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

Kuo-Pei Tsai, Habibullah Uzun, Tanju Karanfil, and Alex T. Chow

ABSTRACT: Wildfires can elevate dissolved organic matter (DOM) levels due to ash input and algal growth in source waters, and consequently impacting disinfection byproduct (DBP) 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. Throughout the entire experiment, C-DBP precursors in the control ranged from 2.41 to 3.09 mmol/mol-C. In the treatment with 10% BE, the amount of C-DBP precursors decreased from 6.8 to 3.0 mmol/mol-C at initial-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.

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. In addition, increases in nutrient concentrations (e.g., nitrogen and phosphorus) concomitant with proliferation of algae also have been observed. Algae are prevailing producers of autochthonous DOM in freshwaters, and the level of DOM released from algae is usually proportional to their population. 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, and excess inputs of nutrient and organic matter in waters are important factors for stimulating its population growth. 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. 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. For example, Wang et al. 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. Regarding quality and quantity of DOMs released from black and white ashes, Wang et al. 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$_3^−$ and NH$_4^+$-N. Quill et al. 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. 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. 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. 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. Previous studies showed that growing algae with an ample supply of nutrients excrete higher amount of DOM to the medium. Using optical indices (e.g., SUVA$_{254}$, HIX, and FI) to examine AOM characteristics, Huang et al. 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. Henderson et al. 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. 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 second 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 southwest of the Cherry Lake in Tuolumne River Watershed within the Stanislaus National Forest (Figure S1, Supporting Information (SI)), 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 h 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 nanoxenically in the medium. The medium composition is provided in SI Table S1. Algal cultures were maintained in the indoor laboratory, at a temperature of 24 ± 2 °C and a 12:12-h light-dark photoperiod illuminated by cool white fluorescent lighting at an intensity of 2100 lx. The experimental 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. Regarding wildfire impacts on streamwater DOC concentration, Hohner et al. 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. Due to postfire thunderstorm or snowmelt, wildfire ash may be flushed into streams causing different levels of ash contamination in receiving water. 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 1 L 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. Afterward, 200 mL of mixtures were distributed separately to 250 mL acid washed Erlenmeyer flasks. Three replicate flasks were included for the control and treatments. All glassware used in the experiment was prewashed by 10% hydrochloric acid and dried in an oven at 50 °C. Subsamples taken from each replicate from the control and treatments (n = 3) were collected on day 0, 7, 28, and 35, which was operationally defined as initial, exponential, stationary, and death phase, respectively. Growth rate during cultivation day t1 and day t2 was calculated as follows:

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

where OD$_{680,t2}$ and OD$_{680,t1}$ refer to OD$_{680}$ values on the cultivation day t2 and day t1.
Chemical and Statistical Analyses. Subsamples collected in the experiment were filtered using prewashed 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 SI. Spectroscopic characteristics of dissolved organic matter were analyzed, including specific UV absorbance at 254 nm (SUVA254), humification index (HIX), E2/E3, and fluorescence.

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) 

<table>
<thead>
<tr>
<th>parameter</th>
<th>control</th>
<th>10% BE</th>
<th>65% BE</th>
<th>10% WE</th>
<th>65% WE</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>9.5 ± 0.1a</td>
<td>9.4 ± 0.0b</td>
<td>8.9 ± 0.1b</td>
<td>9.4 ± 0.1a</td>
<td>8.8 ± 0.0b</td>
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<tr>
<td>conductivity (μS/cm)</td>
<td>342 ± 5a</td>
<td>381 ± 2b</td>
<td>569 ± 8a</td>
<td>390 ± 5a</td>
<td>623 ± 1d</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>2.1 ± 0.2a</td>
<td>3.8 ± 0.1b</td>
<td>23.1 ± 0.4a</td>
<td>3.3 ± 0.1b</td>
<td>17.7 ± 0.4a</td>
</tr>
<tr>
<td>TDN (mg/L)</td>
<td>15.6 ± 0.5a</td>
<td>18.7 ± 0.4a</td>
<td>25.0 ± 0.4b</td>
<td>17.0 ± 0.1a</td>
<td>18.4 ± 0.1a</td>
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<tr>
<td>NH₄⁺-N (mg/L)</td>
<td>0.1 ± 0.0a</td>
<td>0.8 ± 0.1b</td>
<td>3.9 ± 0.2b</td>
<td>0.6 ± 0.1b</td>
<td>0.9 ± 0.0d</td>
</tr>
<tr>
<td>NO₃⁻-N (mg/L)</td>
<td>12.8 ± 0.6a</td>
<td>14.3 ± 0.1b</td>
<td>14.2 ± 0.3b</td>
<td>13.7 ± 0.1a</td>
<td>14.5 ± 0.4b</td>
</tr>
<tr>
<td>DON (mg/L)</td>
<td>2.2 ± 0.6a</td>
<td>3.6 ± 0.2b</td>
<td>7.0 ± 0.3a</td>
<td>2.7 ± 0.1a</td>
<td>3.0 ± 0.3a</td>
</tr>
<tr>
<td>PO₄³⁻ (mg/L)</td>
<td>3.4 ± 0.0a</td>
<td>4.1 ± 0.0b</td>
<td>6.4 ± 0.0b</td>
<td>3.8 ± 0.0b</td>
<td>4.3 ± 0.0b</td>
</tr>
<tr>
<td>Zn (mg/L)</td>
<td>0.02 ± 0.00</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cu (mg/L)</td>
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<td>&lt;0.01</td>
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<td>As (mg/L)</td>
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<tr>
<td>Cd (mg/L)</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cr (mg/L)</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Pb (mg/L)</td>
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<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>OD₆₈₀ on day 0</td>
<td>0.1 ± 0.0a</td>
<td>0.1 ± 0.0a</td>
<td>0.1 ± 0.0a</td>
<td>0.1 ± 0.0a</td>
<td>0.1 ± 0.0a</td>
</tr>
<tr>
<td>OD₆₈₀ on day 7</td>
<td>0.2 ± 0.0a</td>
<td>0.3 ± 0.0b</td>
<td>0.3 ± 0.0b</td>
<td>0.2 ± 0.0e</td>
<td>0.4 ± 0.0e</td>
</tr>
<tr>
<td>OD₆₈₀ on day 28</td>
<td>1.0 ± 0.1a</td>
<td>0.9 ± 0.0a</td>
<td>1.2 ± 0.0b</td>
<td>1.0 ± 0.0e</td>
<td>1.1 ± 0.0e</td>
</tr>
<tr>
<td>OD₆₈₀ on day 35</td>
<td>0.4 ± 0.0a</td>
<td>0.4 ± 0.0a</td>
<td>0.5 ± 0.0b</td>
<td>0.3 ± 0.0e</td>
<td>0.2 ± 0.0e</td>
</tr>
</tbody>
</table>

*aBoth 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 h. Bold numbers indicate the highest number among groups. Lowercase letters refer to the significantly different groups (P < 0.05).
SUVA\textsubscript{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).

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-nitroso-dimethylamine (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 derived precursors and chloramine. The DOM reactivities for DBP formation were expressed as specific DBP formation potential (speci\textit{fi}cD B P - F P ) , which was calculated as the DBP concentration (mmol/L) 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 \textit{M. aeruginosa} Growth Following Exposures to Ash Extracts.

Characteristics of the raw black and white ash extracts (BE and WE) were reported in SI 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 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 (the theoretical values were reported in SI Table S2). The amounts of DOC attributed to the additions of BE and WE were similar to the increments in DOC concentration after thunderstorm or spring snowmelt in the High Park Wildfire-affected river in Colorado. Some metals that have been reported as constituents of wildfire ash, including As, Cd, Cr, and Pb\textsuperscript{3,4} 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\textsubscript{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\textsubscript{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\textsubscript{680} in the control and treatments started to decline. \textit{M. aeruginosa} cultured in the 65% WE revealed the highest growth rate (0.22 ± 0.00 day\textsuperscript{−1}) and decline rate (0.23 ± 0.01 day\textsuperscript{−1}) during initial-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 \textit{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.\textsuperscript{36,37} Moreover, although ammonium

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**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\textsubscript{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. Values and error bars represent the average and standard deviation of experimental triplicates (\( n = 3 \)).
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. 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) and the observed recovery of growth on day 4 and highest OD₆₈₀ 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 toxics. The highest specific growth rate observed in the 65% WE at initial-exponential phase could be ascribed to high nutrient (14.5 mg/L as NO₃⁻⁻⁻⁻) along with high electrical conductivity (623 μS/cm). These findings may be applied to explain why proliferation of algae in streams or ponds is sometimes but not always observed after wildfires.

DOC concentrations in the control and treatments increased over time throughout the experiment (Figure 1C), suggesting that intra- and extracellular algal organic matters were released into solutions. 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 the beginning of experiment, but DOC concentrations in the 10% BE were significantly greater than that in the 10% WE at exponential and stationary phases. During initial-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). SUVA₂₅₄, 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 initial-stationary phase and slightly decreased to 1.0 L/mg/m at death phase (Figure 2A). During initial-exponential phase, SUVA₂₅₄ significantly decreased in the 10% BE (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, SUVA₂₅₄ 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 initial-stationary phase and then increased to 1.5 ± 0.1 at death phase (Figure 2B). The relative high HIX in control on Day 0 compared to other growth phase could be due to undegraded DOM derived from freshly prepared algal solution. Similar trends were also observed in all treatments. Regardless of characteristics and amount of ash extract, SUVA₂₅₄ 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. showing that SUVA₂₅₄ of DOM released from *M. aeruginosa* was less than that from surface waters regardless of its growth phase. The patterns showing that SUVA₂₅₄ 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, and the degree of transformation increases along with growth phase. Detailed catabolic mechanisms and examples for microalgal transforming humic substances, such as oxygenase and dehydrogenase activities along with cleavage pathway, can be found in previous studies.

E2/E3 ratio, an index inversely correlated with DOM molecular weight (MW), in the control decreased from 5.7 to 3.4 during initial-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 initial-exponential phase and started to decrease until death phase. MW of DOM in the 65% WE was higher than the control and other treatments at the beginning of experiment. Noticeably, during initial-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 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.

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 the beginning of experiment 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. A difference in the FI of at least 0.1 may be indicative of a difference in source of fulvic acid, and FI measurement in field studies may augment the interpretation of DOC sources in aquatic ecosystems. DOC in the control decreased from 5.7 to 3.4 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. An increase in FI in the control at initial-exponential phase might suggest reduction of MW; however, changes of E2/E3 value during that period indicated an increase of MW (Figure 2C and D). Although E2/E3 ratio has been used as a proxy for DOM MW in a broad range of bulk DOM samples, its application for algal-derived DOM has not been confirmed yet. The relationship between E2/E3 and DOM MW needs to be further investigated.

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 (Figure 2H, I). For example, in the 10% WE during stationary-death phase, tyrosine-like compounds sig-
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, 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 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 addition, Liu et al. 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 in the proportions of tyrosine-like compound in 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 reducing biotransformation activities. Proportions of soluble microbial byproduct-like compounds continuously increased throughout the experiment in the control and treatments, which could be due to compounds released from growing and lysed algal cells, such as carbohydrates, proteins, lipids, fatty acids, vitamins, and toxins. Similarly to our findings, using XAD-fractionation to characterize DOC, Gough et al. found that DOC during the algal bloom shifted toward to more hydrophilic and aliphatic characteristics with the release of extra- and intracellular organic matters.

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). Values and error bars represent the average and standard deviation of experimental triplicates (n = 3).

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 SI Figure S2. Concentration of each DBP species will be discussed later. In control, the specific C-DBP-FP revealed little changes during initial-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 initial-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., variations in specific THM- and HAA-FPs during M. aeruginosa growth from initial to death phase were also not obvious. In contrast to the control, specific C-DBP-FP significantly decreased during initial-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 (Figure 3C, E) exhibited opposite trends to the 10% BE and WE (Figure 3B, D). For example, specific C-DBP-FP slightly increased from 6.0 ± 0.0 to 7.0 ± 1.1 mmol/mol-C during initial-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. 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. 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. In the 10% BE and WE, decreases in specific C- and O-DBP-FP during initial-exponential phase might be related to changes in algal physiology, such as increases in membrane permeability, allowing release of small molecular weight non-DBP precursors into water solution. Bacteria-produced polysaccharides have been reported as C- and O-DBP precursors. 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. In the 65% BE and WE solutions, increases in specific C- and O-DBP-FP during initial-exponential phase could be due to cell lysis following release of those C- and O-DBP precursors. Gough et al. 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. Elevation of AOM was found related to increases in the propensity of DOM pool to form THMs and HAA*s in San Luis Reservoir, California.* Also, Jack et al. 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*. Moreover, depletion of nutrient could cause reduction in cellular nitrogenous component; and presence of fire-affected DOM may enhance uptake of those DBP precursors by *M. aeruginosa*, leading to continuous declines in the amount of N-DBP precursors in all treatments. Carbohydrate is the major component in extracellular AOM, 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. However, Pivokonsky et al. showed that aging of algal culture was accompanied by an increase in protein-related AOM. Some N-DBP precursors are proteins released from algae. In this study an increase in N-DBP precursors in the control was only observed during initial-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 the beginning of experiment (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 initial-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 beginning of experiment (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 (Figure 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 prefire 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 evidence 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 S5). 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 (SI Table S3). PC 1 showed high positive loadings for SUVA254, 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 NDMA precursors was 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 thunderstorm or spring snowmelt that would carry significant amount of ash-derived DOM into source water during ongoing HABs.

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. 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 on terrestrial DOM quality and quantity. 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.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b01541. Detailed materials and methods, as well as the additional figures and tables referenced in this study (PDF)

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**REFERENCES**


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


