Freshwater Biology

Freshwater Biology (2016) **61,** 1442–1453 doi:10.1111/fwb.12783

Light availability regulates the response of algae and heterotrophic bacteria to elevated nutrient levels and warming in a northern boreal peatland

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SUMMARY

1. Light attenuation associated with elevated levels of dissolved organic matter (i.e. browning) is likely to affect the response of benthic biofilms to nutrient enrichment and warmer temperatures expected for northern aquatic ecosystems with climate change. To examine how these factors will interact to regulate the association between algae and heterotrophic bacteria in northern peatlands, we manipulated light (100%, 60%, 30%, 20% and 5% of ambient), nutrients (unenriched, enriched) and temperature (ambient, warming), in a full factorial design by shading nutrient diffusing substrates inside mesocosms with or without warming (3 °C) in an Alaskan fen.
2. After 12 days of experimental conditions, there was no effect of light or temperature on the abundance of algae or bacteria in the absence of nutrient enrichment. Enrichment with a combination of nitrogen and phosphorus significantly increased the abundance of algae and heterotrophic bacteria at ambient light levels (418 μmol m^{-2} s^{-1}), and warming significantly enhanced the positive effects of nutrients on algal accrual. Although warming enhanced the effects of nutrient enrichment on bacterial growth, the magnitude of the effect was not statistically significant compared to nutrient enrichment alone. The positive influence of nutrient enrichment and the synergistic effects of nutrients and warming on algae and bacteria were lost at ≤30% ambient light (below 125 μmol m^{-2} s^{-1}).
3. We conducted a separate laboratory incubation using recirculating water baths maintained at either ambient or elevated water temperature (warming of 5 °C) to evaluate the effects of warming on the use of algal exudates by heterotrophic bacteria. During the 16-day assay, the abundance of heterotrophic bacteria increased rapidly in the presence of a common solution of algal exudates and the rate of exudate assimilation by bacteria was significantly enhanced by warming compared with ambient temperature.
4. The positive association between light availability and heterotrophic bacteria, coupled with accelerated bacterial growth in the presence of algal exudates, support bacterial dependence on algal subsidies in the presence of recalcitrant organic substrates in this northern peatland. Within a predictive context, our results demonstrate that greater light attenuation associated with browning of surface water may reduce the positive influence of warmer temperatures and elevated nutrient levels on the microbial loop by reducing algal production on submerged surfaces in northern peatlands.

Keywords: algal-bacterial interactions, climate change, decomposition, dissolved organic matter, light attenuation, nutrients, warming

Introduction

In the past few decades, high-latitude regions have experienced greater warming than any other region of the planet, and this phenomenon is predicted to continue throughout this century (Collins *et al.*, 2013). The direct effects of warmer temperatures (e.g. permafrost degradation) as well as the more indirect effects of

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broader climate change (e.g. increased occurrence of drying and flooding cycles associated with changes in the frequency and magnitude of precipitation events), are expected to elevate currently low nutrient levels in northern aquatic ecosystems (Wyatt *et al.*, 2012; Reyes & Lougheed, 2015). The forecast for primary production in northern aquatic ecosystems is not yet clear because elevated nutrient levels are expected to coincide with an overall increase in dissolved organic matter (DOM) in surface waters (Frey & Smith, 2005; Pagano, Bida & Kenny, 2014). This phenomenon, recently described in the literature as 'browning' (Roulet & Moore, 2006; Solomon *et al.*, 2015), is associated with humic substances that can increase light attenuation and alter the quality of light available in the water column (Karlsson *et al.*, 2009). Although elevated nutrient availability and warmer temperatures are expected to promote primary production in northern aquatic ecosystems, greater light attenuation associated with browning may overwhelm the stimulatory influences of nutrients and warming by constraining photosynthesis. The effects of browning may be particularly acute in northern wetlands where much of the primary production occurs on submerged surfaces (Rober *et al.*, 2014) and the effects of darker water may be confounded by greater water depth resulting from accelerated spring snowmelt and surface runoff in low-lying areas (Osterkamp *et al.*, 2000).

Peatlands are a dominant wetland ecosystem within the northern boreal landscape and serve as a global carbon (C) reservoir (Yu, 2012). Although northern peatlands share many characteristics with terrestrial environments, they are often inundated with water (i.e. have a saturated photic zone) for substantial periods of time during the growing season (Glaser, 1998). Shallow open-water pools are a common feature of northern peatlands, covering nearly half of the peatland surface area across the boreal landscape (Roulet *et al.*, 1994; Botch *et al.*, 1995). Despite their broad distribution, information regarding the open-water areas of northern peatlands is sparse, and our current understanding of peatland ecology is derived mainly from studies focusing on submerged and emergent macrophytes and associated detrital pathways of energy transfer to heterotrophic decomposers. Currently, there is relatively little information available on the ecology of algae, including their associations with heterotrophic microorganisms, in northern peatlands. Consequently, it is difficult to adequately evaluate how the aquatic microbial loop within these ecosystems will be altered by environmental disturbance, including processes associated with ongoing climate change.

Microalgae are primary producers in northern wetlands and may be an important energy source for heterotrophic microorganisms in peatlands. Benthic algae (i.e. those attached to surfaces), in particular, can be important primary producers in shallow northern wetlands where sunlight reaches submerged surfaces (Robinson, Gurney & Goldsborough, 2000). Algae release a portion of their photosynthetic products into the surrounding water (Rodríguez *et al.*, 2013). These exudates are often comprised of simple carbohydrates and amino acids (Biddanda & Benner, 1997) and provide energy to heterotrophic microorganisms within the benthic biofilm (Kuehn *et al.*, 2014; Wyatt & Turetsky, 2015). Although algae typically make up a relatively small portion of total above-ground biomass in boreal wetlands (Rober *et al.*, 2014), they have the potential to support a disproportional amount of heterotrophic biomass owing to their rapid turnover rates (e.g. McIntire & Phinney, 1965). Consequently, algae may become increasingly important for heterotrophic metabolism in northern peatlands, where they are expected to become more abundant in conditions associated with climate change and heterotrophic microorganisms are often limited by the availability of labile substrates (Wyatt *et al.*, 2012).

The goal of this study was to examine the independent and interactive effects of warming, nutrient enrichment and light availability on the association between microalgae and heterotrophic bacteria in an Alaskan boreal fen. In previous studies, we demonstrated that warmer temperatures and elevated nutrient levels promote algal and heterotrophic metabolism in shallow Alaskan wetlands and that the presence of heterotrophic bacteria is associated with the release of C exudates by algae during photosynthesis (Wyatt & Turetsky, 2015). In this study, we hypothesised that if light limitation inhibits algal growth in the presence of warming and increased nutrient availability, then bacterial abundance would be indirectly influenced given their dependence on algal exudates. We were also interested in evaluating how a warmer environment would influence bacterial degradation of algal exudates. Therefore, we evaluated degradation of algal exudates by heterotrophic bacteria in both ambient and warming conditions. We predicted that enhanced bacterial growth associated with a warmer climate would accelerate bacterial degradation of algal exudates.

Methods

This study was conducted in the open-water area of a fen peatland located within the Tanana River floodplain, near

the Bonanza Creek Experimental Forest, 35 km southwest of Fairbanks, AK, U.S.A. (64°42N, 148°18W). This region experiences a short growing season (≤135 days) with >21 h day^{-1} sunlight in June and a mean annual precipitation of 269 mm (Hinzman *et al.*, 2006). The peat layer is >1 m in depth within the fen and topography is consistently flat across the site (Rober *et al.*, 2014). The fen lacks trees and is dominated by *Sphagnum* (*S. obtusum, S. platyphyllum*) and vascular plants (*Equisetum, Carex utriculata* and *Potentilla palustris*). Water depth (16.3 ± 1.17 cm) and dissolved organic carbon (DOC) concentration (28.3 ± 0.4 mg L^{-1}) at the fen study site were within the range of other open-water areas of peatlands within the floodplain (Fig. 1). A complete description of physical and chemical conditions within the fen is available in Table 1.

We manipulated the nutrients (enriched or unenriched), temperature (warmed or unwarmed) and light availability (100%, 60%, 30%, 20% or 5% of available light) by shading nutrient-diffusing substrates (NDS) with solar screening and placing the NDS inside mesocosms with or without warming (n = 4 for each treatment combination). Before experimental setup, a raised boardwalk was constructed to avoid disturbing natural wetland conditions during sampling. The experiment was conducted for 12 days beginning 30 May 2014 for colonisation of algae and heterotrophic bacteria on NDS. We assumed that this length of colonisation would allow us to observe the response to nutrient inputs similar to what has been observed following the spring thaw in previous studies within the fen complex (Wyatt *et al.*, 2012), while minimising container effects associated with mesocosm enclosures.

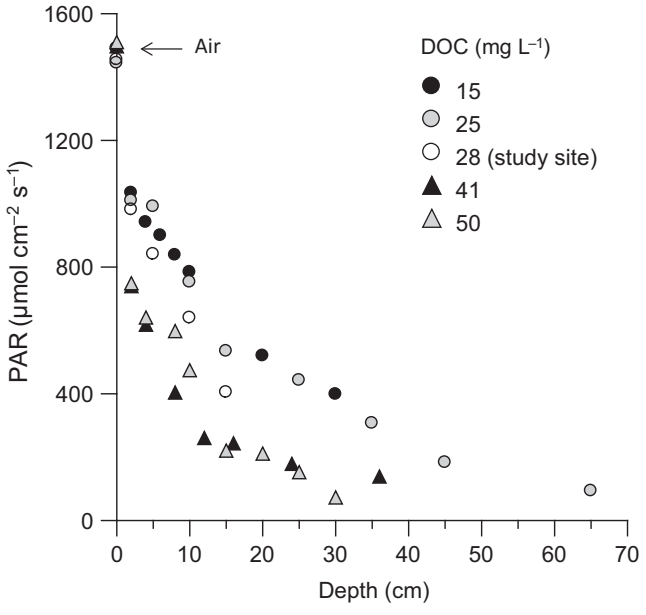

Fig. 1 Light attenuation with water depth in five peatlands with varying concentrations of dissolved organic carbon (DOC) within the Tanana River Floodplain.

Mesocosms (16 total; 25 cm in diameter) were constructed by rolling welded wire mesh into a cylinder and then wrapping each cylinder with a layer of 0.1 mm thick transparent polyvinylidene film (ShurTech, Avon) that transmitted 90% photosynthetically active radiation (PAR) and 80–90% of UV (Rober, Wyatt & Stevenson, 2011; Rober, Stevenson & Wyatt, 2015) (Fig. 2). Mesocosms were evenly spaced throughout an open-water area of the fen (approximately 15 m × 15 m) and the open-bottom of each mesocosm was pushed approximately 10 cm into the peat. Unwarmed mesocosms were trimmed so that 5 cm extended above the water surface. Warmed mesocosms extended approximately 65 cm above the water surface, and were warmed passively (i.e. protection from wind, greenhouse effect) by approximately 3 °C compared to unwarmed mesocosms, which maintained ambient temperatures over 12 days (Fig. 3). To evaluate the potential for side effects from the presence of polyvinylidene film, we also employed a set of mesocosms without polyvinylidene film as a control (open wetland treatment).

Nutrient-diffusing substrates were constructed from polyethylene canisters (5 cm in diameter, 35 mL volume) containing agar enriched with both nitrogen (N) and phosphorus (P) (+NP, enriched) or without nutrients (un-enriched) (Tank, Bernot & Rosi-Marshall, 2007). Because N and P have been shown to co-limit algal growth within the larger wetland complex (Wyatt *et al.*, 2015), both nutrients were supplied as 0.5 mol L^{-1} (N: 50.6 g L^{-1} KNO_3 and P: 68 g L^{-1} KH_2PO_4) (Fairchild, Lowe & Richardson, 1985). These nutrient concentrations have been shown to alleviate nutrient limitation for benthic algae (Borchardt, 1996), and thus were expected to eliminate nutrient limitation and maximise algal growth in the absence of secondary limiting factors. We previously demonstrated that NDS release N and P continuously for 24 days, simulating increased nutrient availability in the water column following spring thaw (Wyatt *et al.*, 2015). A circular piece (2.5 cm in diameter) of the lid of each NDS canister was removed and an organic cellulose sponge (2 mm thick, free of anti-microbial compounds) was placed under the opening and above the agar surface to serve as a substrate for algal and bacterial colonisation (Wyatt *et al.*, 2015).

Five NDS of a single nutrient treatment (enriched or unenriched) were placed inside each mesocosm, with

Table 1 Mean (±SE; $n = 4$) of water temperature (°C), photosynthetically active radiation (PAR; μmol photons m^{-2} s^{-1}), nitrate (NO_3; μg L^{-1}), phosphate (PO_4^3; μg L^{-1}), dissolved oxygen (DO; mg L^{-1}), pH and water depth (cm) among treatments and the open wetland. Treatments with different superscript letters are significantly different ($P < 0.05$).

		Unwarmed		Warmed	
Variable	Open wetland	Unenriched	Enriched	Unenriched	Enriched
Water temperature	14.6 (0.15)[a]	15.3 (0.12)[a]	15.3 (0.13)[a]	18.2 (0.13)[b]	18.2 (0.17)[b]
PAR	418.3 (13.7)[a]	409.0 (12.5)[a]	413.0 (11.5)[a]	422.1 (9.13)[a]	426.4 (11.4)[a]
NO_3	52.3 (6.36)[a]	31.4 (4.47)[a]	630.6 (12.5)[b]	34.2 (5.81)[a]	674.9 (51.6)[b]
PO_4^3	7.06 (1.64)[a]	14.4 (3.22)[a]	1104.2 (59.5)[b]	7.80 (2.37)[a]	1218.7 (66.3)[b]
DO	8.50 (1.07)[a]	8.01 (1.10)[a]	7.61 (0.75)[a]	6.68 (0.24)[a]	9.50 (1.18)[a]
pH	6.13 (0.13)[a]	6.31 (0.20)[a]	6.12 (0.12)[a]	6.44 (0.12)[a]	6.44 (0.04)[a]
Water depth	17.3 (1.65)[a]	13.5 (0.65)[a]	17.5 (0.96)[a]	15.5 (1.32)[a]	17.8 (1.25)[a]

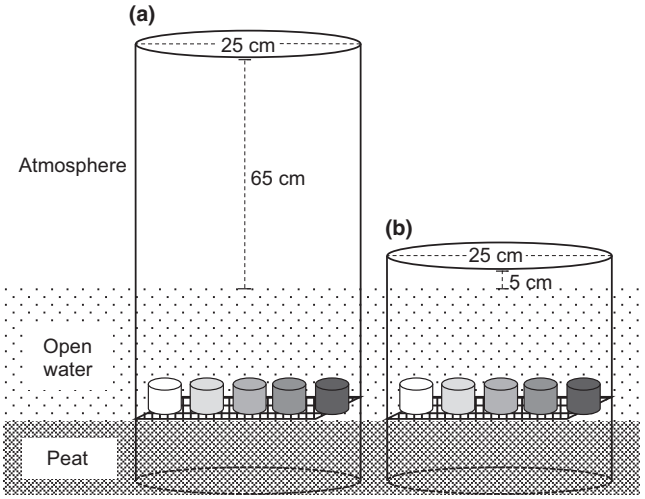

Fig. 2 Schematic of experimental setup in the peatland. Tall (a) and short (b) mesocosms experienced passive warming of 3 °C or ambient temperatures respectively. In each mesocosm, a set of either nutrient enriched or unenriched nutrient diffusing substrates (NDS) were anchored to a wire mesh platform and submerged to the peat surface. Each NDS canister in the set was subject to a different light treatment: 100%, 60%, 30%, 20% or 5% ambient light availability; shading is representative of different light treatments.

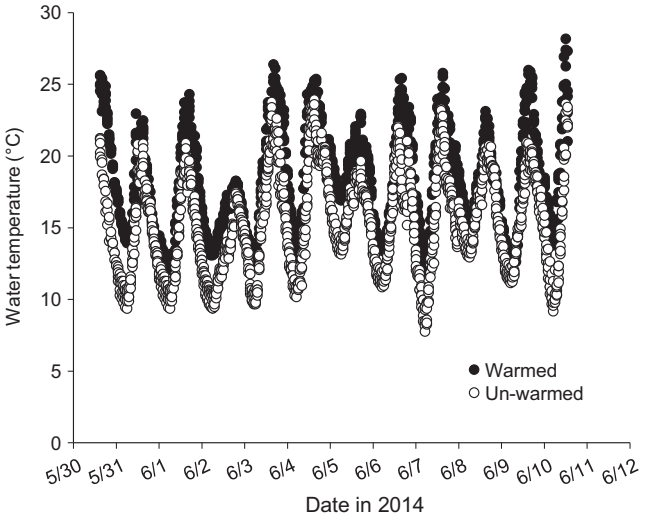

Fig. 3 Daily variation in water temperature ($n = 3$) in warmed and unwarmed treatments during the 12-day field mesocosm experiment.

each NDS canister representing a different light treatment (Fig. 2). Dark solar screening (New York Wire, Mt. Wolf) was loosely wrapped around each NDS canister and attached with zip-ties, forming a canopy approximately 4 cm above each canister in layers to allow for water movement and permit either 60%, 30%, 20%, or 5% ambient light, and no screening served as a control (100% ambient light). NDS were attached to a piece of angle-iron and submerged 4 cm above the peat surface inside each mesocosm.

Water temperature (°C) and light (measured as Lux and converted to μmol photons m^{-2} s^{-1} PAR according to the manufactures specifications) were measured at hourly intervals at a depth of 15 cm below the water surface using HOBO Pendant TEMP/Light data loggers (model # UA-002-64; Onset Computer Corporation, Bourne). After 12 days, a single water sample was collected from 5 cm below the water surface inside each mesocosm with a syringe and then filtered through a 0.45-μm pore-size filter into 60 mL sterile polyethylene bottles for measures of dissolved nutrients. Filtered samples were frozen and later analysed for NO_3^- and PO_4^{3-} using a Dionex ICS-3000 ion chromatograph (Dionex Corporation, Sunnyvale). Dissolved oxygen (DO) and pH were measured using a calibrated Hach model 40d multiprobe (Hach Company, Loveland), and water depth inside mesocosms was measured with a meter stick.

After 12 days of colonisation, sponges (80 total) were removed from NDS for analysis of algal chlorophyll *a* (chl *a*) concentration, algal cell density and heterotrophic

bacterial density. Each sponge was carefully removed with forceps and then cut in half with scissors in the field. One-half of each sponge was placed into a 20 mL sterile centrifuge tube with 90% ethanol and transported (within approximately 1 h) to the lab in a dark cooler. Sponges were then incubated in the dark at 4 °C for 24 h and analysed for chl *a* with a Shimadzu UV-Mini model 1240 spectrophotometer (Shimadzu Scientific Instruments, Columbia) after correction for phaeophytin (APHA, 1998). The other half of each sponge was preserved in the field with 2% buffered formalin in a sterile 20 mL glass scintillation vial for analysis of algal cell density and bacterial abundance. Algal and bacterial cells were removed from sponges by brushing. Algal cell density was determined by counting ≥300 natural units per sample at 400× magnification using a Palmer-Maloney nanoplankton counting chamber (Charles, Knowles & Davis, 2002). Algal abundance (cells cm^{-2} of substrate) was determined using the equation provided by Lowe & LaLiberte (2007). An aliquot of each sample was stained with 4′,6-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980) and vacuum-filtered onto a 0.2-μm pore-size black filter (OSMONIC Inc., Livermore) to enumerate bacterial cells. Bacterial abundance was quantified by counting a minimum of 300 bacterial cells or 25 fields per filter at 1000× magnification using a Leica DM 4000 microscope with fluorescence (Leica Microsystems, Wetzlar).

In an effort to extrapolate our results to other peatlands within the region, we evaluated the interactions between bathymetry (i.e. water depth) and DOM concentration on light attenuation at five adjacent peatlands (Fig. 1) that captured the range of DOM concentrations and water depths of peatlands within the floodplain (Rober *et al.*, 2014). Within each peatland, we measured light attenuation as PAR beginning just above the water surface (air) and then at 5 cm intervals below the water surface until the bottom was reached using a LI-COR submersible quantum sensor and LI-250 light meter (LI-COR, Lincoln, NE) attached to a meter stick. We used the slope of the linear regression of the natural logarithm of PAR and depth to calculate the vertical light-extinction coefficient (k_d) for each peatland (Wetzel & Likens, 2000). We filtered a water sample from each wetland site for analysis of DOC through a 0.45-μm Millex®-HA syringe-driven filter unit (Millipore Corporation, Bedford) into 120 mL sterile polyethylene bottles. Samples were acidified in the field and stored on ice until returning to the laboratory, where samples were analysed for DOC using a Shimadzu TOC-V carbon analyzer (Shimadzu Scientific Instruments, Columbia).

Laboratory incubation

We conducted a separate laboratory incubation in the dark to evaluate the effects of warming on the use of algal exudates by heterotrophic bacteria. Filamentous algal material that was loosely associated with plant material (i.e. metaphyton) was carefully removed with a syringe and then transferred to the laboratory in a dark cooler within 1 h of collection (Wyatt *et al.*, 2012). Algal exudates were collected by incubating algal material in a 2000 mL flask with 0.2-μm filtered fen water in ambient sunlight (PAR approximately 1200 $\mu mol\ m^{-2}\ s^{-1}$) for 12 h (Wyatt *et al.*, 2014). After the incubation period, we filtered exudates through a 0.2-μm filter (VacuCap; Pall Life Sciences, Ann Arbor) and then poured the homogenised solution of algal exudates into six 300 mL acid-washed flasks. A 10 mL sample of unfiltered fen water was added to each flask as a bacterial inoculum (Wyatt *et al.*, 2012). Each flask was then placed inside a jacketed beaker (Ace Glass Inc., Vineland, NJ) that circulated temperature-regulated water around each flask from one of two external water baths, maintaining either ambient (15 °C) or warming (20 °C) water temperatures ($n = 3$ for each treatment) for 16 days (Fig. S1). The flasks were wrapped with aluminium foil to exclude light and oxygenated with air stones during the incubation period. Each flask was brought to a final concentration of 5 μM P and 50 μM N by adding KH_2PO_4 and KNO_3, respectively, to prevent nutrient limitation of bacteria (Fagerbakke, Heldal & Norland, 1996). Our goal was to evaluate the effect of temperature on bacterial degradation of algal exudates without the confounding variable of nutrient limitation of bacterial growth. We sampled flasks at 0, 2, 4, 8 and 16 days for measures of DOC concentration and bacterial abundance. Subsamples for bacterial abundance were fixed with formaldehyde and subject to the same procedures as described above for determining bacterial density. Samples were analysed for DOC as described above.

Statistical analyses

Differences in mean water temperature, PAR, NO_3, PO_4^3, DO, pH and water depth among treatments were evaluated with analysis of variance (ANOVA) models. A nested ANOVA was used to evaluate the effects of temperature, nutrients and light availability on algal chl *a*, algal cell density and heterotrophic bacterial abundance. When ANOVA indicated significant differences among treatments, Tukey's *post hoc* comparison of means tests were used to discriminate between different factor

levels. Linear regression analysis was used to evaluate the relationship between algal cell density and bacterial cell density across all treatments and to evaluate the influence of DOC on light attenuation among peatlands that varied in DOC concentration and water depth. Differences in DOC degradation and bacterial abundance between temperature treatments during the degradation experiment were analysed using a two-way general linear model and Tukey's comparison of means tests that included the effects of temperature, incubation time and their interaction. The distributions of variables were log (x + 1)-transformed as necessary to correct for non-normal distribution and unequal variances among treatments prior to analysis. Statistical analyses were performed using IBM SPSS Statistics (Version 20; IBM Corporation, Armonk).

Results

Mesocosm experiment

Experimental manipulation of water temperature and nutrients successfully altered environmental conditions within mesocosms. Water temperature varied across all treatments (Fig. 3), but was significantly elevated (by approximately 3 °C) in the warmed treatments compared to the unwarmed treatments ($F_{3,2865}$ = 157.8, $P \leq 0.0001$; Table 1) and water temperature was similar between unwarmed treatments and the open wetland (P = 0.29). Nutrient concentrations were significantly greater in enriched treatments compared to unenriched treatments (NO_3: $F_{4,20}$ = 194.47; PO_4^3: $F_{4,20}$ = 251.22, $P < 0.0001$; Table 1). Nutrient concentrations were higher in the open wetland compared to unenriched treatments, but differences were not statistically significant (P = 0.15; Table 1). Mean DO, pH, water depth and PAR (ambient light levels reaching the peat surface) were similar among treatments and between treatments and the open wetland ($P > 0.05$; Table 1).

Temperature, nutrients and light had a significant interactive effect on benthic algal chl *a* and cell density. After 12 days of experimental conditions, there were no main effects of light or temperature on the abundance of algae in the absence of nutrient enrichment ($P \geq 0.54$). At ambient light levels (418 μmol m^{-2} s^{-1}), nutrient enrichment significantly elevated algal chl *a* and cell density compared to unenriched treatments (chl *a*: $F_{1,60}$ = 111.1, $P \leq 0.0001$; cell density: $F_{1,60}$ = 136.3, $P \leq 0.0001$; Fig. 4a, b), and the positive effects of nutrients were enhanced by warming (chl *a*: $F_{1,60}$ = 18.5, $P \leq 0.0001$; cell density: $F_{1,60}$ = 21.5, $P \leq 0.0001$; Fig. 4a, b). The synergistic effects

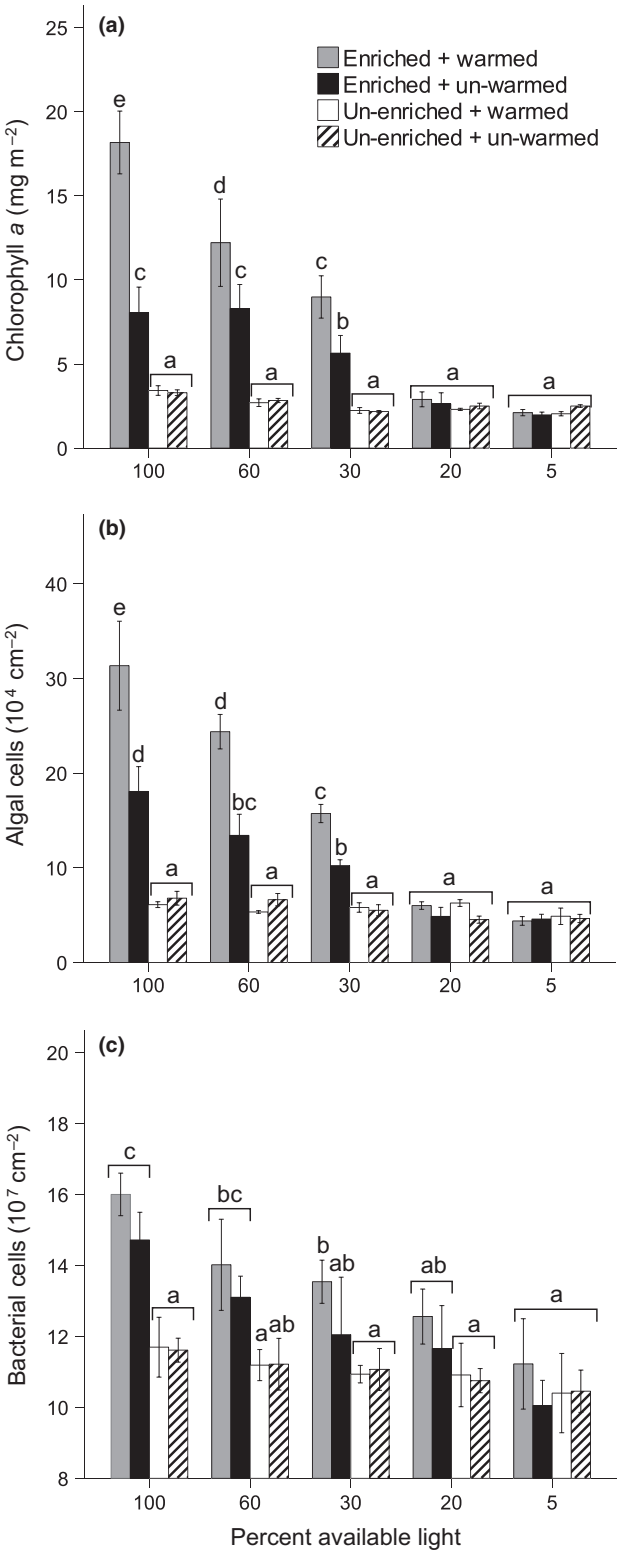

Fig. 4 Benthic algal chlorophyll *a* (a), algal cell density (b) and bacterial cell density (c) in warmed or unwarmed treatments with nitrogen and phosphorus (enriched) or without (unenriched) in the presence of different levels of ambient light (100%, 60%, 30%, 20% and 5%). Bars are the mean of four replicates ± 1 SE. Significant differences are indicated by different letters ($P < 0.05$).

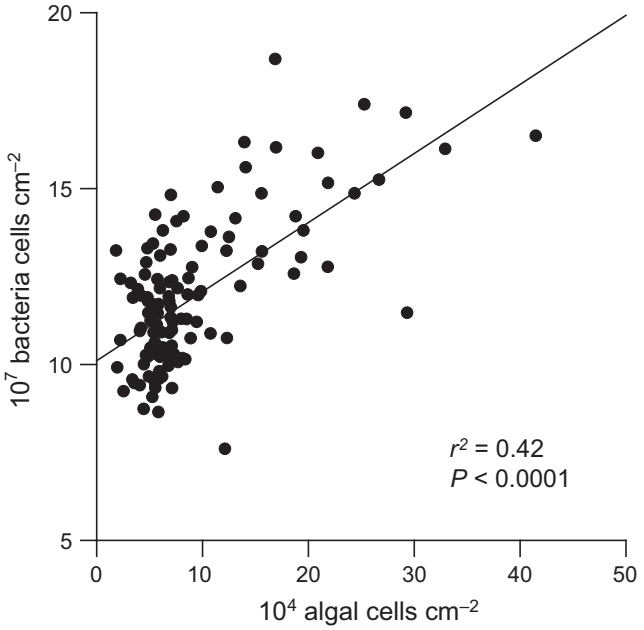

Fig. 5 Relationship between algal cell density and bacterial cell density across all treatments after 12 days of experimental manipulation.

of nutrients and warming were gradually reduced by light attenuation (Fig. 4a, b), and algal chl *a* and abundance were significantly reduced at ≤30% of ambient light (chl *a*: $F_{8,60} = 4.52$, $P \leq 0.0001$; cell density: $F_{8,60} = 4.22$, $P \leq 0.0001$).

Temperature, nutrients and light had a similar effect on bacterial cell density as they had on the algae. There were no main effects of temperature or light availability on bacterial cell density in the absence of nutrient enrichment ($P > 0.05$), but the abundance of bacteria was significantly elevated by nutrient enrichment at ambient light levels ($F_{1,60} = 23.8$, $P \leq 0.0001$; Fig. 4c). Although bacterial cell density was further elevated by warming in the presence of nutrient enrichment (Fig. 4c), the magnitude of the effect was not statistically significant compared to nutrient enrichment alone ($P = 0.14$). The positive effects of nutrients on bacterial cell density gradually declined with increasing light attenuation (Fig. 4c), and bacterial cell density was reduced at ≤30% of ambient light ($F_{4,60} = 2.29$, $P = 0.07$). Bacterial cell density was positively related to algal cell density across all treatments ($r^2 = 0.42$, $P \leq 0.0001$; Fig. 5).

Field survey of peatland sites

Dissolved organic carbon concentration and water depth interacted to influence light attenuation across a wide range of conditions at adjacent peatland sites (Fig. 1). Maximum water depth ranged from 15 to 65 cm and DOC concentration ranged from 15 to 50 mg L^{-1} among peatlands (Fig. 1) and the light-extinction coefficient (k_d) increased with DOC concentration across all sites ($r^2 = 0.81$, $P < 0.0001$). A comparison of light attenuation between our study site and adjacent peatlands showed that a 30% reduction in light (i.e. achieved by our manipulation) could be obtained by either an increase in DOC concentration from 28 to 50 mg L^{-1} or an increase in water depth from 16 to 45 cm (Fig. 1).

Laboratory incubation

The degradation of algal exudates by heterotrophic bacteria was enhanced by warming during the laboratory incubation. Bacterial growth (10^6 cells mL^{-1}) increased rapidly in the presence of algal exudates at both ambient and elevated (warming) temperatures (Fig. 6a), and the rate of increase was significantly greater in the warming treatment compared to the ambient temperature treatment (ANOVA, $F_{1,20} = 14.8$, $P = 0.001$). The degradation of algal exudates (measured as DOC) increased in step with bacterial density (Fig. 6b), and the rate of degradation was significantly greater in the warming treatment compared to the ambient temperature treatment (ANOVA, $F_{1,20} = 20.6$, $P \leq 0.0001$).

Discussion

Global mean temperature is predicted to increase throughout this century with exacerbated warming across high latitudes where minimum temperatures have constrained energy flow in aquatic ecosystems (Moore & Basiliko, 2006; Collins *et al.*, 2013). Accelerated warming will be coupled with permafrost thaw, which is expected to elevate currently low nutrient levels in surface waters throughout the boreal landscape (Wyatt *et al.*, 2012; Reyes & Lougheed, 2015). Although these conditions are expected to stimulate primary production in northern aquatic ecosystems (Rouse *et al.*, 1997; Wyatt *et al.*, 2012, 2015), the magnitude of the response may be subdued by greater light attenuation associated with an influx of humic substances in the water column (e.g. Ask *et al.*, 2009; Karlsson *et al.*, 2009; Pagano *et al.*, 2014). It is within this predictive context that our goal was to evaluate how a reduction in light availability may influence the growth of benthic algae and their association with heterotrophic bacteria in northern peatlands.

Our results demonstrate that elevated nutrient levels stimulate algal development on peat surfaces and that the positive effects of nutrients are enhanced by

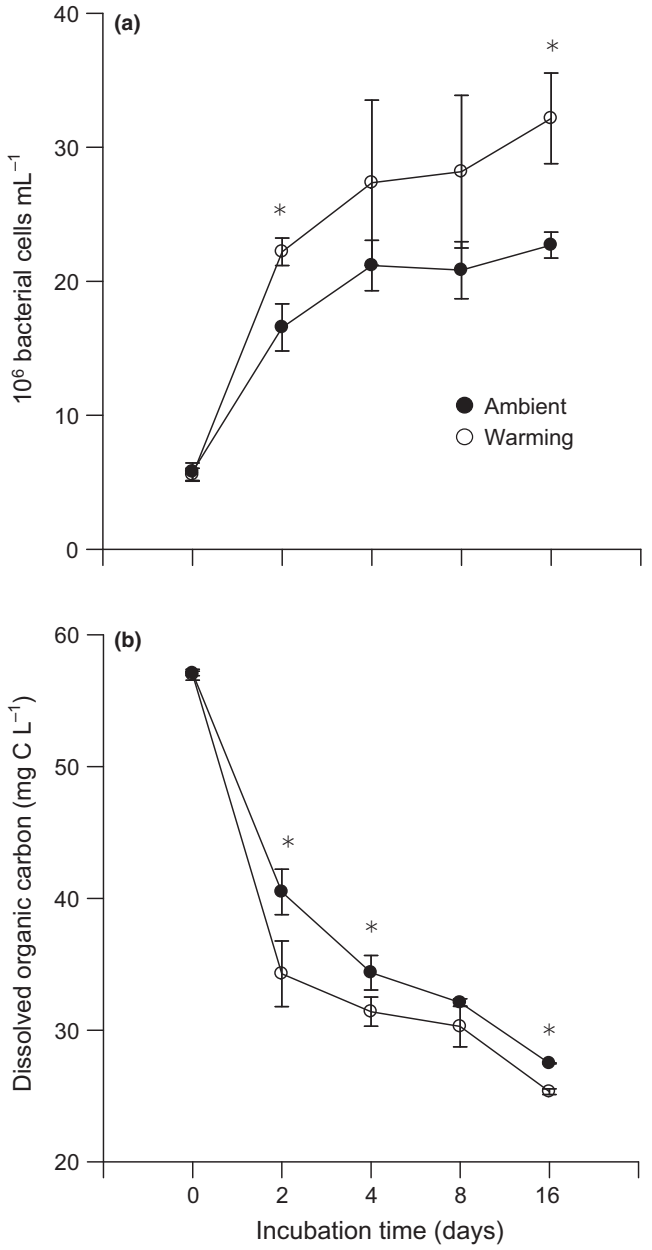

Fig. 6 Bacterial density (10^6 cells mL^{-1}) (a) and dissolved organic carbon (DOC) uptake (b) in the ambient (15 °C) and warming (20 °C) treatments during a 16-day laboratory incubation in the dark. Each data point represents the mean of 3 replicates ± 1 SE. Significant differences between ambient and warming treatments are represented by an asterisk ($P < 0.05$).

warming. Other studies have demonstrated the importance of nutrient limitation to inhibit algal growth in northern wetlands (Robinson *et al.*, 2000) and for warmer temperatures to enhance the effects of nutrient enrichment on algal biomass when sufficient light reaches submersed surfaces (Wyatt *et al.*, 2015). Our findings are also in agreement with the broader literature, which has demonstrated that algal growth rates are an exponential function of temperature in the presence of nutrient saturation (e.g. Goldman & Carpenter, 1974; Bothwell, 1988; Marcarelli & Wurtsbaugh, 2006), possibly owing to accelerated nutrient uptake rates in the presence of elevated temperatures (Rasmussen *et al.*, 2011; Cross *et al.*, 2015). Since elevated nutrient levels are likely to coincide with warmer temperatures at northern latitudes, our results demonstrate the need to consider elevated temperatures when evaluating the effects of greater nutrient availability on algal production in boreal wetlands.

The stimulatory effects of nutrients and warming on biofilm development declined with light attenuation and were absent at light levels ≤125 μmol m^{-2} s^{-1} (≤30% of ambient light). Algal accrual was greatest at ambient light levels (418 μmol m^{-2} s^{-1}) in the presence of warming and elevated nutrients, which is consistent with previous studies demonstrating saturation of benthic algal growth at 200–400 μmol m^{-2} s^{-1} across a range of aquatic ecosystems (Hill, 1996; Baulch *et al.*, 2005). Despite warming and nutrient enrichment, algal accrual declined as light availability (μmol m^{-2} s^{-1}) decreased from 418 (100% available ambient light) to 125 (30% ambient). Below approximately 125 μmol m^{-2} s^{-1}, benthic algae became limited by light, such that algal growth was no longer stimulated by the presence of nutrient enrichment and warming. These findings are in line with those derived from lotic studies, which have shown algal biomass to be limited by light availability at levels less than 100 μmol m^{-2} s^{-1}, despite saturating nutrient supplies (Lamberti *et al.*, 1989; Steinman *et al.*, 1989; DeNicola & McIntire, 1991). More specifically, our results are consistent with previous work demonstrating that light limitation associated with humic waters (e.g. Karlsson *et al.*, 2009) may inhibit the strong positive relationship typically observed between increasing nutrient levels and algal biomass in other regions (e.g. Flanagan *et al.*, 2003).

Although algal accrual was significantly reduced by greater light attenuation, it is important to note that algae were present (at just under 5×10^4 cells cm^{-2}) at even the lowest experimental light levels (approximately 21 μmol m^{-2} s^{-1}). This finding corroborates other studies which have found algal photosynthesis to occur at light levels as low as 10 μmol m^{-2} s^{-1} (Carlton & Wetzel, 1987, 1988), particularly in microalgae living under ice in polar regions (Cota, 1985; Palmisano, Soohoo & Sullivan, 1985). Studies within these ecosystems have found that freshwater benthic algae can adapt to limited light availability by enhancing photosynthetic efficiency and reaching photosynthetic saturation at lower

irradiances when compared to algae subject to greater irradiances (110–1100 μmol m^{-2} s^{-1}) (McIntire & Phinney, 1965; Hill, Ryon & Schilling, 1995). Studies within other wetland ecosystems (e.g. the Everglades) have also shown that algae can acclimate to reductions in ambient light, such that a prolonged reduction in ambient light has no influence on algal production (Thomas, Gaiser & Tobias, 2006). Similarly, a previous study found that light levels of approximately 80 μmol m^{-2} s^{-1} (10–12% of ambient light) do not inhibit benthic algal growth in northern marshes, but instead, increase the chlorophyll to biovolume ratio in the presence of nutrient enrichment (Rober *et al.*, 2015). Therefore, it is important to consider the potential for algal communities to adapt to low light levels during extended periods of colonisation associated with prolonged inundation, which some models predict will occur with more frequent flooding associated with ongoing climate change.

The abundance of heterotrophic bacteria was positively related to benthic algae across all treatments, and the response of heterotrophic bacteria to nutrient enrichment and warming was reduced by light attenuation. Although the biofilm bacterial community was elevated by a combination of nutrient enrichment and warming, the magnitude of the synergistic effect was not high enough to make it statistically significant, possibly owing to nutrient uptake rates exceeding diffusing rates in the presence of elevated temperatures (White *et al.*, 1991). Since bacteria are heterotrophic microorganisms, the positive relationship between light availability and bacterial density suggests that bacteria were supported by the presence of algae. In a previous study within the larger wetland complex (Wyatt & Turetsky, 2015), we found that bacteria were limited by C, which was alleviated in the presence of algal exudates. Applying this information to our current study, we can infer that the increase in bacteria in the presence of nutrient enrichment and ≥30% ambient light was likely the result of greater algal abundance in the presence of elevated nutrients and sufficient light for photosynthesis (Rier & Stevenson, 2001). Our laboratory incubations support this hypothesis, demonstrating that bacteria rapidly use algal exudates and that the uptake of algal exudates by heterotrophic bacteria was enhanced by warming. Consequently, warming will probably increase the rate at which heterotrophic bacteria use algal exudates on the peat surface, a result that has been demonstrated in polar surface waters (Robarts, Sephton & Wicks, 1991). Other characteristics of the biofilm complex are likely important for the strong algal-bacterial link in this boreal fen, including increased surface area for bacterial colonisation as part of greater algal biomass (Rier & Stevenson, 2002; Carr, Morin & Chambers, 2005). Our results do not aim to single out the role of exudates as the predominant ingredient for support of heterotrophic metabolism, but our findings do help to explain why the density of heterotrophic bacteria was most elevated when levels of ambient light and nutrients were sufficient to stimulate algal photosynthesis.

Conclusions

As temperatures continue to increase in response to growing atmospheric CO_2, other associated environmental changes are predicted to occur, such as elevated nutrient availability and browning of surface water in high latitude regions. Although it is predicted that the combined effects of warmer temperatures and increased nutrient availability will promote biofilm development in northern peatlands, our findings demonstrate that increased light attenuation associated with elevated levels of DOM (Freeman *et al.*, 2004; Fenner *et al.*, 2007) could quickly constrain biofilm development even in a warmer, more nutrient-rich environment. Within a more predictive context, our results show that biofilm development in this shallow peatland pool (<16 cm in depth) will begin to be limited by light attenuation if current DOM levels are doubled by browning. Our results also demonstrate that an increase in water depth associated with accelerated the spring snowmelt (e.g. from 16 to 45 cm) could have a similar effect on light attenuation as browning alone. Consequently, the response of benthic biofilms to elevated warming and nutrient levels will depend on physical aspects of northern peatlands (e.g. bathymetry) as well as changes in light attenuation associated with future climate change. However, this is not to suggest that other aspects of the biofilm will not respond positively to warmer temperatures and nutrient enrichment within the complex structure of northern peatlands. For example, extended wet periods may result in greater abundance of floating algal mats (i.e. metaphyton) rather than benthic forms of algae. Metaphyton grow in close association with plant stems at the water surface, and thus would be unaffected by changes in light attenuation. Additionally, different algal species may be more adept at acclimation to low light, or variations in light quality, and changes in community composition over time may allow the persistence of benthic algae in northern peatlands. Ultimately, these considerations necessitate continued research to better elucidate the role of algae in northern peatlands, including their role in biofilm development and their potential to act as

a priming agent for organic matter decomposition within the microbial landscape (e.g. Danger *et al*., 2013; Kuehn *et al*., 2014). Such research, coupled with a better grasp of top-down controls on biofilm structure, will improve our ability to predict the consequences of a changing environment on northern peatland ecosystems.

Acknowledgments

This research was funded by The Wetland Foundation, the Phycological Society of America, Sigma Xi and the ASPiRE Program at Ball State University. We thank A Rober, D DeColibus and A Sampson for field and laboratory assistance, and the faculty and staff at the Bonanza Creek LTER for logistical support while working in Alaska.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Schematic of (a) circulating water bath during the laboratory temperature manipulation and (b) jacketed beakers. An internal pump circulates temperature-regulated water around the inner walls of each beaker, maintaining constant temperature of water inside each incubation flask. Two water baths (each incubating 3 flasks) were set to either ambient (15 °C) or warming (20 °C) temperatures in the laboratory. Modified from M.G. Stillwagon, unpubl. data.

(Manuscript accepted 9 May 2016)