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

Kuo-Pei Tsai*†‡ and Alex T. Chow†‡

1Department of Forestry and Environmental Conservation, Clemson University, Clemson, South Carolina 29634, United States
2Department of Environmental Engineering and Earth Sciences, Clemson University, Anderson, South Carolina 29625, United States

Supporting Information

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 SUVA254) 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. Postfire algal blooms can increase chlorine reactivity of fire-affected terrestrial DOM for DBP formation.

INTRODUCTION

Wildfire can affect streamwater 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 comcomitant 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., SUVA254 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.13 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. For example, Bruun et al. 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. 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. Previous studies showed that extent and rate of DOM biodegradation were closely related to DOM fluorescence characteristics. It is expected that biodegradation of TA-DOM would substantially alter DOM composition and characteristics and consequently affect chlorine reactivity in DBP formation.

Planktonic algae are ubiquitous microorganisms playing important roles in DOM cycles in freshwaters. 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. Both species also have been proven to be able to remove nutrients from a wide variety of wastewaters. Since bacterial C/N ratios are lower than those for phytoplankton, cyanobacteria may need more nitrogen per unit biomass than green algae. In addition, it has been demonstrated that the addition of biochar water extractable substances or humic acids has a promoting effect on algal growth. 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, 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. 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. 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. 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 ninth, 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 g of dried litter were put in an aluminum foil and placed in a preheated muffle furnace at 250 and 400 °C underoxic 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. 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 h using an orbital shaker at 250 rpm and remained another 24 h without shaking. Extracts were filtered using 0.45 μm poly(ether sulfone) membrane filters (Supor-450, Pall Galman 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. The composition of culture medium was reported in Supporting Information (SI Table S1). The algal culture conditions were followed by the previous study. Algal cultures were maintained 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. 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. 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 OD680 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. 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 poly(ether sulfone) 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 (SUVA254, HIX, E2/E3, FI, β/α, and EEM) and DBP (trihalomethanes, haloacetonitriles, chloral hydrate, and haloketones) formation, were published previously. Detailed descriptions are presented in the SI. 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 SI. 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 raw litter extracts with the cultural medium. The initial OD680 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. 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 poly(ether sulfone) membrane filters (Supor-450, Pall Gelman Science) for chemical analyses.

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microorganisms resulted in a decrease in DOC. 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. 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. 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. The increases in SUVA_{254} and HIX in this study could be due to the uptake of nonaromatic DOM such as carbohydrates by growing algae, and the biotic transformation of labile DOM to products containing more unsaturated carbon bonds such as polyunsaturated fatty acids. 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 nonaromatic compounds instead of humic substances.\textsuperscript{23} 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.\textsuperscript{24} 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.\textsuperscript{27,28} 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.\textsuperscript{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.\textsuperscript{44} 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 culture medium.\textsuperscript{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–C). 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–I). 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,D,G). 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,I).

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.\textsuperscript{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).\textsuperscript{50} In addition, Fellman et al.\textsuperscript{27,49} 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\textsubscript{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.\textsuperscript{50} The proportion of autochthonous and allochthonous DOM in the

![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.](Image)
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.31 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 fluorescent material or quenching of humic substances caused by organic molecules formed during degradation.52,54

In addition 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 Haines31 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-Cl2/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 and 250 °C-extracts, SCD exhibited no significant difference (1.26 ± 0.05 and 1.18 ± 0.10 mg-Cl2/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,B), suggesting that chlorine reactivity of DOM was closely related to the proportion of aromatic compound or humic substance. Beggs and Summers31 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 nonchlorine 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, nonaromatic 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 precursors58,59 and SHAN-FP for the organic matters produced from green algae and cyanobacteria are different. For example, Oliver60 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 basilensis. Yang et al.61 showed that the yield of dichloroacetoniitrile 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 (SI 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,60 and...
extracellular oxidation and hydrolysis of amino acids and proteins have been reported as common pathways as these compounds were assimilated by algae. 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 litter by growing algae. After the inoculations with algae, SHK-FP for 50 and 400 °C-extracts significantly increased (Figure 4E). The formations of halo ketones may originate from reactions between chlorine and compounds containing carbonyl functional groups. Increases in carbonyl groups during humification of fulvic acid as well as oxidation of primary amino groups by chlorine 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 (SI 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 and were observed after inoculations with algae (SI 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. 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. Beggs and summers found that aromaticity of pine leachates was a strong indicator of THM formation. 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 S). During water chlorination processes, studies demonstrated that bromine incorporation of DBPs was associated with DOM characteristics and bromide concentration in water. Decreases of SUVA254 and MW were observed for the 400 °C-extract after inoculations with algae (Figures 2A,C). 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 SUVA254 and low MW of DOM. 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, the increases in BIF could also be explained by the findings that hydrophilic fractions of DOM were generally more reactive with bromine than chlorine. Maes et al. found that exposure of the green alga *Desmodesmus subspicatus* 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, occurrences of wildfire and seasonal proliferation of algae as a result of global warming are more likely observed in spring and summer, 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. 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.

**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.
ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01578.

(1) Detailed descriptions of analyses of water quality, DOM characteristics, and DBPs; (2) Cultural medium water quality; (3) DBP-FP before and after inoculations with algae; (4) The composition of cultural medium; (5) Water quality before and after inoculations with algae; and (6) Correlation analyses of SDBP-FP, DBP-FP, and DOM spectroscopic indices (PDF)

AUTHOR INFORMATION

Corresponding Author
Phone: +1 843 546 1013; fax: +1 843 546 6296; e-mail: kuopei@post.harvard.edu.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was funded by Joint Fire Science Program (14-1-06-19 and 14-1-06-11) and USDA NIFA (SCN-2013-2784), under project number SC-1700489 Technical Contribution No. 6444 of the Clemson University Experiment Station. We thank Dr. Alexander M. Ruecker, Dr. Jun-Jian Wang, Jennifer E. Untener, and the editor and three reviewers for their review and comments.

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