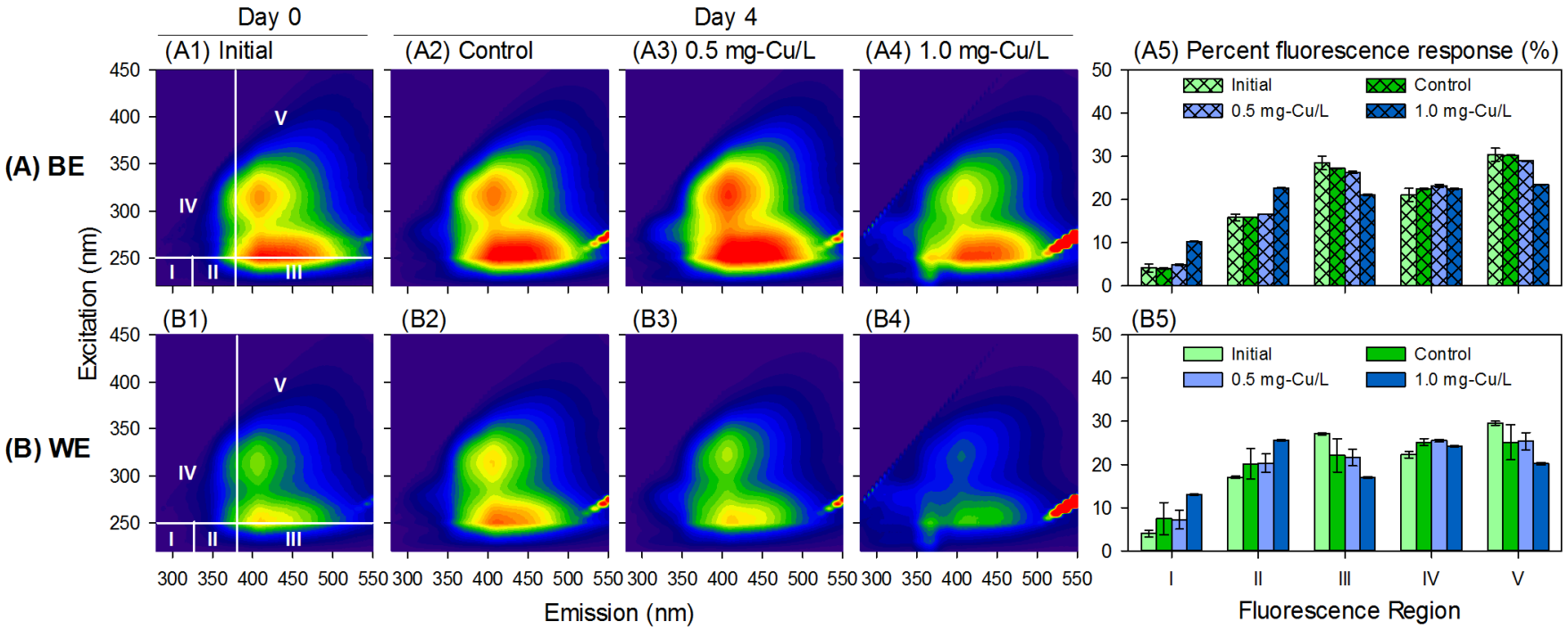


Figure 3. 3D excitation and emission matrices (EEM) and fluorescence regional integration for *M. aeruginosa* growing in the presence of (A) black ash extract (BE, A1-A5) and (B) white ash extract (WE, B1-B5) before (initial) and after 4-days exposures to copper sulfate (0.5 and 1.0 mg-Cu/L as nominal concentrations). Roman numerals I, II, III, IV, and V represent proportion of tyrosine-like, tryptophan-like, fulvic acid-like, soluble microbial byproduct-like, and humic acid-like components analyzed by fluorescence EEM, respectively. Error bars represent the standard deviation (n = 3).

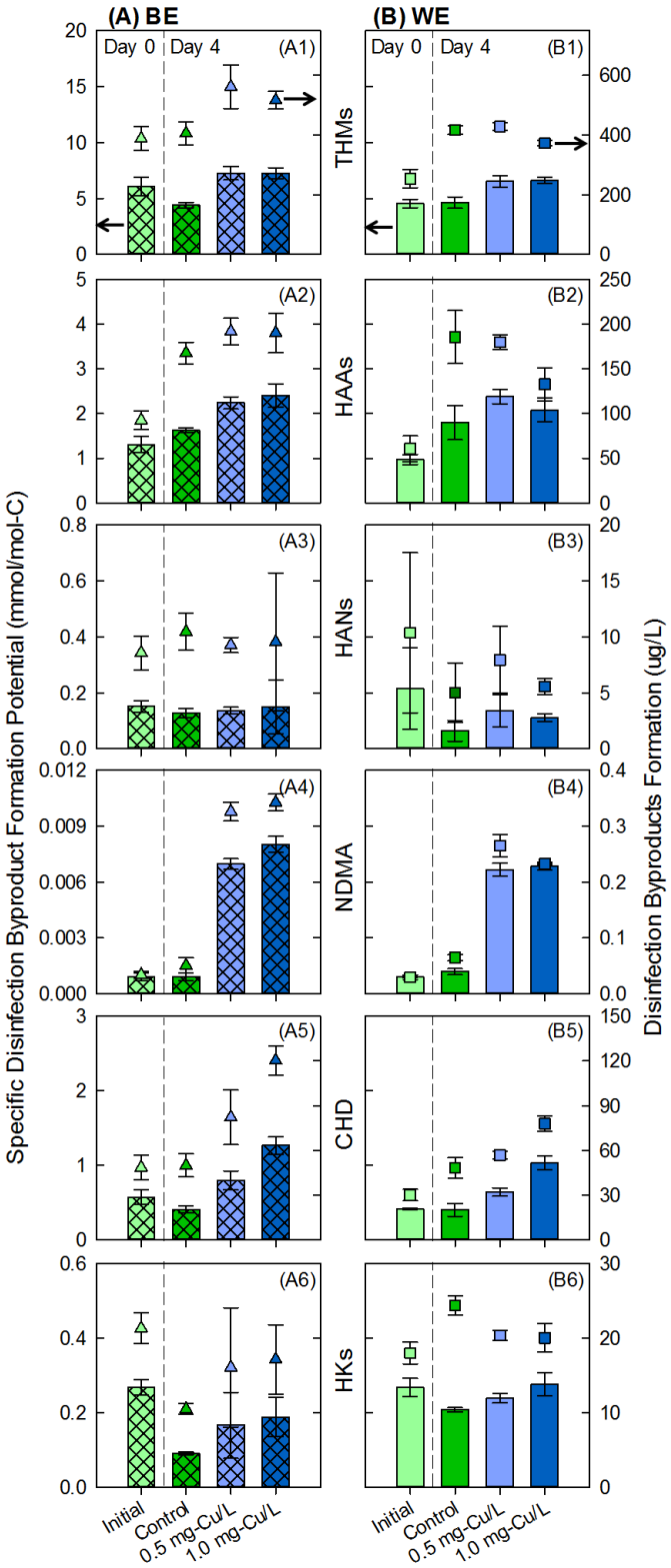

(A) BE
(B) WE
Day 0
Day 4
(A1)
(B1)
(A2)
(B2)
(A3)
(B3)
(A4)
(B4)
(A5)
(B5)
(A6)
(B6)
THMs
HAAs
HANs
NDMA
CHD
HKs
Specific Disinfection Byproduct Formation Potential (mmol/mol-C)
Disinfection Byproducts Formation (ug/L)
Initial
Control
0.5 mg-Cu/L
1.0 mg-Cu/L

Figure 4. Specific DBP-FP (grid and non-grid bars, left y-axis) and DBP formation (triangle and square symbols, right y-axis) for *M. aeruginosa* growing in the presence of (A) black ash extract (BE, A1-A6) and (B) white ash extract (WE, B1-B6) before (initial) and after 4-days exposures to copper sulfate (0.5 and 1.0 mg-Cu/L as nominal concentrations). THMs, trihalomethanes; HAAs, haloacetic acids; HANs, haloacetonitriles; NDMA, N-nitrosodimethylamine; CHD, chloral hydrate; HKs, haloketones. Error bars represent the standard deviation (n = 3).

CHAPTER FIVE

ENVIRONMENTAL SIGNIFICANCE AND IMPLICATIONS

In order to accurately predict impacts of wildfire on downstream water quality as well as to take precautions against negative impacts on community drinking water supply, it is essential for responsible agencies to understand how the quality and quantity of DOM from fire impacted areas are changed during its transport from forested watersheds to water treatment facilities. This dissertation presents evidence that growing algae alter fire-affected bulk DOM composition and chlorine reactivity. The fraction of biodegradable DOM exported from forested watersheds decreases with an increase in fire intensity (i.e., fire temperature), implying that DOMs exported from mildly burned (≤ 250 °C) areas are more likely subjected to biotic transformations in downstream compared to that from severely burned (≥ 400 °C) areas. Fire-affected DOMs can accelerate proliferations of phytoplankton in drinking water resources. Algal blooms may have an ephemeral effect on decreasing DBP precursors in fire-affected terrestrial DOM; but as algal populations increase, concomitant productions of algal organic matters will likely outweigh that loss, resulting in increases of DOM and chlorine reactivity for DBP formations as well as increases of the proportion of brominated THMs.

Additionally, this dissertation demonstrates dynamic changes of DBP precursors by incorporating different quality and quantity of DOMs released from ash and noxious algae *M. aeruginosa* during its growth. Dynamic changes in DOM reactivities and associated DBP concentrations would provide useful information for water resource managers, regarding the timing for treating waters impaired by ongoing harmful algal bloom and wildfire. The conceptual model in Chapter III shows that the pattern of

specific DBP-formation potential during bloom process is more influenced by the concentration than composition of ash solution. Despite of color of ash, the results also suggest that input of high amount of ash solution into source water can significantly increase DBP formation during a developing algal bloom. Forest and water resource managers shall put efforts on reducing postfire ash load in forested watersheds to minimize DBP precursors in source water. Authorities need to be particularly cautious about postfire extreme weather conditions (e.g., heavy rain storms or strong wind events) that would result in large input of ash into source water. Furthermore, this study suggests that postfire DBP precursors can be dynamically changed during an entire algal life cycle, and the changes are different in C-, N-, and O-DBP species. The concentrations of US EPA regulated DBPs such as THMs and HAAs would reveal highest concentrations during a decline of algal bloom, regardless of ash solution concentration.

Public concerns regarding the potential risks created by applying copper algaecide, such as inducing excess toxin release into source water and impacts on aquatic ecosystem, have been extensively studied.[8,22,60] However, information about its influence on DBP formation during drinking water disinfection is not available. Understanding influences of copper algaecide application on DBP formation is important for making risk-based decisions regarding management of drinking water resource affected by wildfire. This study demonstrates changes of DBP formation as well as algal population following exposures of toxin-producing *M. aeruginosa* to copper sulfate, when *M. aeruginosa* was cultured with black and white ash extracts to simulate growing in ash-contaminated waters with scenarios of low and high burning temperature. It was found that low copper concentration (0.5 mg-Cu/L) was as effective as high concentration (1.0

mg-Cu/L) in controlling *M. aeruginosa* while minimizing promotion of DBP formation. Although DBP concentrations in the control were generally lower than or similar to the treatments, OD_{680} value and DOM concentration were higher. The results may serve to support risk evaluations of algal population and DBP concentration when wildfire-induced *M. aeruginosa* bloom is left untreated and when it is treated by copper sulfate.

APPENDIX I - Supporting Information for Chapter II

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^{TM} 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 ^oC in dark for 24 hours without headspace.[9] The chlorine concentration added to the sample was calculated

according to the equation [Cl_2] = [3 × (DOC) + 7.6 × (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.

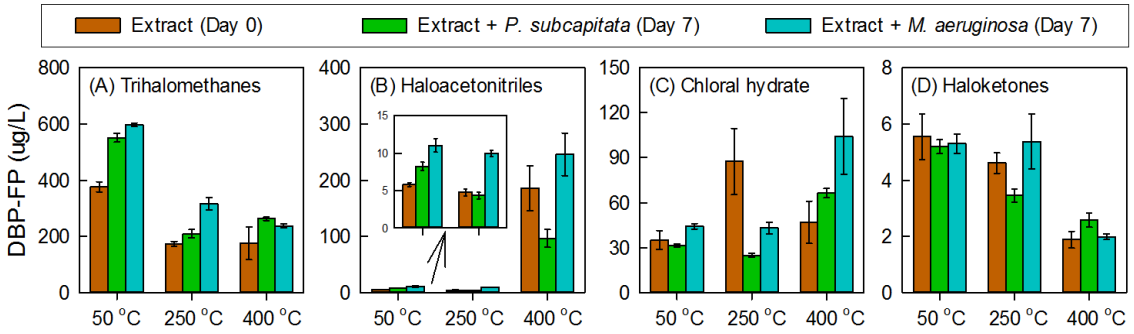


Figure S1. Disinfection byproduct formation potential (DBP-FP) of thermally-altered extracts before (Day 0) and after (Day 7) inoculations with *P. subcapitata* and *M. aeruginosa*. Error bars represent the standard deviation.

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
$MgSO_4 \cdot 7H_2O$	7.5	75
$CaCl_2 \cdot 2H_2O$	3.6	36
Na_2CO_3	2	20
Micronutrients		
$Na_2EDTA \cdot 2H_2O$	2	2
H_3BO_3	2.86	2.86
$FeCl_3 \cdot 6H_2O$	1	1
$MnCl_2 \cdot 4H_2O$	1.81	1.81
$ZnSO_4 \cdot 7H_2O$	0.22	0.22
$Na_2MnO_4 \cdot 2H_2O$	0.39	0.39
CuSO4·5H2O	0.079	0.079
$Co(NO_3)_2 \cdot 6H_2O$	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.

APPENDIX II - Supporting Information for Chapter III

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-N (NH_4^+-N), and nitrate/nitrite (NO_x^--N), 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^+-N and NO_x^--N were measured using a Systea® Easychem^{TM} 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. The orthophosphate (PO_4^{3-}) concentration was measured using DR 900 spectrophotometric PhosVer 3 ascorbic acid method (Hach Method 8048). Metal concentrations (Zn, Cu, As, Cd, Cr, and Pb) were measured using inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Arcos, Ametek).

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]

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 ^oC in dark for 24 hours without headspace, except that the samples for haloaceticacids analysis were incubated

for 5 days.[1] The chlorine concentration added to the sample was calculated according to the equation $[Cl_2] = [3 \times (DOC) + 7.6 \times (TDN)]$.[1] The residual chlorine was quenched by a 10% Na_2SO_3 solution and DBPs were extracted and quantified by Agilent 6890 GC-ECD with an auto sampler equipped with Phenomenex ZB-1 column (30 m × 0.25 mm × 1μm) following USEPA methods 551.1 and 552.2.[1] We quantified four trihalomethanes (THMs; trichloro-, dichlorobromo-, dibromochloro-, and tribromo- methanes), five haloacetic acids (HAAs; chloro-, dichloro-, trichloro-, bromo-, and dibromoacetic acids), four haloacetonitriles (HANs; trichloro-, dichloro-, bromochloro-, and dibromo-acetonitriles), chloral hydrate (CHD), and three haloketones (HKs; 1,1-dichloro-2-, 1,1,1-trichloro-2-, 1,2,3-trichloro- propanones). The minimum reporting limits (MRLs) for all the above DBP species were approximately 0.1-0.3 μg/L.

N-Nitrosodimethylamine (NDMA) FP test was designed to determine the maximum formation of NDMA in the presence of excess chloramines. NDMA was extracted and analyzed by following the USEPA Method 521. EPA 521 nitrosamine mix (2000 μg/ml of each component, 98.6-99.9%) in methanol, nitrosamine calibration mix of N-nitrosodimethylamine-d6 (NDMA-d_6, 98%) as a surrogate and N-nitrosodi-n-propylamine-d_{14} (NDPA-d_{14}, 99%) as an internal standard (1000 μg/ml of each in dichloromethane [DCM]) were purchased form Sigma Aldrich and Restek and used to prepare primary diluted solutions (PDS), respectively. These PDS solutions in DCM were used to generate calibration curves for each measurement. Typical calibration curves were generated from eleven (1 to 400 ng/L) standard points. Samples (500 mL) were oxidized with excessive chloramine (100 mg/L as Cl_2) at pH 8.0 (borate buffered) and stored in the dark at 22 °C. A pH near 8.0 was used for chloramination to simulate the

actual pH environment in drinking water distribution systems. After 5 days of contact time, residual chloramines were quenched with excess amount Na_2SO_3 in the samples. 40 ng/L NDMA-d_6 was always added to the samples as a surrogate before extraction. A laboratory quality control blanks (10 ng/L and 50 ng/L NDMA spiked samples) were extracted simultaneously with each batch of samples. Samples were passed through cartridges pre-packed with 2 g of coconut charcoal purchased from UCT. Prior to sample extraction, cartridges were pre-conditioned with DCM, methanol, and distilled and deionized water (DDW). After solid phase extraction, cartridges were dried with air, and then eluted with DCM. Eluted samples were passed through column pre-packed with 6 g of sodium sulfate (Na_2SO_4) and concentrated to 1 ml under high purity nitrogen gas. Then the extracts were spiked with NDPA-d_{14} as an internal standard (target concentration = 40 ng/L), and analyzed using Agilent 7000C GC/MS/MS equipped with DB1701 (30 m × 0.25 mm × 1 μm) column under the electron ionization mode.

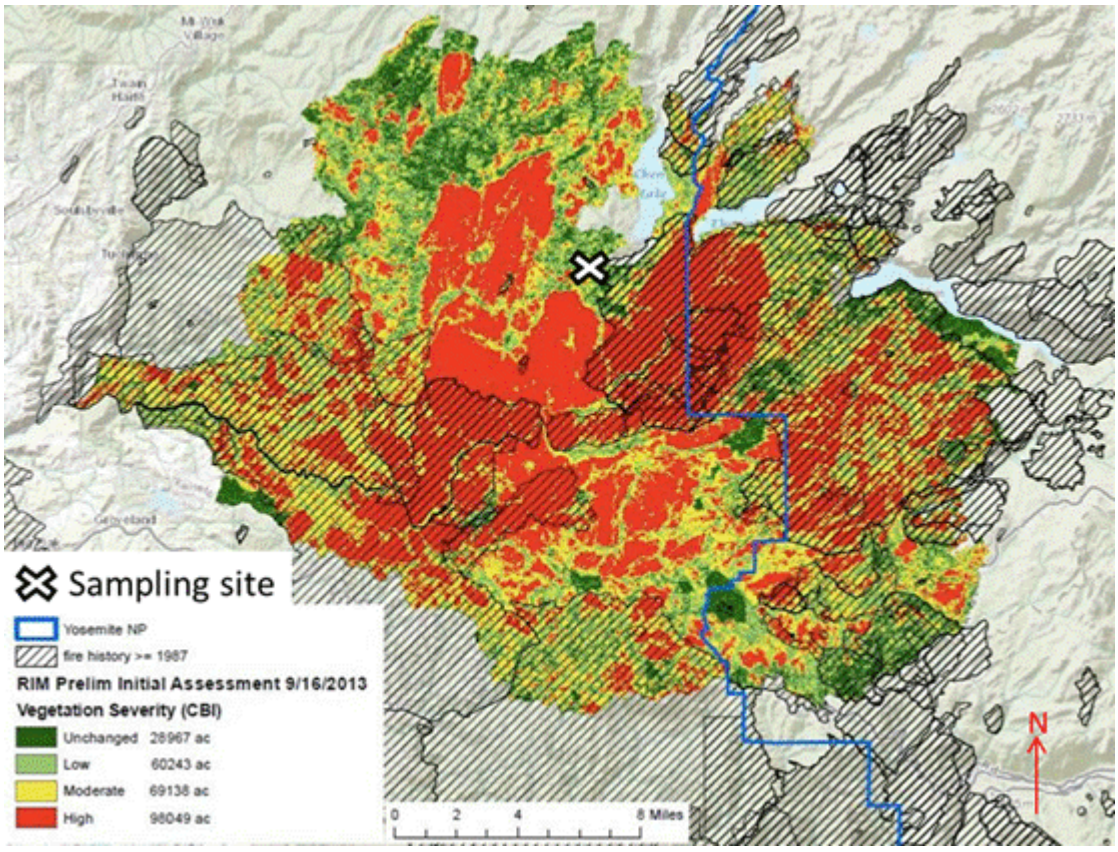


Figure S1. The location of sampling site (approximately 2 km south west of the Cherry Lake). The original map was accessed from the website (http://inciweb.nwcg.gov/incident/map/3726/0/).

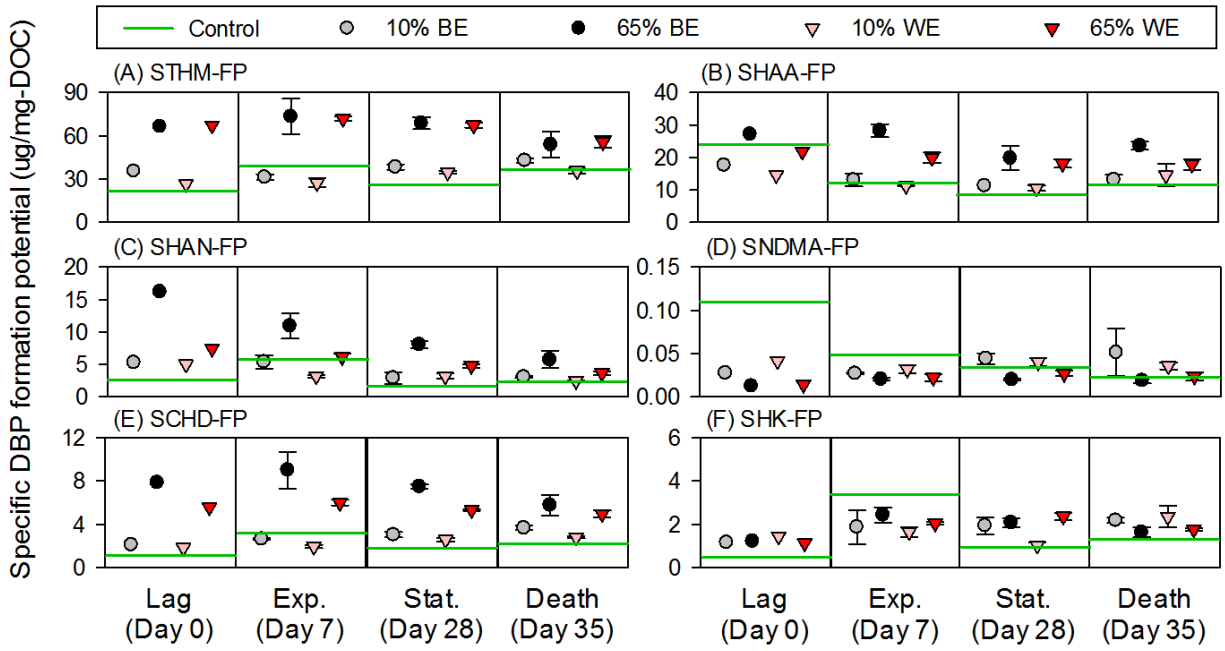


Figure S2. Specific DBP formation potential (SDBP-FP) in the absence (control) and presence of black and white ash extracts (10% and 65% BE and WE) at different algal growth phases. THM, trihalomethane; HAA, haloacetic acid; HAN, haloacetonitrile; NDMA, N-nitrosodimethylamine; CHD, chloral hydrate; HK, haloketone. Error bars represent the standard deviation (n = 3).

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
$MgSO_4 \cdot 7H_2O$	7.5	75
$CaCl_2 \cdot 2H_2O$	3.6	36
Na_2CO_3	2	20
Micronutrients		
$Na_2EDTA \cdot 2H_2O$	2	2
H_3BO_3	2.86	2.86
$FeCl_3 \cdot 6H_2O$	1	1
$MnCl_2 \cdot 4H_2O$	1.81	1.81
$ZnSO_4 \cdot 7H_2O$	0.22	0.22
$Na_2MnO_4 \cdot 2H_2O$	0.39	0.39
CuSO4·5H2O	0.079	0.079
$Co(NO_3)_2 \cdot 6H_2O$	0.0494	0.0494

Table S2. Characteristics of the raw black ash and white ash 72-h water extracts (average ± standard deviation, n =3).

	Black ash extract (BE)	White ash extract (WE)
pH	8.1 ± 0.1	8.3 ± 0.0
OD_{680}	0.0 ± 0.0	0.0 ± 0.0
Conductivity (µS/cm)	377 ± 8	482 ± 2
DOC (mg/L)	31.0 ± 0.1	23.8 ± 0.2
TDN (mg/L)	14.9 ± 0.1	5.5 ± 0.2
NH_4^+-N (mg/L)	5.0 ± 0.2	1.0 ± 0.0
NO_x^--N (mg/L)	2.4 ± 0.1	0.2 ± 0.0
DON (mg/L)	7.5 ± 0.2	4.4 ± 0.0
PO_4^{3-} (mg/L)	1.0 ± 0.0	1.0 ± 0.0
Zn (mg/L)	< 0.01	< 0.01
Cu(mg/L)	< 0.01	< 0.01
As (mg/L)	< 0.02	< 0.02
Cd (mg/L)	< 0.01	< 0.01
Cr (mg/L)	< 0.01	< 0.01
Pb (mg/L)	< 0.03	< 0.03

Table S3. Coefficients of optical indices and specific DBP-FP on the principal components.

	Coefficients of PC 1	Coefficients of PC 2
SUVA	0.30	0.26
HIX	0.33	0.01
E2/E3	0.22	-0.19
FI	-0.28	0.26
I	-0.32	0.18
II	-0.30	0.28
III	0.32	-0.17
IV	-0.18	-0.09
V	0.33	-0.18
STHM-FP	0.30	0.22
SHAA-FP	0.15	0.09
SHAN-FP	0.21	0.45
SNDMA-FP	-0.12	-0.08
SCHD-FP	0.26	0.38
SHK-FP	-0.01	0.49

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.

APPENDIX III - Supporting Information for Chapter IV

MATERIALS AND METHODS

Sampling Site and Ash Collection

Ash samples were collected on October 2nd from the 2013 Rim Fire, which started on August 17th and is recorded as the third largest wildfire in California history covering more than 100,000 ha in watersheds. The sampling site was located approximately 2 km south west of the Cherry Lake in Tuolumne River Watershed within the Stanislaus National Forest (Figure S1), where the dominate vegetation type was ponderosa pine. Based on the visual color of ash, postfire ash samples including black ash and white ashes were collected using a 7.6 cm diameter × 5.0 cm depth metal coring device. Each type of ash samples consisted of three subsamples collected within a 10 m radius.

Analyses of Water Chemistry, Soluble Copper, and DOM spectroscopic characteristics

Characteristics of water extracts and algal solution, including pH, specific conductivity, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), ammonium-N (NH_4^+-N), and nitrate/nitrite (NO_x^--N), 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^+-N and NO_x^--N 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. Soluble copper was measured following U.S. EPA

Method 3005A (U.S. EPA, 1992).[2] Samples were acidified by HNO_3 (pH < 2) for 24 hours and analyzed by a graphite furnace atomic absorption spectrometer (AA280FS, Agilent Technologies). The method detection limit was 5 µg-Cu/L.

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.[3] 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).[4] 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).[5] 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)].[4] 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. Two fluorescence spectroscopic indices were used to describe DOM characteristics.[6] 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.[7] 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.[8]

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 ^oC in dark for 24 hours without headspace, except that the samples for haloaceticacids analysis were incubated for 5 days.[1] The chlorine concentration added to the sample was calculated according to the equation $[Cl_2] = [3 \times (DOC) + 7.6 \times (TDN)]$.[1] The residual chlorine was quenched by a 10% Na_2SO_3 solution and DBPs were extracted and quantified by Agilent 6890 GC-ECD with an auto sampler equipped with Phenomenex ZB-1 column (30 m × 0.25 mm × 1μm) following USEPA methods 551.1 and 552.2.[1] We quantified four trihalomethanes (THMs; trichloro-, dichlorobromo-, dibromochloro-, and tribromo- methanes), five haloacetic acids (HAAs; chloro-, dichloro-, trichloro-, bromo-, and dibromoacetic acids), chloral hydrate (CHD), and three haloketones (HKs; 1,1-dichloro-2-, 1,1,1-trichloro-2-, 1,2,3-trichloro- propanones). The minimum reporting limits (MRLs) for all the above DBP species were approximately 0.1-0.3 μg/L.

Haloacetonitrile (HAN) formation potential was tested under chloramination condition. During HAN and N-nitrosodimethylamine (NDMA) formation potential tests, pH was maintained at 8 with 10 mM borate buffer. Pre-formed NH_2Cl stock solution at

Cl_2/N mass ratio of 4:1 was generated by dropping sodium hypochlorite slowly to $(NH_4)_2SO_4$ solution at pH 9, and pre-determined volume of monochloramine (NH_2Cl) stock solution was spiked to samples to achieve 100 mg/L of initial NH_2Cl in the bottles (125 and 500 mL amber bottles were used for HAN and NDMA FP tests, respectively). After 5 days of contact time at room temperature, residual chloramines were quenched by ascorbic acid,[11] and sodium thiosulfate solutions for HAN and NDMA samples, respectively. Then, samples were extracted with USEPA methods 551.1 for HANs, and 521 for NDMA. Finally, four HANs (i.e., dichloro-, trichloro-, bromochloro-, and dibromoacetonitriles) were quantified by Agilent 6890 GC-ECD with an auto sampler equipped with Phenomenex ZB-1 column (30m × 0.25mm × 1μm), and NDMA analyzed using Agilent 7000C GC/MS/MS equipped with DB1701 (30m × 0.25mm × 1 um) column under the electron ionization mode.

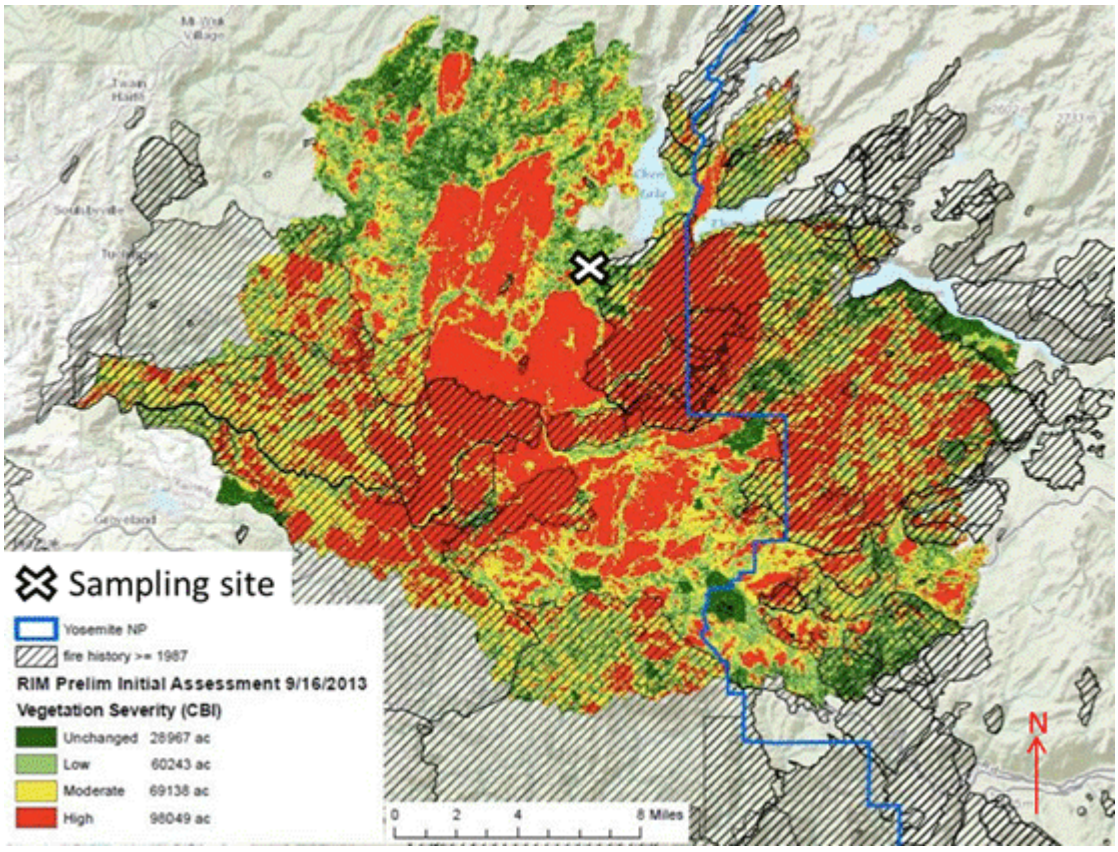


Figure S1. The location of sampling site (approximately 2 km south west of the Cherry Lake). The original map was accessed from the website (http://inciweb.nwcg.gov/incident/map/3726/0/).

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
$MgSO_4 \cdot 7H_2O$	7.5	75
$CaCl_2 \cdot 2H_2O$	3.6	36
Na_2CO_3	2	20
Micronutrients		
$Na_2EDTA \cdot 2H_2O$	2	2
H_3BO_3	2.86	2.86
$FeCl_3 \cdot 6H_2O$	1	1
$MnCl_2 \cdot 4H_2O$	1.81	1.81
$ZnSO_4 \cdot 7H_2O$	0.22	0.22
$Na_2MnO_4 \cdot 2H_2O$	0.39	0.39
CuSO4·5H2O	0.079	0.079
$Co(NO_3)_2 \cdot 6H_2O$	0.0494	0.0494

Table S2. Copper-to-DOC ratio (mg-Cu^{2+}/mg-DOC) and $SUVA_{254}$ for the control and treatments on day 4 (average ± standard deviation, n =3).

	BE			WE		
	Control	Treatment		Control	Treatment	
Nominal Cu^{2+}	0	0.5	1.0	0	0.5	1.0
Cu^{2+}/DOC	0.003 ± 0.000	0.069 ± 0.014	0.125 ± 0.001	0.003 ± 0.000	0.086 ± 0.006	0.136 ± 0.007
$SUVA_{254}$	2.58 ± 0.06	3.95 ± 0.27	5.16 ± 0.06	2.61 ± 0.24	3.76 ± 0.52	4.87 ± 0.09

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) United States Environmental Protection Agency (US EPA). Acid-digestion of waters for total recoverable or dissolved metals for analysis by FLAA or ICP spectroscopy. Method 3005A. **1992**.

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

(4) 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.

(5) 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.

(6) 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.

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

(8) 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.

(9) Yu, Y.; Reckhow, D. A. Kinetic Analysis of Haloacetonitrile Stability in Drinking Waters. *Environ. Sci. Technol.* **2015**, *49* (18), 11028–11036.

(10) Bond, T.; Huang, J.; Templeton, M. R.; Graham, N. Occurrence and control of nitrogenous disinfection by-products in drinking water - A review. *Water Res.* **2011**, *45* (15), 4341–4354.

(11) Kristiana, I.; Lethorn, A.; Joll, C.; Heitz, A. To add or not to add: The use of quenching agents for the analysis of disinfection by-products in water samples. *Water Res.* **2014**, *59*, 90–98.