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Warming enhances the stimulatory effect of algal exudates on dissolved organic carbon decomposition

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Abstract

1. The current paradigm in peatland ecology is that the organic matter inputs from plant photosynthesis (e.g. moss litter) exceed that of decomposition, tipping the metabolic balance in favour of carbon (C) storage. Here, we investigated an alternative hypothesis, whereby exudates released by microalgae can actually accelerate C losses from the surface waters of northern peatlands by stimulating dissolved organic C (DOC) decomposition in a warmer environment expected with climate change. To test this hypothesis, we evaluated the biodegradability of fen DOC in a factorial design with and without algal DOC in both ambient (15°C) and elevated (20°C) water temperatures during a laboratory bioassay.

2. When DOC sources were evaluated separately, decomposition rates were higher in treatments with algal DOC only than with fen DOC only, indicating that the quality of the organic matter influenced degradability. A mixture of substrates (½ algal DOC + ½ fen DOC) exceeded the expected level of biodegradation (i.e. the average of the individual substrate responses) by as much as 10%, and the magnitude of this effect increased to more than 15% with warming.

3. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), a proxy for aromatic content, was also significantly higher (i.e. more humic) in the mixture treatment than expected from $SUVA_{254}$ values in single substrate treatments.

4. Accelerated decomposition in the presence of algal DOC was coupled with an increase in bacterial biomass, demonstrating that enhanced metabolism was associated with a more abundant microbial community.

5. These results present an alternative energy pathway for heterotrophic consumers to breakdown organic matter in northern peatlands. Since decomposition in northern peatlands is often limited by the availability of labile organic matter, this mechanism could become increasingly important as a pathway for decomposition in the surface waters of northern peatlands where algae are expected to be more abundant in conditions associated with ongoing climate change.

KEYWORDS
algae, climate change, peatland, priming effect, temperature

1 | INTRODUCTION

The potential for increases in atmospheric carbon dioxide (CO_2) following the release of organic carbon (C) stored in northern peatlands is among the most concerning aspects of climate change. Although peatlands occupy just 10% of the ice-free surface of our planet, they store approximately one-third of the world's soil C stocks (Gorham, 1991). The fate of this C reservoir is uncertain as northern regions are warming rapidly in a changing climate (IPCC, 2013). Increasing ground temperatures at northern latitudes have already accelerated permafrost thaw (Grosse, Goetz, McGuire, Romanovsky, & Schuur, 2016), resulting in deeper seasonally thawed active layers and subsurface flow paths that redistribute C to surface waters as dissolved organic C (DOC; Abbott, Jones, Godsey, Larouche, & Bowden, 2015; Wickland et al., 2018). The degradability of this material in a warming climate has potential implications for aquatic CO_2 emissions to the atmosphere (Lapierre, Guillemette, Berggren, & del Giorgio, 2013). Consequently, a better understanding of how organic matter is processed in the surface waters of northern peatlands is important to understanding future C cycling associated with warming in northern ecosystems.

Although peatlands share many characteristics with terrestrial ecosystems, a large portion of peatland-rich landscapes throughout the boreal biome can be inundated with water (i.e. a saturated photic zone) during all or part the growing season (Glaser, 1999; Pelletier, Strachan, Garneau, & Roulet, 2014; Turner et al., 2016). Despite the broad distribution of open water areas, information regarding the aquatic components of northern peatlands is sparse, especially as they pertain to decomposition dynamics (i.e. the aquatic microbial loop). However, we know that wet and dry phases of northern peatlands operate differently because dry surfaces typically sequester CO_2 while wet surfaces (i.e. pools) act as a source of CO_2 to the atmosphere (Pelletier et al., 2014; Waddington & Roulet, 2000). This fundamental difference between wet and dry conditions in northern peatlands may be due in part to currently unknown drivers of heterotrophic metabolism during the wet phase, including the presence or absence of aquatic microautotrophs (i.e. algae), and their ability to stimulate the microbial loop in surface waters.

Microalgae can be abundant in the saturated photic zone of northern peatlands, and current levels of algal biofilm production may increase in conditions expected with ongoing climate change. At the Alaska Peatland Experiment, for example, long-term studies have shown that algae can contribute as much as 30% of above-ground biomass when water covers peat surface layers (Wyatt et al., 2012), and similar levels of algal production can persist in peatlands with both short and long-term hydroperiods throughout lowlands of interior Alaska (Rober, Wyatt, Stevenson, & Turetsky, 2014). Conditions associated with climate warming, including thicker active layers as well as warmer soil conditions (Schuur et al., 2015), are expected to accelerate nutrient mineralisation in northern aquatic ecosystems (Reyes & Lougheed, 2015; Wickland et al., 2018). Greater nutrient availability and warmer temperatures will probably elevate current levels of algal production on peat surface layers during periods of surface water inundation (DeColibus et al., 2017; Wyatt, Bange, Fitzgibbon, Bernot, & Rober, 2015). Although algal material is labile and not likely to contribute substantially to the structural formation of peat, algae have the potential to facilitate energy flow through the microbial loop in northern peatlands by influencing the metabolic activity of heterotrophic microorganisms in the surrounding environment (Wyatt et al., 2012).

Algae form complex biofilms on decaying plant matter and have strong associations with heterotrophic microorganisms on peat surface layers (Wyatt & Turetsky, 2015). Algae release a portion (between 1 and 99.9%) of photoassimilated C into the environment as exudates (Bertilsson & Jones, 2003). These compounds, which are typically of low molecular weight (e.g. simple carbohydrates), have been shown to serve as a source of energy for decomposers in the surrounding water column (Guillemette, Leigh McCallister, & del Giorgio, 2016; Wagner, Bengtsson, Findlay, Battin, & Ulseth, 2017; Wyatt et al., 2012). Since DOC in the surface waters of peatlands is often composed of a complex mixture of high molecular weight aromatic compounds that are resistant to decomposition (Hansen et al., 2016; Olefeldt, Turetsky, & Blodau, 2013), fresh organic matter from algal sources may produce hotspots of microbial activity (McClain et al., 2003), possibly facilitating the breakdown of more refractory compounds. This process, which is often referred to as a *priming* (Bengtsson, Attermeyer, & Catalán, 2018) or *synergistic* (Farjalla et al., 2009) effect, has not been studied with algae in northern peatlands, although previous studies have demonstrated that heterotrophic metabolism tends to increase with elevated algal production on peat surface layers (Wyatt & Turetsky, 2015).

The goal with this study was to determine if free exudates released during periods of elevated algal production interact with refractory fen organic matter to influence heterotrophic metabolism and organic matter reactivity (i.e. time frame of consumption) in the surface waters of northern peatlands. We were also interested in knowing if warming expected for the northern boreal region would influence the rate of organic matter biodegradation. To answer these questions, we evaluated the biodegradability of fen DOC and algal-derived DOC in a full factorial design under both ambient and warmed water temperatures during a laboratory bioassay. We hypothesised that a mixture of algal DOC and refractory fen DOC would enhance the ability for heterotrophic bacteria to breakdown DOC in the surface waters of northern peatlands and this synergistic effect would be enhanced by warming.

2 | METHODS

2.1 | Study area and experimental design

Water for the bioassay was collected from a fen peatland located within the floodplain of the Tanana River, 35 km southwest of Fairbanks, Alaska, USA (64°42N, 148°18W). The peatland has a plant community composed primarily of *Sphagnum* moss species with sparsely distributed vascular species (*Equisetum fluviatile*, *Potentilla palustris*, and *Eriophorum vaginatum*) that surround an area of open

water with a depth of approximately 15 cm and pH of 5.83 ± 0.37. Background surface water levels of nitrate (NO_3^-) and phosphate (PO_4^{3-}) were 53.2 ± 0.02 and 37.4 ± 0.01 µg/L, respectively. Surface water was poured through a 10-µm mesh plankton net into sterile polyethylene carboys and transported to the laboratory (within 1 hr of collection) where most of the solution was filtered through a 0.2-µm pore-size VacuCap® filter (Pall Life Sciences) into sterile flasks. A portion of the 10-µm-filtered fen water was not filtered through a 0.2-µm pore-size filter so that it could be used as a microbial inoculum. All fen water was kept dark at room temperature until the start of the bioassay (2 hr). Sample aliquots of filtered fen water were analysed for specific ultraviolet absorption at 254 nm (SUVA$_{254}$) and DOC concentration.

A concentrated solution of algal exudates was lyophilised prior to the start of the bioassay. Tufts of filamentous green algae growing associated with submerged senescent plant stems (i.e. metaphyton) were collected from an adjacent wetland in sterile Whirl-Pak® bags (Nasco) containing filtered fen water and transported to the laboratory in a dark cooler (within 1 hr of collection). In the laboratory, algal filaments were rinsed with filtered fen water to remove debris and incubated under constant light (500 µmol m^{-2} s^{-1} photosynthetically active radiation) in an open-top polyethylene container filled with sterile Milli-Q water and aerated vigorously to provide mixing and to facilitate gas exchange for 12 hr (Wyatt, Tellez, Woodke, Bidner, & Davison, 2014; Wyatt et al., 2012). We found in previous studies that this length of incubation time provides a solution of highly concentrated exudates while minimising the potential for the build-up of microbes during the incubation process (Wyatt et al., 2012). Following the leaching process, the concentrated algal exudate solution was filtered through a 0.2-µm pore-size VacuCap® filter into sterile flasks and lyophilised using a Labconco® freeze dryer (Labconco Corp.). Our experimental goal was to make treatments for relative comparisons of biodegradation rates between fen DOC and algae DOC. Therefore, we prepared a stock solution of algal DOC (1 hr prior to the start of the bioassay) with approximately the same

DOC concentration as the fen water (68.6 ± 0.18 mg/L DOC) by dissolving the lyophilised exudate powder in sterile Milli-Q water.

To set up the biodegradation experiment, we made three treatment solutions in 3 L sterile flasks for relative comparisons of degradation rates: (a) algae DOC only (ALGAE); (b) fen DOC only (FEN); and (c) a ½:½ mixture: ALGAE + FEN (MIXTURE). Each treatment solution was poured into sterile 60 ml biological oxygen demand (BOD) bottles, which served as incubation flasks for the biodegradation experiment. Six flasks (i.e. subsamples) of a single treatment solution were placed inside each of 24 jacketed beakers (Ace Glass Inc.) that circulated temperature-regulated water from one of two external Isotemp® water baths (Thermo Fisher Scientific), maintaining either ambient (15°C) or warmed (20°C) water temperatures (*n* = 4 for each treatment) for 192 hr (Figure 1). In this experimental design, a jacketed beaker represented one replicate and each flask represented a subsample that allowed us to evaluate degradation at each of six time intervals (0, 12, 24, 48, 96, and 192 hr) without disturbing any other time intervals.

Once the beakers were in place, we inoculated each flask with 1 ml of 10-µm-filtered peatland water to introduce a natural microbial community and initiate the degradation process. The low inoculum-to-sample ratio had no measurable effect on DOC concentrations within incubation flasks. At the beginning of the experiment, each flask was amended with nutrients, increasing background levels by 70 µM NO_3^- and 3 µM PO_4^{3-}, respectively, to relieve potential nutrient limitation of bacteria. Cultures were incubated in the dark and each flask was continuously aerated at low pressure to prevent anoxic conditions. We removed flasks at 0, 12, 24, 48, 96, and 192 hr for measures of biodegradable DOC (BDOC), SUVA$_{254}$, and bacterial biomass. We analysed filtered (0.2 µm) aliquots for DOC and calculated BDOC as the percent DOC mineralised or taken up at each time interval using the formula: [(DOC$_{initial}$ − DOC$_{final}$)/(DOC$_{initial}$)] (100), where DOC$_{initial}$ is the starting concentration and DOC$_{final}$ is the concentration at each time interval. Because filtering samples removes microbial biomass prior to measuring DOC, BDOC represents DOC loss due to both mineralisation and microbial uptake.

We calculated the expected values for each response variable in the mixture solution (i.e. MIXTURE) as the average of the individual measurements (ALGAE, FEN) with and without warming (MIXTURE$_{WARMING}$ and MIXTURE$_{AMBIENT}$, respectively). For instance, BDOC$_{expected}$ = (BDOC$_{ALGAE}$ + BDOC$_{FEN}$)/2, where BDOC$_{expected}$ is the expected BDOC in the MIXTURE treatment, BDOC$_{ALGAE}$ is the observed BDOC in the ALGAE treatment, and BDOC$_{FEN}$ is the observed BDOC in the FEN treatment. If the observed response in the MIXTURE treatment exceeded the calculated *expected* response, the substrates were considered to be acting synergistically (Farjalla et al., 2009).
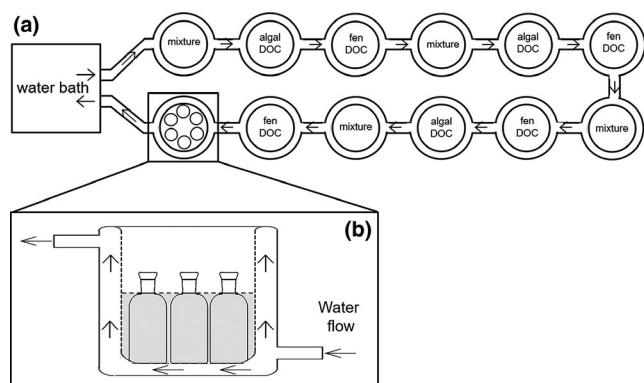

FIGURE 1 Schematic of biodegradation experimental design with a recirculating water bath plumbed to (a) 4-L jacketed beakers that circulated either ambient (15°C) or warmed (20°C) water around 60 ml biological oxygen demand bottles placed inside (b) jacketed beakers that contained either algal dissolved organic carbon (DOC) only, fen DOC only, or a ½:½ mixture of algal and fen

2.2 | Sampling and analytical procedures

We analysed filtered samples for DOC concentration with a Shimadzu TOC-V carbon analyser (Shimadzu Scientific Instruments) and for ultraviolet absorption at 254 nm with a Shimadzu UV-Mini

model 1240 spectrophotometer (Shimadzu Scientific Instruments). We calculated $SUVA_{254}$ by dividing ultraviolet absorption at 254 nm by DOC concentration and reported $SUVA_{254}$ in units of L mg C^{-1} m^{-1}. The $SUVA_{254}$ value gives an average molar absorptivity for all the molecules contributing to the DOC in a sample, and it has been shown to be a useful measure of DOC aromatic content (Weishaar et al., 2003) and molecular weight (Chowdhury, 2013).

Bacterial density was quantified by direct counts with epifluorescence microscopy. Unfiltered sample aliquots stained with 4′,6-diamino-2-phenylindole (DAPI; Porter & Feig, 1980) were vacuum-filtered through 0.2-μm pore-size black polycarbonate filters (Whatman Inc.) and bacteria were counted with a Leica DM 4000 microscope with fluorescence (Leica Microsystems). A minimum of 300 cells or 25 fields were counted per filter at 1,000× magnification. Bacterial biomass was calculated by converting bacterial abundance to biomass using a conversion factor of 35 fg C per cell (Theil-Nielsen & Søndergaard, 1998).

2.3 | Statistical methods

Differences in BDOC, $SUVA_{254}$, and bacterial biomass among ALGAE, FEN, and MIXTURE treatments over time and between ambient and warmed temperatures were analysed with three-way analysis of variance (ANOVA) tests. Time was included as an independent factor in ANOVAs owing to unequal variance among sampling events (Abbott, Larouche, Jones, Bowden, & Balser, 2014). When ANOVA indicated significant differences among treatments ($p < 0.05$), post hoc least significant differences were used to make pairwise comparisons among factor levels. Levels of BDOC, $SUVA_{254}$, and bacterial biomass in the MIXTURE treatment were compared to the calculated *expected* values using unpaired *t* tests. The magnitude of the effect of warming on BDOC, $SUVA_{254}$, and bacterial biomass was calculated at each time interval using the log response ratio (ln *R*). The log response ratio quantifies the proportionate change resulting from experimental manipulation and is calculated as the natural log (ln) of the treatment mean (i.e. warmed) divided by the control mean (i.e. ambient) or $\ln(X_{WARMED}/X_{AMBIENT}$; Hedges, Gurevitch, & Curtis, 1999). The direction of the effect size indicates whether warming had a positive or negative effect on the response variable. All statistical analyses were performed using SPSS 20 (SPSS).

3 | RESULTS

Overall initial ($t = 0$; mean ± *SE*; $n = 24$) DOC concentration (68.4 ± 0.11 mg/L) and bacterial biomass (0.01 ± 0.002 μg C/ml) were similar among all treatment combinations ($p ≥ 0.97$; Figure 2a,c). Initial ($t = 0$; mean ± *SE*; $n = 4$) $SUVA_{254}$ (L mg C^{-1} m^{-1}) was highest in the $FEN_{AMBIENT}$ treatment (3.91 ± 0.11) and lowest in the $ALGAE_{AMBIENT}$ treatment (1.90 ± 0.04). Initial $SUVA_{254}$ values in the $MIXTURE_{AMBIENT}$ treatment (2.84 ± 0.03) reflected the combination of individual substrates and was significantly lower than the $FEN_{AMBIENT}$ treatment ($p < 0.0001$), higher than the $ALGAE_{AMBIENT}$

treatment ($p < 0.0001$), and similar to the calculated *expected* value ($p = 0.26$; Figure 2b). Initial $SUVA_{254}$ values were similar between all ambient and warming treatments at $t = 0$ ($p ≥ .68$).

3.1 | Effects of individual substrates on biodegradation and heterotrophic activity

Organic matter quality influenced degradability (Table 1). Biodegradable DOC increased over time in the $ALGAE_{AMBIENT}$ treatment, reaching a maximum of 46% after 96 hr (Figure 2a). The elevated level of biodegradation in the $ALGAE_{AMBIENT}$ treatment was significantly greater than the $FEN_{AMBIENT}$ treatment ($p < 0.0001$), where BDOC remained below 2% during the assay (Figure 2a). Values of $SUVA_{254}$ increased with BDOC but remained significantly lower in the $ALGAE_{AMBIENT}$ treatment compared to the $FEN_{AMBIENT}$ treatment ($p < 0.0001$), where $SUVA_{254}$ values remained relatively constant over time (Figure 2b). Bacterial biomass reflected patterns of BDOC and was significantly greater in the $ALGAE_{AMBIENT}$ treatment compared to the $FEN_{AMBIENT}$ treatment throughout the assay ($p < 0.0001$; Figure 2c).

3.2 | Combined effects of substrates on patterns of biodegradation and heterotrophic activity

The mixture of substrates had a synergistic effect on organic matter composition and degradability (Table 1). Biodegradable DOC increased over time in the $MIXTURE_{AMBIENT}$ treatment and was significantly greater than the single-substrate treatments ($ALGAE_{AMBIENT}$ or $FEN_{AMBIENT}$) during the first 24 hr ($p ≤ 0.02$; Figure 2a). Although BDOC in the $ALGAE_{AMBIENT}$ treatment surpassed the $MIXTURE_{AMBIENT}$ treatment after 24 hr (Figure 2a), BDOC in the $MIXTURE_{AMBIENT}$ treatment continued to be greater than the calculated expected value throughout the duration of the assay ($p ≤ 0.006$; Figure 2a). Values of $SUVA_{254}$ in the $MIXTURE_{AMBIENT}$ treatment increased with BDOC and were significantly greater than expected after 48 hr ($p = 0.02$), but remained consistently lower than the $FEN_{AMBIENT}$ treatment ($p < 0.0001$) and higher than the $ALGAE_{AMBIENT}$ treatment during the assay ($p < 0.0001$; Figure 2b). Similar to patterns of BDOC, bacterial biomass in the $MIXTURE_{AMBIENT}$ treatment was greater than the best performing single culture (i.e. $ALGAE_{AMBIENT}$) during the first 24 hr ($p < 0.0001$; Figure 2c) and remained greater than the calculated expected value after 24 hr ($p ≤ 0.03$; Figure 2c).

3.3 | Effects of warming on biodegradation of individual and combined substrates

Warming had a stimulatory effect on organic matter degradability and the magnitude of the effect varied with substrate composition and time (Table 1 and Figure 3). Warming significantly elevated levels of BDOC, $SUVA_{254}$, and bacterial biomass compared to ambient temperatures in all treatments except for the $FEN_{WARMING}$ treatment (Table 1), where levels of each response variable were relatively
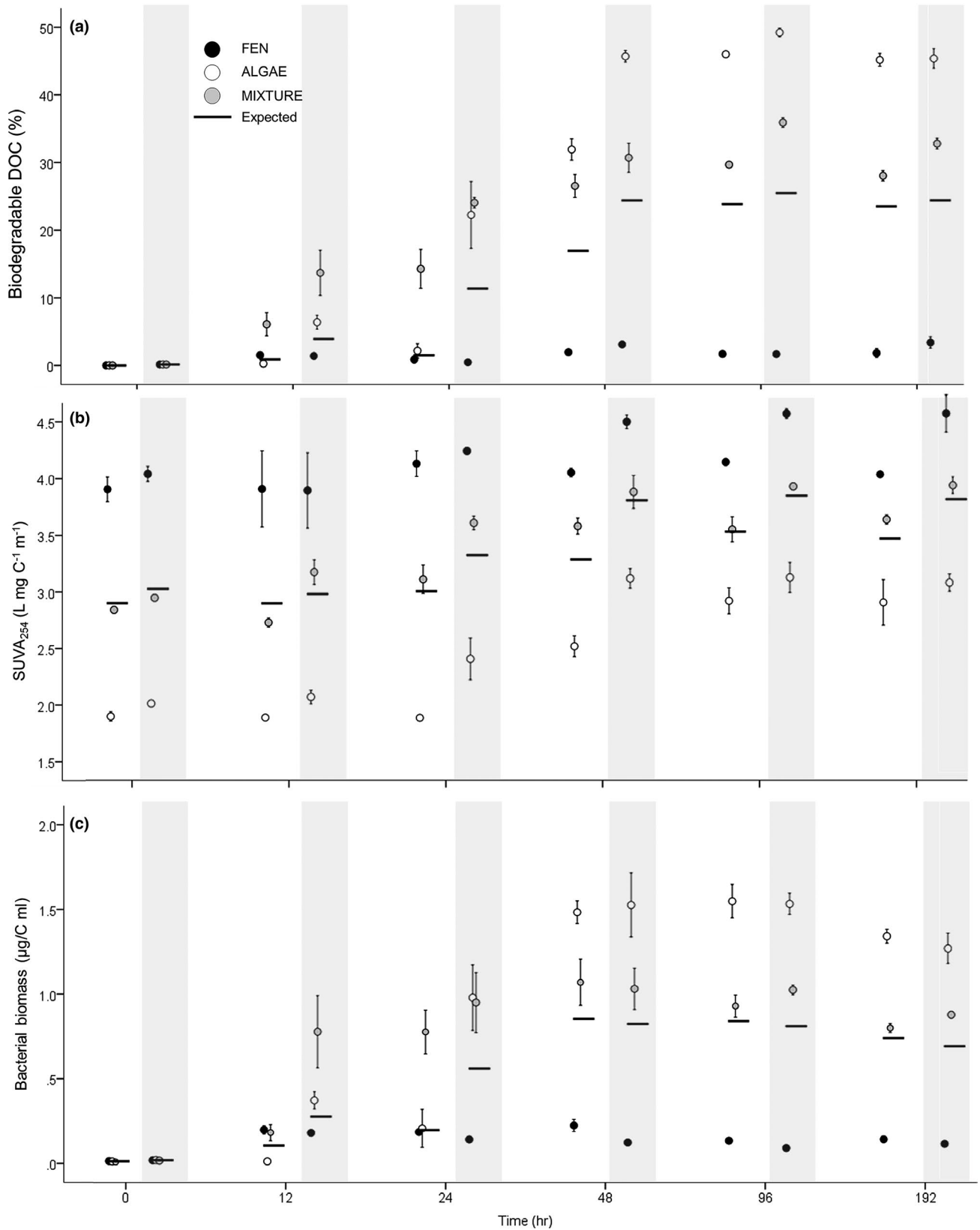
FIGURE 2 Comparison of (a) percent biodegradable dissolved organic carbon (DOC), (b) specific UV absorbance at 254 nm (SUVA$_{254}$), and (c) heterotrophic bacterial biomass among treatments with either algal DOC only (ALGAE), fen DOC only (FEN), or a ½:½ mixture: ALGAE + FEN (MIXTURE) in ambient and warmed (shaded portion) conditions during a 192-hr laboratory assay. Solid lines are the *expected* values for each parameter calculated as the average from the single substrate treatments (i.e. ALGAE, FEN). Points are mean ± *SE*, *n* = 4 for each treatment

TABLE 1 Results of three-way ANOVA to determine the independent and interactive effects of dissolved organic carbon (DOC) composition (i.e. treatments with fen DOC only, algal DOC only, or a 1:1 mixture of fen and algal DOC), warming, and time on biodegradable DOC (BDOC), specific UV absorbance at 254 nm (SUVA$_{254}$ L mg C^{-1} m^{-1}), and bacterial biomass (µg C/ml)

Effect	df	SS	F	p-value
BDOC				
Time	5	15,535.9	400.9	<0.0001
Treatment	2	13,665.7	881.5	<0.0001
Warming	1	618.3	79.8	<0.0001
Time × treatment	10	8,593.4	110.9	<0.0001
Time × warming	5	329.9	8.51	<0.0001
Treatment × warming	2	289.7	18.7	<0.0001
Treatment × warming × time	10	397.9	5.13	<0.0001
Error	105	806.9		
SUVA$_{254}$				
Time	5	14.2	53.3	<0.0001
Treatment	2	60.8	572.1	<0.0001
Warming	1	1.09	20.4	<0.0001
Time × treatment	10	2.47	4.64	<0.0001
Time × warming	5	0.29	1.10	0.367
Treatment × warming	2	0.10	0.94	0.394
Treatment × warming × time	10	0.68	1.27	0.255
Error	105	5.53		
Bacterial biomass				
Time	5	17.1	86.8	<0.0001
Treatment	2	17.2	218.1	<0.0001
Warming	1	1.74	44.1	<0.0001
Time × treatment	10	10.9	27.6	<0.0001
Time × warming	5	0.80	4.04	0.002
Treatment × warming	2	1.10	13.9	<0.0001
Treatment × warming × time	10	0.85	2.16	0.026
Error	105	4.15		

Note: Significance was determined at the $p < 0.05$ level.

constant over time (Figure 2). Biodegradable DOC and bacterial biomass were significantly greater in the MIXTURE$_{WARMING}$ treatment compared to the best performing single culture (i.e. ALAGE$_{WARMING}$) during the first 12 hr ($p \leq 0.001$) and remained greater than the calculated expected value thereafter ($p \leq 0.03$; Figure 2a,c). Values of SUVA$_{254}$ in the MIXTURE$_{WARMING}$ treatment were greater than expected based on calculations of each substrate (ALGAE$_{WARMING}$ or FEN$_{WARMING}$) throughout the assay ($p \leq 0.04$; Figure 2b). The magnitude of the effect of warming on each response variable was greatest during the first 48 hr of incubation, where, with exception of SUVA$_{254}$ values in the MIXTURE treatment at 12 hr, the effect

size was most elevated in the ALGAE treatment followed by the MIXTURE and FEN treatments (Figure 3).

4 | DISCUSSION

Across high latitudes, ecosystem and hydrology changes related to climate warming are expected to elevate currently low levels of algal production in aquatic ecosystems (DeColibus et al., 2017; Lougheed et al., 2015; Wyatt et al., 2015). Algae release a wide range of C substrates into the environment as a by-product of photosynthesis (Bertilsson & Jones, 2003), yet it is not clear how these exudates influence heterotrophic activity in the surface waters of northern peatlands. Using a factorial design with and without algal DOC and warming, we were able to evaluate how heterotrophic bacteria process a mixture of algal exudates and refractory fen organic matter as it occurs during periods of elevated algal production. The results of our study show that heterotrophic activity and organic matter biodegradability were greater in solutions containing a mixture of algal DOC and fen DOC than expected based on calculations of each substrate individually and these effects were significantly enhanced by warming. These findings move past simply showing that exudates from algal sources are an important energy source for decomposers and demonstrate how these secondary metabolites interact synergistically with refractory organic matter to influence heterotrophic metabolism and organic matter reactivity in northern peatlands.

When evaluated separately, algal DOC and fen DOC followed very different degradation dynamics, with the algal pool being more rapidly degraded compared to its terrestrial counterpart. The relative degree of exudate biodegradation was somewhat expected given that algal exudates have been shown to be an excellent source of energy for decomposers across a wide range of aquatic ecosystems (Guillemette et al., 2016; Wagner et al., 2017; Wyatt et al., 2012). In contrast to exudates, DOC from the surface waters of peatlands is typically composed of a complex mixture of high molecular weight compounds that are recalcitrant in nature (Hansen et al., 2016; Wickland, Neff, & Aiken, 2007). The rapid rate of short-term degradability of algal exudates is important to consider in the context of other studies as it provides insight as to why free exudates are often so difficult to detect in surface waters, even during periods of elevated algal production (Wyatt et al., 2012). Consequently, heterotrophic parameters (e.g. bacterial production) may be a more useful indicator of free exudates associated with elevated algal production in the surface waters of northern peatlands, a point that has been made following the investigation of algal-bacterial associations in other aquatic ecosystems (Søndergaard, Hansen, & Markager, 1995).

There was a constant synergistic effect of algal exudates and fen organic matter on heterotrophic activity and organic matter reactivity. Since the degree of organic matter reactivity in the mixture solution exceeded levels calculated from individual measurements, we can conclude that the substrates were interacting synergistically, a situation known as non-transgressive overyielding (Loreau, 1998). Accelerated decomposition in the presence of exudates was coupled
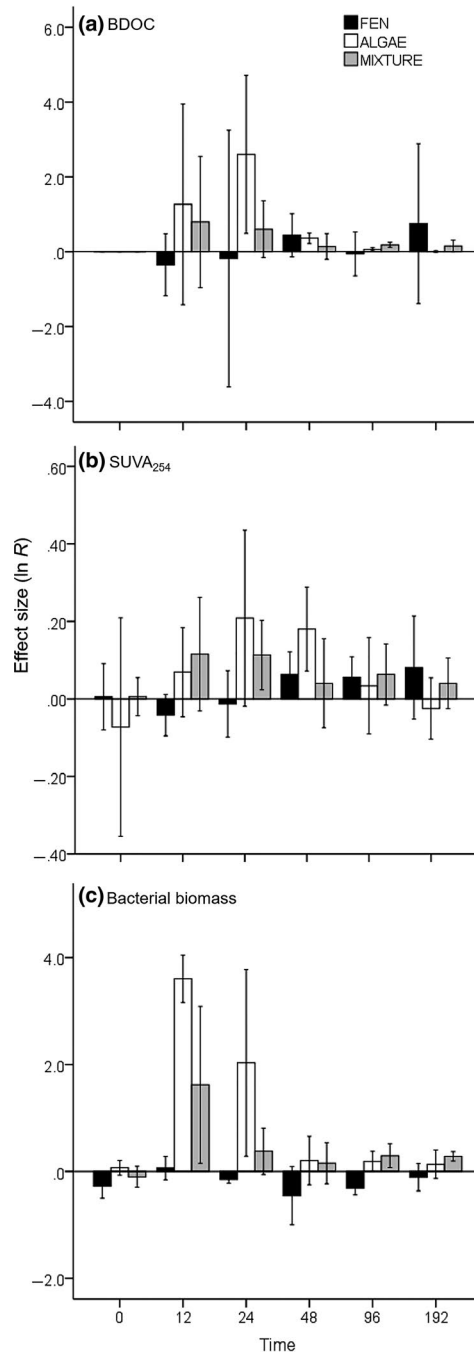
FIGURE 3 Comparison of effect sizes (log response ratio ± 95% confidence interval) of warming on (a) percent biodegradable dissolved organic carbon (BDOC), (b) specific UV absorbance at 254 nm (SUVA$_{254}$), and (c) heterotrophic bacterial biomass among treatments with either algal DOC only (ALGAE), fen DOC only (FEN), or a ½:½ mixture: ALGAE + FEN (MIXTURE)

with a higher than expected level of bacterial biomass, demonstrating that enhanced metabolism was associated with a more abundant microbial community. Evidence for a synergistic effect was particularly strong during the initial phase of the assay when biodegradation in the mixture solution was not only more elevated than the calculated expected value, but it was also significantly greater than the best performing single culture. This condition is referred

to as transgressive overyielding (Farjalla et al., 2009) and can be interpreted as evidence for complimentary metabolism between decomposers of labile organic matter and decomposers of recalcitrant organic matter (Caliman et al., 2007; Loreau, 1998). Complimentary metabolism often involves extracellular enzymatic degradation (Münster & De Haan, 1998) and is widely considered to be a mechanism for the so-called *priming effect*, where recalcitrant organic matter is mineralised following the addition of more labile sources (Guenet, Danger, Abbadie, & Lacroix, 2010). Although our study was not specifically designed to test for a priming effect, the accelerated level of organic matter reactivity observed in our study is similar to reports from temperate wetlands and rivers (Danger et al., 2013; Hotchkiss, Hall, Baker, Rosi-Marshall, & Tank, 2014; Kuehn, Francoeur, Findlay, & Neely, 2014) as well as the sediments of marine ecosystems (Bianchi, 2011), where organic matter can be rapidly decomposed following the addition of fresh substrates from algal sources. Other mechanisms may also explain the synergism in our study, including the potential for a mixture of substrates to support a more diverse microbial community with the capacity to breakdown aromatic compounds (Ward et al., 2018).

Compared to the number of studies that have examined the role of organic matter quality on metabolic activity in aquatic ecosystems, far fewer have examined how substrate quality influences the role of temperature on organic matter reactivity. When each substrate was evaluated individually, warming had a disproportionate effect on the reactivity of algal exudates compared to the recalcitrant fen organic matter. This interaction further exacerbated the discrepancy between fen organic matter and exudate biodegradability observed at ambient temperatures. Similarly, the relative importance of warming for synergistic activity in the mixture treatment was most apparent during the early stages of biodegradation and then declined over time as the most labile organic matter became more recalcitrant. This result compliments our knowledge of belowground processes (Dieleman, Lindo, McLaughlin, Craig, & Branfireun, 2016) and highlights the role of above-ground energy pathways for regulating the influence of temperature on heterotrophic activity in northern peatlands. Similar conclusions regarding the interactive effects of temperature and substrate quality have been made by other investigators (Jane & Rose, 2018), although warming has also been shown to favour the reactivity of refractory organic matter more often than bioavailable forms which tend to be highly reactive regardless of temperature (Ylla, Romaní, & Sabater, 2012).

Values of SUVA$_{254}$ reflected differences in organic matter composition among treatments and changed over time with biodegradation. Values of SUVA$_{254}$ typically range from 1.0 to 6.0 L mg C^{-1} m^{-1} in surface waters, with higher numbers indicative of more humic compounds with a larger molecular weight (Hansen et al., 2016). At the start of the assay, SUVA$_{254}$ values for the fen DOC were within the range of those reported from other peatlands, which tend to be made up of high molecular weight aromatic compounds (Hansen et al., 2016; Hribljan, Kane, Pypker, & Chimner, 2014; Olefeldt et al., 2013). Values of SUVA$_{254}$ for algal DOC were lower than fen DOC, but slightly higher than values reported for plant and algal leachates from other

studies, which are typically below 1.0 L mg C^{-1} m^{-1} (Hansen et al., 2016; Pellerin, Hernes, Saraceno, Spencer, & Bergamaschi, 2010). Discrepancies between initial $SUVA_{254}$ values for fen DOC and algal DOC were expected given that the low molecular weight compounds that make up exudates do not absorb at 254 nm (Hansen et al., 2016). The increase in $SUVA_{254}$ values over time in all treatments is consistent with organic matter becoming more humic with biodegradation. Similarly, other studies have shown that the reactive pool of organic matter becomes less reactive as aromatic compounds increase with microbial processing (i.e. Giroldo, Vieira, & Paulsen, 2003; Wickland et al., 2007; Wyatt et al., 2012). A greater percent increase in $SUVA_{254}$ values in the algal DOC treatment highlights the preferential removal of low molecular weight organic compounds and the production of high molecular weight material during biodegradation (Hansen et al., 2016; Moran, Sheldon, & Zepp, 2000; Pellerin et al., 2010). The higher than expected $SUVA_{254}$ values in the mixture solution indicate that the synergistic effects of biodegradability influence the chemical composition of the organic matter (Hansen et al., 2016), which could have implications for how organic matter reacts in the environment (Creed et al., 2018).

Inland waters, which were once considered to be passive conduits for the transport of organic C from terrestrial sources to the ocean, are now widely recognised to be metabolically active components of the global C cycle (Cole et al., 2007). Our results provide insight into how substrate composition and temperature interact to regulate organic matter cycling in the surface waters of northern peatlands. Exudates released by algae present a novel energy pathway for heterotrophic consumers to breakdown organic matter in northern peatlands and the importance of the algae energy pathway will probably be exacerbated by future warming. These findings represent a dramatic shift in the current paradigm in peatland ecology where primary producers act not only as agents of photosynthesis and C storage but also as promoters of decomposition. The net effects of the algae energy pathway on ecosystem C exchange are not entirely clear and need additional study. For example, accelerated decomposition in the presence of labile organic matter from algal sources may be offset by CO_2 uptake during periods of elevated algal photosynthesis, at least in the short term. Future studies should aim to link the reactivity of organic matter in the presence of algal exudates to CO_2 emissions from surface waters during periods of elevated algal production. Doing so would help to better understand linkages between climate change and C flux from northern aquatic ecosystems.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (Kevin Wyatt; khwyatt@bsu.edu) upon request.

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