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Algae alleviate carbon limitation of heterotrophic bacteria in a boreal peatland

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Summary

1. In most high-latitude wetlands, carbon accumulation as peat represents a balance between plant net primary productivity and heterotrophic decomposition. We hypothesized that this assessment of ecosystem metabolism is incomplete as it does not include information on energy inputs from microalgae, which form complex biofilms with heterotrophic micro-organisms on the peat surface.

2. To investigate the potential for algae (and associated exudates) to support heterotrophic metabolism under ambient and elevated nutrient levels, we conducted a factorial enrichment of nutrients (nitrogen and phosphorus) and carbon (glucose) in mesocosms with and without the presence of algae (using light-transparent and dark treatments, respectively) in an Alaskan fen. We measured respiration rates and changes in bacterial biomass to characterize the response of heterotrophic bacteria to our experimental treatments.

3. During incubation assays, algae released up to 38% of their net productivity as exudates and there was a positive relationship between algal production and concentrations of dissolved organic carbon inside mesocosms.

4. Elevated algal productivity in the presence of nutrient enrichment stimulated heterotrophic bacterial respiration and biomass. These responses did not occur with nutrient enrichment in the dark (without algae). The response of bacteria to algae was similar in magnitude to bacterial responses to glucose enrichment.

5. *Synthesis.* We conclude that bacteria in this boreal fen were primarily limited by labile carbon, and this constraint was alleviated in the presence of elevated algal production. Consequently, algae may facilitate hotspots of microbial activity in northern peatlands, especially in conditions of greater nutrient availability associated with more variable hydrology expected for this region with ongoing climate change.

Key-words: Alaska, algal–bacterial interactions, aquatic plant ecology, climate change, decomposition, dissolved organic carbon, nutrients, priming effect, resource subsidies, wetland

Introduction

Northern peatlands are a critical component of the global carbon (C) cycle (Gorham 1991), and their response to climate change likely will play a key role in determining future concentrations of atmospheric carbon dioxide (CO_2) (McGuire *et al.* 2007). Throughout the Holocene, peatlands in northern latitudes have served as a net sink for atmospheric CO_2 (Harden *et al.* 1992). As a result of this C sequestration, it is estimated that boreal peatlands contain about one-third of the world's soil C stocks (Gorham 1991). Carbon accumulates as peat where net primary productivity (NPP) at the peat surface exceeds decomposition, which generally occurs because of a low metabolic environment (i.e. anoxic soils) and organic matter that is resistant to decomposition (Moore & Basiliko 2006). The potential for northern peatlands to continue to be a sink for CO_2 is uncertain and will depend largely on the nature of organic matter entering peatlands and the metabolic environment for heterotrophic metabolism in conditions associated with future climate change (Laiho 2006).

Much effort has been made to quantify organic matter turnover and to identify linkages between primary producers and heterotrophic micro-organisms in northern peatlands (Basiliko *et al.* 2012; Waldrop *et al.* 2012; Fan *et al.* 2013). As in many other ecosystems, photosynthetic activity in peatlands can influence decomposition by controlling the quality of 'new' organic material available for heterotrophic metabolism (Wieder 2006). Given that surface peat is often comprised of

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plant material that is inherently resistant to decomposition, plant inputs of labile C likely have substantial impacts on microbial population dynamics and organic matter decomposition. For example, research has demonstrated that labile exudates from plant roots can promote energy transfer in surface peat by stimulating heterotrophic respiration in the rhizosphere (Crow & Wieder 2005; Basiliko *et al.* 2012). Compared to plants, we know relatively little about energy inputs from microautotrophs (i.e. algae) in northern peatlands, even though algae can be abundant in these ecosystems (Rober *et al.* 2014) and probably interact with heterotrophic microorganisms to influence metabolic activities and possibly organic matter decomposition at the peat surface.

Algae, which are part of complex biofilms that form on the surfaces of decaying organic matter (Battin *et al.* 2007), may be central to energy transfer in peatland ecosystems by providing resource subsidies to, and exchanging resources with, heterotrophic micro-organisms (i.e. consumer–producer codependency) during photosynthesis (i.e. Marcarelli *et al.* 2011). Algal exudates typically are rich in carbohydrates (Myklestad 1995; Biddanda & Benner 1997), which can be readily consumed by heterotrophic micro-organisms in the overlying water column (Rodríguez *et al.* 2013). Consequently, algal subsidies have been shown to stimulate energy flow in a wide range of benthic ecosystems (Danger *et al.* 2013; Kuehn *et al.* 2014), though our understanding of their influence on heterotrophic metabolism in northern peatlands is limited to laboratory incubations (Wyatt *et al.* 2012).

The role of algae in boreal wetlands, including their abundance and interactions with heterotrophic micro-organisms, likely will be altered by future climate change, including both the degree of warming and precipitation fluctuations (i.e. Hinzman *et al.* 2005; McGuire *et al.* 2007). One potential consequence of warming is the release of nutrients from the expanded active layer following permafrost thaw (Davis 2001). Increased decomposition in thicker active layers as well as warmer and drier surface soil conditions likely will increase near-surface nutrient concentrations during periods of inundation (Kane *et al.* 2010). Although algae are expected to increase in abundance with elevated nutrient levels in northern wetlands (Wyatt, Stevenson & Turetsky 2010; Rober *et al.* 2014), it is not yet clear how elevated algal production will affect heterotrophic bacteria, making it difficult to accurately predict how energy flow within these ecosystems will be altered by processes associated with ongoing climate change.

The goal of this study was to investigate the potential for algae to support heterotrophic bacteria under ambient and elevated nutrient levels in a northern peatland. To do this, we evaluated the biomass and respiration of heterotrophic bacteria in response to a factorial enrichment of nutrients and glucose inside mesocosms with and without the presence of algae (using light-transparent and dark treatments, respectively). We hypothesized that algae were limited by nutrients and that heterotrophic bacteria were limited by a combination of nutrients and labile organic matter provided by the algae. We predicted that (i) greater nutrient availability would promote heterotrophic bacteria on the peat surface by increasing C subsidies available during periods of elevated algal production and that (ii) nutrient enrichment would not promote heterotrophic metabolism on the peat surface in the absence of algae (i.e. dark treatments) or in the absence of glucose enrichment.

Materials and methods

SITE DESCRIPTION

This study was conducted in a poor fen located within the floodplain of the Tanana River positioned just outside of the Bonanza Creek Experimental Forest and approximately 35 km southwest of Fairbanks, Alaska, USA (64°42′N, 148°18′W). The region of interior Alaska is classified as continental boreal and has a growing season of 135 days or less with more than 21 h of light per day in June. The region has a mean annual temperature of −3.1 °C and precipitation of 287 mm. The fen lacks trees and the plant community is dominated by emergent vascular taxa, including *Equisetum*, *Carex* and *Potentilla*, and brown and *Sphagnum* mosses. Peat depth exceeds 1 m at the centre of the site and there is little microtopography across the site. Concentrations of nitrate (NO_3) and phosphate (PO_4) are frequently below 23 and 5 μg L^{-1} at this fen site, respectively, though levels were more elevated during this current study. pH at the site ranges from 5.5 to 6.5.

EXPERIMENTAL DESIGN

We established a mesocosm experiment within the fen to examine mechanisms driving interactions between algae and heterotrophic bacteria on submersed plant litter. Prior to beginning the study, we constructed a boardwalk to prevent the disturbance of sediments during experimental set-up and regular sampling. We constructed 32 open-top and open-bottom mesocosms by rolling wire mesh into a cylinder (50 cm in diameter) and then wrapping each cylinder with a 0.1 mm thick layer of clear plastic (Wyatt, Stevenson & Turetsky 2010). Enclosures were evenly spaced throughout a 20 × 20-m area of the fen and pushed into the peat so that the top extended 10 cm above the water surface. The open-bottom design allowed for water inside enclosures to be in contact with the peat to maintain hydrologic connectivity. Over-wintered standing-dead stems of *Carex utriculata* were used as a standard substrate for measures of algal and bacterial growth. Stems were collected from a common location within the fen, air-dried and cut into equal sections (10-cm long) in the laboratory. Stems were placed inside 10 × 10-cm clear polyethylene mesh (1 mm) bags (Nitex; Dynamic Aqua-Supply Ltd., Surrey, BC, Canada), and a single bag containing six stems was fixed to the peat surface (approximately 30 cm below the water surface) within each mesocosm 1 week prior to the initiation of the study.

We used a full factorial design with and without nutrients (nitrogen and phosphorus in combination, NP), with and without carbon (C), and with and without sunlight [light-transparent (L) and dark (D) conditions, respectively]. Each enclosure was randomly assigned to one of eight treatments, with four replicates for each treatment. The top and sides of the dark treatment enclosures were covered with a black shroud made of polyester fabric that blocked more than 99% of incoming PAR (hereafter dark treatments) to inhibit algal photosynthesis, and light-transparent treatment enclosures were left uncovered to

allow for passage of ambient sunlight to promote algal growth. Nutrient enrichments were added as a pre-mixed stock solution to achieve final concentrations of 100 μg L^{-1} KH_2PO_4 and 1000 μg L^{-1} KNO_3 for nutrients and 5 mg L^{-1} $C_6H_{12}O_6$ for C every 2 days, beginning on 7 June 2013, and continued for 16 days. Our goal was to saturate nutrient levels beyond the combined energetic requirements for algae and bacteria to evaluate whether bacteria use algal exudates as a carbon source without the confounding variable of nutrient limitation of bacterial growth. We assumed these concentrations would saturate algal growth because they exceed those reported to be limiting in studies reviewed by Borchardt (1996). We expected that algal photosynthesis would be maximized in conditions of nutrient enrichment in the light $(NP)_L$ and for algae to be absent in the dark $(NP)_D$. Consequently, plant litter (and associated micro-organisms) in the $(NP)_L$ treatment would be exposed to algal exudates, while substrates in the $(NP)_D$ treatment would be exposed to nutrient enrichment in the absence of algae. Dark treatments [$(C)_D$, $(NP)_D$, $(NP + C)_D$] allowed us to evaluate the importance of C for heterotrophic bacteria in the absence of algal photosynthesis. If C was the primary limiting factor for heterotrophic bacteria, we predicted that measures of heterotrophic activity would be greater in the $(C)_D$ treatment compared to the $(NP)_D$ treatment and similar to the $(NP + C)_D$ treatment. By comparing heterotrophic bacteria in the $(NP)_L$ treatment (with elevated algal production) to the $(C)_D$, $(NP)_D$ and $(NP + C)_D$ treatments, we were able to isolate the importance of algae for heterotrophic bacteria independent of nutrients. If heterotrophic bacteria were using algal subsidies, we expected to find a similar response of heterotrophic bacteria between the $(NP)_L$ and $(NP + C)_D$ treatments. We also compared each treatment to a control without nutrient enrichment in light-transparent $(control)_L$ and dark $(control)_D$ conditions, and we evaluated container effects by placing substrates in four random locations within the fen without enclosures under both light and dark conditions. In this case, dark conditions were established by suspending black fabric above the open water with a wooden dowel (inserted vertically into the peat), making a 'tent' that extended to the peat surface (i.e. covering all sides) and held in place at the bottom with metal pins.

SAMPLING AND ANALYTICAL METHODS

We monitored physical and chemical conditions inside each enclosure during the experiment. The height of the water-table above the peat surface was measured with a meter stick, and surface water temperature (°C), dissolved oxygen (DO) and pH were measured every 2 days with a calibrated Hach model 40d multi-probe (Hach Company, Loveland, CO, USA). On day 16, water was collected from 5 cm below the water surface with a syringe and filtered for dissolved nutrient analysis with a 0.45-μm pore-size filter (Millipore Corporation, Bedford, MA, USA) into 120 mL sterile polyethylene bottles. Samples were stored on ice until returning to the laboratory, where a portion of each filtered sample was analysed for dissolved organic carbon (DOC) with a Shimadzu TOC-V analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). The remaining portion of each sample was frozen and later analysed for nitrate (NO_3) and phosphate (PO_4) with a Dionex (Dionex Corporation, Sunnyvale, CA, USA) ion chromatograph (APHA 1998).

After 16 days of experimental manipulation, six stems were removed from each enclosure to measure the accumulation of algae and heterotrophic bacteria. Two stems were immediately placed into a 300 mL clear biological oxygen demand bottle filled with fen water for measures of NPP for 1 h in the light and then wrapped with aluminium foil for measures of oxygen consumption (respiration, R_d) in the dark for an additional hour using a luminescent DO probe (Hach Company). Bottles were incubated at a water depth of approximately 25 cm during midday hours with an average light level of 225 μmol m^{-2} s^{-1}. We measured exudate release on stems collected from the $(NP)_L$ and $(control)_L$ treatments during a second set of light-bottle incubations. These treatments spanned the range of algal productivity observed in the study and allowed us to calculate the proportion of exudates released by algae at each end of the nutrient spectrum. These samples were incubated as described above except that pore water was filtered through a 0.2-μm pore-size filter (VacuCap; Pall Life Sciences, Ann Arbor, MI, USA) to remove most bacteria from solution prior to incubations. A 60-mL water sample was collected immediately following each incubation and acidified in the field for measures of DOC concentration (mg L^{-1}). The initial pre-incubation DOC concentration was subtracted from the post-incubation concentration to calculate net exudate release. Net primary productivity was calculated according to Wetzel & Likens (2000) and exudate release was reported as a percentage of NPP (Wyatt *et al.* 2014).

A separate set of stems was collected from each bag for measures of algal biomass as chlorophyll *a* concentration. Two stems were placed directly into a 20-mL centrifuge tube filled with filtered wetland water, wrapped with aluminium foil and transported to the laboratory on ice where the biofilm was removed by scraping and brushing. The resulting algal slurry was filtered onto a glass fibre filter (0.7 μm Whatman GF/F, Springfield Mill, UK) and then placed back into a 20 mL centrifuge tube with 90% ethanol and steeped in the dark overnight in a refrigerator. Chlorophyll *a* was measured from the extract with a Shimadzu UVmini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 665 and 750 nm after acidification to correct for phaeopigments (APHA 1998).

Bacterial biomass was determined from stems by direct counts using epifluorescence microscopy. Two stems were collected from each mesocosm and placed into sterile 20-mL glass scintillation vials and preserved in the field with formalin. Bacterial cells were detached from stems by probe sonication followed by active scraping and brushing. Samples were stained with 4′, 6-diamino-2-phenylindole (DAPI) (Porter & Feig 1980) and vacuum filtered onto a 0.2-μm pore-size black filter (Osmonic Inc., Livermore, CA, USA). A minimum of 300 cells or 25 fields were counted per filter at 1000× magnification with a Leica DM 4000 microscope with fluorescence (Leica Microsystems, Wetzlar, Germany). Cell volume (μm^3) was calculated from measurements of length (l) and width (w) using the formula: $V = w^2/4 \times (l - w) \times \pi + w^3/6 \times \pi$. Bacterial biomass was calculated using the formula: bacterial dwt (fg) = $435 \bullet V^{0.86}$ (Loferer-Krößbacher, Klima & Psenner 1998) and assuming that C made up 50% of bacterial dry weight. Bacterial biomass was related to oxygen consumption (R_d) in dark treatments (i.e. in the absence of autotrophic respiration) and used as an estimate of biomass-specific bacterial respiration.

STATISTICAL ANALYSES

Differences in mean water depth, pH, temperature, DO, NO_3, PO_4 and DOC concentration among treatments were evaluated with analysis of variance (ANOVA) models. A three-way ANOVA was used to determine the independent and interactive effects of nutrients (with and without), C (with and without) and light (light-transparent and dark) on algal parameters, R_d, and bacterial biomass. In instances when ANOVA indicated significant differences among treatments, a Tukey's *post hoc* comparison of means tests was used to discriminate between different factor levels. *Post hoc* comparisons of means were

performed using Tukey's tests. All statistical analyses were conducted using SPSS 18 software (SPSS Inc., Chicago, IL, USA) with a significance level of $P < 0.05$.

Results

Physical and chemical parameters were similar between the open wetland and treatment controls ($P > 0.05$). Overall mean water depth (29.2 ± 2.24 cm), pH (6.52 ± 0.09) and temperature (22.4 ± 0.69 °C) were similar among treatments ($P > 0.05$) and were relatively constant over time.

Algae responded positively to nutrient enrichment in the light-transparent treatments and elevated algal production altered the physical and chemical conditions in the water column. The nutrient enrichment treatments [$(NP)_L$, $(NP)_D$, $(NP + C)_L$, $(NP + C)_D$] significantly increased dissolved nutrient concentrations (NO_3: $F_{1,24} = 1867.2$, $P < 0.001$; PO_4: $F_{1,24} = 324.6$, $P < 0.001$; Table 1). However, in the light-transparent treatments only, algal biomass and productivity increased significantly in response to nutrient enrichment (chlorophyll *a*: $F_{1,24} = 116.3$, $P < 0.001$; NPP: $F_{1,24} = 125.3$, $P < 0.001$; Table 1) and elevated algal production subsequently reduced nutrient concentrations in the water column compared with dark treatments. For example, the $(NP)_L$ and $(NP + C)_L$ treatments, which both had elevated algal biomass and productivity, were characterized by 50% lower nutrient levels (Table 1). Mean ± SE DO concentration (mg L^{-1}) was positively related to algal production and significantly greater in the $(NP)_L$ (6.75 ± 0.43) and $(NP + C)_L$ (6.58 ± 0.42) treatments than all other treatments ($F_{7,216} = 428.8$, $P < 0.001$). Dissolved oxygen concentration declined in all other treatments over time and was significantly lower ($P < 0.001$) in the $(control)_D$ (1.85 ± 0.20), $(C)_D$ (1.7 ± 0.16) and $(NP + C)_D$ (1.43 ± 0.25) treatments compared to the $(control)_L$ (4.26 ± 0.37) and $(C)_L$ (3.94 ± 0.40) treatments, which were not significantly different from each other ($P > 0.05$).

Algae released a substantial amount of fixed C as exudates and DOC concentrations were elevated in mesocosms with accelerated algal production. Exudate production by algae during the incubation assays was 21% (0.21 ± 0.02 mg C h^{-1} mg^{-1}) and 38% (0.38 ± 0.03) of NPP in the $(NP)_L$ and $(control)_L$ treatments, respectively. At this rate, algae were contributing 0.91 ± 0.07 mg DOC m^{-2} h^{-1} in the $(control)_L$ treatment and 5.29 ± 0.50 mg DOC m^{-2} h^{-1} in the $(NP)_L$ treatment. Concentrations of DOC were greater in the light-transparent treatment with nutrient enrichment [$(NP)_L$] than in the $(control)_L$ treatment ($F_{1,24} = 5.23$, $P = 0.03$; Table 1). Concentrations of DOC in the $(NP)_L$ treatment were similar to concentrations in the dark treatments with glucose enrichment [$(C)_D$ and $(NP + C)_D$, that is treatments without algal productivity] ($P > 0.05$).

Both algae and glucose additions had a stimulatory effect on heterotrophic bacteria. Algal effects on bacterial metabolism were independent of nutrient enrichment, as nutrient enrichment did not stimulate heterotrophic bacteria in the absence of glucose or in the dark (without algae). Measures

Table 1. Measures of dissolved organic carbon (DOC, mg L^{-1}), nitrate (NO_3, μg L^{-1}), phosphate (PO_4, μg L^{-1}), algal chlorophyll *a* concentration (mg m^{-2}), algal net primary productivity (NPP, mg C m^{-2} h^{-1}), bacterial respiration (in dark treatments) (R_d) (mg C m^{-2} h^{-1}) and bacterial biomass (mg C m^{-2}) on plant stems among treatment enclosures following the 16-day mesocosm experiment (mean ± SE, $n = 4$)

	Treatment							
	Control		NP		C		NP + C	
	Light $(control)_L$	Dark $(control)_D$	Light $(NP)_L$	Dark $(NP)_D$	Light $(C)_L$	Dark $(C)_D$	Light $(NP + C)_L$	Dark $(NP + C)_D$
Environmental								
DOC	60.3 ± 1.9[a]	61.4 ± 1.7[a]	68.3 ± 0.8[b]	63.0 ± 0.9[a]	70.8 ± 1.8[b]	70.1 ± 1.4[b]	71.9 ± 1.8[b]	68.1 ± 1.2[b]
NO_3	39.0 ± 5.5[a]	36.0 ± 4.0[a]	783.0 ± 101.7[b]	1839.5 ± 98.0[c]	35.7 ± 4.6[a]	42.7 ± 3.8[a]	838.0 ± 100.1[b]	1733.3 ± 145.7[c]
PO_4	12.5 ± 2.0[a]	11.8 ± 4.1[a]	112.2 ± 5.2[b]	227.2 ± 16.9[c]	10.4 ± 4.7[a]	13.7 ± 3.6[a]	100.2 ± 5.2[b]	239.5 ± 6.9[c]
Algae								
Chlorophyll *a*	4.49 ± 0.22[a]	1.35 ± 0.13[a]	59.8 ± 6.05[b]	1.86 ± 0.19[a]	4.81 ± 0.55[a]	1.92 ± 0.37[a]	48.8 ± 6.90[b]	1.41 ± 0.17[a]
NPP	2.14 ± 0.31[a]	0.50 ± 0.15[a]	25.2 ± 2.05[c]	0.39 ± 0.05[a]	−6.90 ± 0.27[b]	−9.07 ± 0.36[b]	18.5 ± 3.60[c]	−10.1 ± 1.36[b]
Bacteria								
Respiration	0.76 ± 0.11[a]	0.62 ± 0.08[a]	13.3 ± 1.61[b]	0.72 ± 0.05[a]	11.4 ± 0.44[b]	12.2 ± 1.2[b]	15.3 ± 1.71[b]	13.0 ± 1.64[b]
Biomass	111.6 ± 2.7[a]	104.3 ± 2.9[a]	148.2 ± 5.8[b]	118.6 ± 3.1[a]	138.3 ± 1.9[b]	135.9 ± 1.7[b]	162.3 ± 9.7[b]	158.6 ± 13.1[b]

Values having the same letter are not significantly different (Tukey's test, $P < 0.05$).

of dark respiration and bacterial biomass were similar among the $(NP)_L$ treatment and treatments enriched with glucose [$(C)_L$, $(C)_D$, $(NP + C)_L$, $(NP + C)_D$], which were all significantly greater than the $(NP)_D$ and control treatments (R_d: $F_{1,24} = 6.46$, $P = 0.018$; bacterial biomass: $F_{1,24} = 6.99$, $P = 0.014$; Table 1). Measures of heterotrophic bacteria were similar between the $(NP)_D$ and control treatments (Table 1). Using estimates of respiration in dark treatments (without autotrophic respiration) as an estimate of bacterial respiration, we calculated that bacteria were assimilating up to 12.15 mg C m^{-2} h^{-1} in the $(NP)_L$ treatment (Table 1).

Discussion

Given that warming at northern latitudes is occurring more quickly than in other regions and in general is expected to increase nutrient mineralization rates, northern wetlands may be subject to changing nutrient availabilities under a warming climate. Our results demonstrate that algae, which typically occur in low abundance (relative to higher plants) in northern peatlands, increase rapidly in the presence of elevated nutrient levels in our study fen. By doing so, our results add to a growing line of evidence for the importance of nutrients as a limiting factor for algal productivity in northern ecosystems (Elser *et al.* 2007) and suggest that greater nutrient availability expected for this region will enhance the presence of algae on the peat surface during periods of inundation. Given the reduction in nutrient levels observed in the light-transparent treatments as compared to the dark treatments (without and without algae, respectively), our results also highlight the capacity for benthic algae to rapidly sequester available nutrients in the water column, likely making them unavailable for uptake by plants. These results also demonstrate the difficulty of evaluating the levels of nutrients released following disturbance events (e.g. rewetting of dry sediments) as nutrients are assimilated by biofilms and the actual amount of nutrients released is probably greater than amounts detected from measures of dissolved nutrient analysis alone (e.g. Wyatt *et al.* 2012).

Compared to other ecosystems, the involvement of nutrients in the interplay between algae and heterotrophic bacteria is not well understood in northern peatlands. Our results show that elevated algal productivity in the presence of nutrient enrichment stimulated heterotrophic bacteria respiration and biomass. Although algae and bacteria compete for the same inorganic nutrients (Bratbak & Thingstad 1985), both groups are expected to coexist if nutrient levels surpass their combined energetic requirements (Currie & Kalff 1984) or if bacteria are limited by C subsidies provided by the algae (Daufresne & Loreau 2001). Consequently, we expected that competition between algae and heterotrophic bacteria would decline in more nutrient-rich conditions, making it difficult to evaluate the interactions between algae and bacteria under elevated nutrient levels. However, our factorial experimental design made it possible to disentangle the effects of nutrients versus algae on bacterial metabolism. We found that nutrient enrichment alone (i.e. in the dark treatments with no algae) did not enhance heterotrophic bacteria. This finding demonstrates that bacteria were not simply responding to elevated nutrient levels but instead that algae played a role in stimulating heterotrophic bacteria independent of nutrient enrichment.

Previous studies have demonstrated that algae can enhance bacteria growth on both organic and inorganic surfaces and in pelagic mats (Neely & Wetzel 1995; Scott & Doyle 2006; Kuehn *et al.* 2014). The potential mechanisms for the association between algae and heterotrophic bacteria vary from increased surface area for colonization associated with biofilm structure (Rier & Stevenson 2001; Carr, Morin & Chambers 2005) to mutualistic interactions, whereby algae provide bacteria with resource subsidies and vice versa (Kuehn *et al.* 2014). Previous work on the composition of algal exudates and patterns of bacterial uptake during laboratory assays (Wyatt *et al.* 2012) points to the importance of algal subsidies for supporting bacteria growth on plant litter. During the field incubations in this current study, we measured rates of exudate release by algae that increased DOC concentrations inside mesocosms by approximately 12% relative to the control treatment. Bacteria were stimulated by glucose enrichment in the dark treatments independent of nutrient enrichment, suggesting that heterotrophic metabolism in these fen soils was limited by labile organic matter. This response was similar to treatments that had elevated algal production rates [i.e. $(NP)_L$] with no glucose additions. Taken together, these results indicate that algae can alleviate C limitation on bacterial metabolism through the release of C subsidies during periods of elevated nutrient availability. It is important to note that abiotic factors, including photolysis, probably played a role in the production of organic molecules assimilated by heterotrophic micro-organisms (Paul *et al.* 2012). However, minimal amounts of heterotrophic bacteria observed in light treatments with low levels of algal production reduce the likelihood that our results were due to photolysis alone.

We found that algae released a smaller fraction of exudate in conditions of nutrient enrichment. This result offers support for the hypothesis that exudate production by algae operates as a protection mechanism when photosynthesis exceeds cellular growth because of nutrient limitation (Fogg, Nalewajko & Watt 1965). Even with a lower rate of release, DOC concentration was greater in treatments with nutrient enrichment (and elevated algal production) than in treatments without nutrient enrichment (with a greater per cent exudate release but lower overall algal production). This is noteworthy because discussions about the importance of algal exudates to ecosystem processes have focused on low-nutrient environments where algae release a large fraction of photosynthate as exudates (e.g. Danger *et al.* 2013; Wyatt *et al.* 2014), possibly in exchange for nutrients mineralized by heterotrophic bacteria in a mutualistic interaction (Daufresne & Loreau 2001). Our results indicate that exudate production is important for heterotrophic bacteria during periods of nutrient limitation and in conditions of elevated nutrient levels due to overall greater algal production, though the percentage of release is less in these environments.

Bacteria play an important role in the turnover of soil C in boreal peatlands (Waldrop *et al.* 2012), and our results show that their presence on plant litter is associated with algal production. This suggests that algae likely produce hotspots of microbial activity where heterotrophic microbial decomposers are provided subsidies that aid in their ability to breakdown organic matter, possibly through a priming effect (i.e. Guenet *et al.* 2010). The priming effect is not well studied in aquatic ecosystems, but it is a common response in terrestrial ecosystems where a relatively small amount of labile organic matter results in a disproportionate turnover of more recalcitrant organic matter (Blagodatsky *et al.* 2010). The priming effect may be particularly important in peatland ecosystems where decomposition is often limited by the availability of labile organic matter (Bergman, Svensson & Nilsson 1998). Although our experiment was not designed to test for a real priming effect (i.e. Kuzyakov, Friedel & Stahr 2000), respiration rates in treatments with elevated algal production appear to exceed contributions from autotrophic exudate production, suggesting a possible turnover of soil organic matter. In doing so, the results of this current study demonstrate the importance of algae as an energy source for microbial decomposers in this northern peatland and point to avenues of future research on algal priming within the context of ongoing climate change in boreal ecosystems.

In conclusion, our findings demonstrate that nutrients promote algal production on the peat surface and the release of algal exudates promotes heterotrophic bacteria on plant detritus. Consequently, the release of nutrient constraints on algal production may provide a new and relatively unexplored mechanism to enhance heterotrophic metabolism in boreal peatlands. It is important to note that the strength of the relationship between algae and heterotrophic bacteria observed in our study could be influenced by the natural variation in environmental conditions that occur among northern peatlands. For example, variation in pH has been shown to have a strong influence on the binding capacity of nutrients and on algal community composition, with implications for overall production (Wyatt & Stevenson 2010). Spatial variability in plant community composition will also shape the chemical nature of organic substrates and have consequences for biofilm formation and overall decomposability of litter in surface peat layers (Limpens *et al.* 2008). Future studies should consider these factors as well as others including 'brownification' associated with increased inputs of terrestrial organic matter which may limit algal responses to nutrient enrichment through its effects on light penetration. Additional study in these areas coupled with a better understanding of top-down effects (i.e. food-web dynamics) on algal structure and function will improve our understanding of ecosystem dynamics in boreal peatlands. Considering the degree to which algae influenced the presence of heterotrophic bacteria in this current study, future research may benefit by including measurements of algal abundance (i.e. per cent cover) when evaluating spatial and temporal variability in CO_2 flux associated with variable hydrology in boreal peatlands.

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Data accessibility

Data used for analysis available at http://www.lter.uaf.edu.

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