

# Nutrients and temperature interact to regulate algae and heterotrophic bacteria in an Alaskan poor fen peatland

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**Abstract:** Permafrost thaw associated with warmer temperatures is expected to elevate nutrient levels in northern aquatic ecosystems, including peatlands. To evaluate these effects on algae and heterotrophic bacteria, we manipulated nutrients (nitrogen (N) and phosphorus (P)) and temperature (ambient and warmed) in a factorial design using nutrient diffusing substrates inside warming chambers in an Alaskan peatland. After 16 days, there was no effect of warming on the abundance of algae or heterotrophic bacteria in the absence of nutrient enrichment. Algal production and bacterial biomass were substantially elevated by N with and without P (NP and N, respectively), independent of warming. Warming significantly enhanced the effect of nutrient enrichment on the abundance of algae and heterotrophic bacteria compared with ambient temperatures. Rates of N fixation increased with the presence of heterocyst-forming cyanobacteria, which represented a greater proportion of algal taxonomic composition in the absence of N enrichment in both ambient and warmed conditions. Our results indicate that warmer temperatures and nutrient enrichment will elevate algal and heterotrophic metabolism in northern peatlands, and the magnitude of increase will depend on the combination of nutrients available during periods of inundation.

**Résumé :** La fonte du pergélisol associée à la hausse des températures devrait entraîner une augmentation des concentrations de nutriments dans les écosystèmes aquatiques nordiques, notamment les tourbières. Afin d'évaluer ces effets sur les algues et les bactéries hétérotrophes, nous avons manipulé les nutriments (azote (N) et phosphore (P)) et la température (ambiante et élevée) dans un schéma factoriel faisant appel à des substrats qui diffusent les nutriments, à l'intérieur d'enceintes de réchauffement dans une tourbière alaskienne. Après 16 jours, il n'y avait aucun effet du réchauffement sur l'abondance d'algues ou de bactéries hétérotrophes en l'absence d'enrichissement en nutriments. La production d'algues et la biomasse de bactéries étaient significativement élevées par l'enrichissement en N avec ou sans P (NP et N, respectivement), indépendamment du réchauffement. Ce dernier rehaussait de manière significative l'effet de l'enrichissement en nutriments sur l'abondance d'algues et de bactéries hétérotrophes par rapport aux températures ambiantes. Les taux de fixation de N augmentaient en présence de cyanobactéries formant des hétérocystes, qui représentaient une proportion plus importante de la composition taxinomique des algues en l'absence d'enrichissement en N à des températures tant ambiantes qu'élevées. Nos résultats indiquent que des températures plus élevées et l'enrichissement en nutriments entraîneront une augmentation du métabolisme algal et hétérotrophe dans les tourbières nordiques, l'ampleur de cette augmentation dépendant de la combinaison de nutriments disponibles durant les périodes d'inondation. [Traduit par la Rédaction]

## Introduction

Peatlands cover extensive portions of northern boreal regions and store large amounts of soil carbon (C) as partially decomposed organic matter (Gorham 1991; Bridgham et al. 2006). The location of boreal peatlands within high latitude regions makes them vulnerable to climate change, and climate models predict temperature increases of 1.4–5.8 °C during this century (IPCC 2007). The current paradigm in peatland ecology is that energy flows primarily through plant detrital pathways and that energy flow is constrained by a low metabolic environment and recalcitrant organic matter (Wieder 2006). Consequently, efforts to evaluate the effects of climate change on aspects of primary production have focused on plants, especially mosses, which produce a large fraction of annual biomass and tend to form litter that decomposes slowly (Turetsky 2003). Comparatively, we know less about the ecology of other primary producers in northern peatlands, including benthic algae, which grow as part of a complex network of microorganisms on plant litter.

Algae can be abundant under a variety of soil moisture conditions in northern peatlands (Rober et al. 2013, 2014). Although algae are not likely to contribute substantially to the structural formation of peat (given their labile nature), they may influence the metabolic environment for heterotrophic metabolism by regulating the fate of nutrients and organic matter at the peat surface (Wyatt et al. 2012). Algae can do this in several ways, including through the release of exudates (Bertilsson and Jones 2003), which typically consist of simple carbohydrates and amino acids (Wyatt et al. 2012) and promote heterotrophic metabolism in the surrounding biofilm (Kuehn et al. 2014). Other important functions of algae in wetlands are dependent on species membership, including nitrogen (N) fixation, which is limited to a few groups of heterocyst-forming cyanobacteria (i.e., blue-green algae). In conditions of low N availability, cyanobacteria can contribute substantially to N cycling in northern peatlands (Solheim et al. 2006), even when standing water is absent from the surface (DeLuca et al. 2002).

Processes associated with ongoing climate change may alter algal production and community structure in northern peatlands.

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In particular, more variable hydroperiods and permafrost degradation (Hinzman et al. 2005; Schuur et al. 2013) are anticipated to promote nutrient availability in surface waters across the boreal landscape (Flanagan et al. 2003). In temperate and subtropical regions, nutrient enrichment tends to increase algal growth and alter species composition (Goldsborough and Robinson 1996), with consequences for ecosystem function (Richardson 2010). Similar information is not available for algae in northern peatlands, where nutrient levels are typically low (Sorensen et al. 2012) and elevated nutrient levels are likely to coincide with warmer temperatures. Since many of the enzymatic reactions involved in photosynthesis are temperature-dependent (Davison 1991), warmer temperatures may enhance the influence of elevated nutrient levels on algal production in northern ecosystems where low mean annual temperatures tend to constrain metabolic activity (Rosa et al. 2013).

The goal of this study was to examine how nutrient supply and warming interact to influence aspects of algal and heterotrophic metabolism in an Alaskan poor fen. We manipulated nutrients (N and phosphorus (P)) and temperature (ambient and warmed) in a completely crossed experimental design using nutrient diffusing substrates (NDS) inside warming chambers to test the hypothesis that nutrient availability and temperature interact to regulate both algae and heterotrophic parameters in northern peatlands. We predicted that conditions of elevated nutrient availability and warming would accelerate algae and heterotrophic metabolism and that unbalanced nutrient supply would promote N fixation by cyanobacteria.

## Methods

### Site description

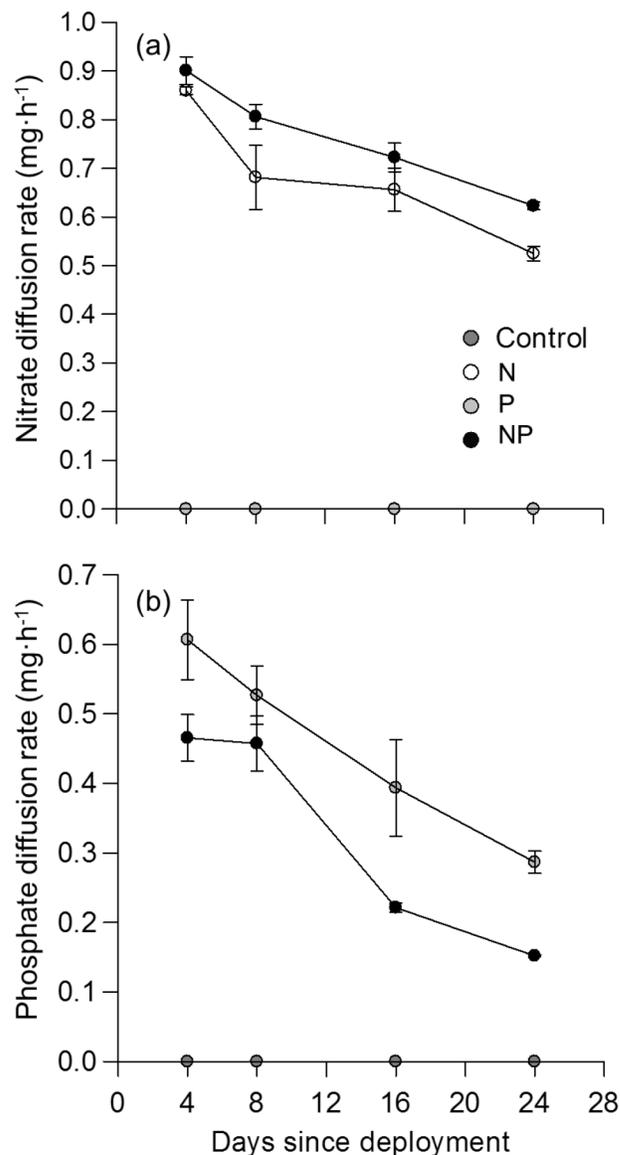
This study was conducted in a poor fen located within the floodplain of the Tanana River positioned just outside of the Bonanza Creek Experimental Forest and approximately 35 km southwest of Fairbanks, Alaska, USA (64°42'N, 148°18'W). This region within interior Alaska has a relatively short growing season ( $\leq 135$  days) with  $>21$  h of light per day in June. The fen site is characteristic of other peatlands within the region with a bryophyte community composed of *Sphagnum* species (*Sphagnum obtusum*, *Sphagnum platyphyllum*) and emergent vascular plants *Equisetum fluviatile*, *Carex* spp., and *Potentilla palustris*. There is minimum topographic variability across the fen, and peat depth exceeds 1 m at the center of the site. Concentrations of nitrate ( $\text{NO}_3^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ) are frequently below 23 and 5  $\mu\text{g}\cdot\text{L}^{-1}$ , respectively, and pH typically ranges from 5.48 to 6.54 during the summer growing season (Rober et al. 2014). A detailed description of the physical and chemical characteristics of the study site and comparisons among similar sites in the region are described by Rober et al. (2014).

### Experimental design

NDS were used to manipulate nutrient levels available for algae and heterotrophic bacteria within the fen (Tank et al. 2006). NDS were constructed from polyethylene canisters (35 mL volume) filled with agar enriched with one of three nutrient treatments (N, P, and N + P (NP)) as 0.5 mol·L<sup>-1</sup> N (50.6 g·L<sup>-1</sup> KNO<sub>3</sub>) and 0.5 mol·L<sup>-1</sup> P (68 g·L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>) (Fairchild et al. 1985) or a control with deionized water only ( $n = 3$  for each treatment). Our goal with enrichments was to determine which nutrient could be limiting, and we assumed the diffusion rates observed during laboratory assays (Fig. 1) would saturate algal growth rates because they exceeded those reported to be limiting for benthic algae in studies reviewed by Borcardt (1996). A 2.5 cm circular hole was cut into the lid of each canister, and a sponge disk was placed on the surface of the agar to serve as a substrate for algal and bacterial growth.

Prior to deployment, nutrient diffusion rates from the NDS were evaluated in the laboratory for 24 days. A single NDS cup was placed inside a 2 L beaker with 1 L deionized water at room temperature ( $n = 3$  for each nutrient treatment), and water was sam-

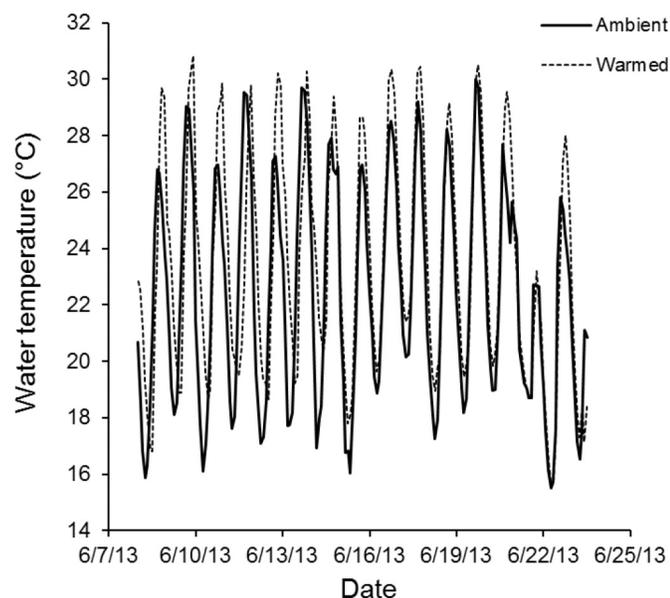
Fig. 1. Nitrate (a) and phosphate (b) diffusion rates from nutrient diffusing substrates ( $n = 3$  per treatment) incubated for 24 days prior to deployment.



pled for released  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  at 0, 4, 8, 16, and 24 days using a 0.45  $\mu\text{m}$  syringe-driven filter unit and analyzed on a Dionex ICS-3000 Ion Chromatograph (Dionex Corporation, Sunnyvale, California, USA). Plastic wrap was placed over each beaker to minimize evaporative water loss during the experiment, and water removed during sampling was replaced with deionized water (Fairchild et al. 1985). Release rates were calculated by multiplying the volume of water in each beaker by N or P concentration and then dividing by time elapsed between samples (Rugenski et al. 2008). Nutrient diffusion rates declined over time, but the NDS continued to release both N and P throughout the 24-day experimental period; control substrates released no measurable N or P (Fig. 1).

Nutrient diffusing substrates were placed inside cylinder mesocosms representing two temperature treatments, ambient (A) and warmed (W). Prior to the initiation of the study, a boardwalk was constructed to prevent the disturbance of sediments during experimental setup and regular sampling. Mesocosms were constructed of welded wire mesh rolled into a cylinder (40 cm in diameter) and wrapped with a layer of 0.1 mm thick transparent

**Fig. 2.** Daily variation in water temperature ( $n = 3$ ) in warmed and ambient treatments during the experiment. Date format on  $x$  axis is month/day/year.



plastic (Wyatt and Stevenson 2010). Mesocosms were evenly spaced throughout a 20 m × 20 m area of the fen, pushed into the sediments, and the top portion of mesocosms was trimmed to a height of 65 cm above the water surface for the warmed temperature treatment and 5 cm above the water surface for the ambient temperature treatment. The taller (65 cm) set of mesocosms facilitated passive warming by approximately 3 °C compared with the shorter (5 cm) mesocosms, which maintained ambient temperatures over 16 days (Fig. 2). The open-bottom and open-top design maintained connectivity between water inside mesocosms (approximately 24 L) and the pore water to better simulate natural fen conditions. A set of five NDS cups representing a single nutrient treatment (i.e., subsamples) were attached to a single piece of angle iron (40 cm long) and placed inside each mesocosm using hooks that could be repositioned as necessary to maintain the NDS at a constant depth of 15 cm below the water surface (approximately 4 cm above the peat surface). Nutrient diffusing substrates were allowed to colonize for 16 days beginning 8 June 2013. Each mesocosm represented a single replicate in statistical analysis ( $n = 3$  for each treatment), and each NDS cup was used as a subsample in subsequent analysis of algae and heterotrophic parameters.

### Sampling and analytical methods

Water temperature (°C) and photosynthetic active radiation (PAR,  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) were continuously monitored at a depth of 15 cm below the water surface inside mesocosms and in the open wetland using data loggers (HOBOTEMP, Onset Computer Corporation, Bourne, Massachusetts, USA) at 60 min intervals for the duration of the experiment. The height of the water table above the peat surface was measured with a metre stick, and dissolved oxygen (DO) and pH were measured with a calibrated Hach model 40d multiprobe (Hach Company, Loveland, Colorado, USA) every 2 days. After 16 days, water was collected from 5 cm below the water surface inside mesocosms and in the open wetland with a syringe and filtered for measures of dissolved nutrients with a 0.45  $\mu\text{m}$  pore-size filter into 120 mL sterile polyethylene bottles. Samples were frozen and later analyzed for  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  with an ion chromatograph (APHA 1998). Sponge disks were removed from the NDS with forceps for subsequent measurements of algae and heterotrophic bacteria after 16 days of incubation in experimental conditions.

Measures of algal parameters included biomass and productivity, taxonomic composition, exudate release, and N fixation. A single disk from each treatment was placed into a 300 mL clear biological oxygen demand bottle filled with filtered wetland water (collected from inside each respective treatment mesocosm) for measures of net primary production for 1 h in the light and then wrapped with aluminum foil for measures of oxygen consumption in the dark for an additional hour using a luminescent DO probe (Hach Company). Care was taken during this procedure to limit disruption of the intact biofilm, and bottles were incubated at a water depth of approximately 15 cm during midday hours (10 am and 2 pm) with a mean light level of 250  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . A 60 mL water sample was collected before and after each light-bottle incubation with a syringe and filtered through a 0.45  $\mu\text{m}$  pore-size filter for measures of exudate release as dissolved organic carbon (DOC) concentration ( $\text{mg}\cdot\text{L}^{-1}$ ) following Wyatt et al. (2014). Change in DO concentration in light and dark bottles was used to calculate net productivity and respiration, respectively, and net productivity values were converted into carbon units according to Wetzel and Likens (2000). Productivity rates were expressed per unit area, and exudate release was calculated as a percentage of gross primary productivity (GPP).

A separate set of disks was collected for measures of chlorophyll *a* (Chl *a*) concentration, algal cell abundance, and taxonomic composition. One subsample was placed into a 50 mL centrifuge tube and extracted with 90% ethanol for measures of Chl *a* ( $\text{mg}\cdot\text{m}^{-2}$ ) using a Shimadzu UV-Mini model 1240 spectrophotometer (Shimadzu Scientific Instruments, Columbia, Maryland, USA) after correcting for phaeophytin (APHA 1998). A second subsample was preserved in the field with a 2% formalin solution, and algal cells were later detached from sponges by probe ultrasonication followed by active scraping and brushing in preparation for algal composition analysis. Algal taxonomic composition was characterized by counting and identifying at least 300 natural units per sample using a Palmer-Maloney nanoplankton counting chamber and identifying algae at 400× magnification. We quantified benthic algal abundance ( $\text{cells}\cdot\text{cm}^{-2}$ ) using the formula provided by Lowe and Laliberte (2006).

Nitrogen fixation was estimated using the acetylene-reduction method (Stewart et al. 1967). One sponge from each treatment was placed into a 60 mL gas-tight screw-cap glass jar with septa filled with 45 mL of wetland water. Each incubation jar was injected with 3 mL of acetylene ( $\text{C}_2\text{H}_2$ ) gas generated from calcium carbide to achieve a headspace of approximately 20% acetylene gas (Stewart et al. 1967) and incubated in situ for 2 h. Acetylene is reduced to ethylene ( $\text{C}_2\text{H}_4$ ) by nitrogenase in N-fixing organisms and can therefore be used as an indirect estimate of  $\text{N}_2$  fixation (Sorensen et al. 2012). We collected 3 mL gas from each jar for an initial, middle, and final concentration after 5 min, 1 h, and 2 h incubation, respectively, in cleaned, pre-evacuated 3 mL blood serum vials (Tyko Healthcare Group, Mansfield, Massachusetts, USA). We replaced the headspace in each incubation jar after incremental sampling with a 20% acetylene gas mixture. Ethylene concentration in each sample was measured with a Hewlett Packard 5890A gas chromatograph (Hewlett Packard Company, Palo Alto, California, USA) equipped with a Poropak Q column and a flame-ionization detector using He as a carrier gas.

Bacterial abundance was determined from a subsample by direct counts with epifluorescence microscopy. A sponge was placed in a sterile 20 mL glass scintillation vial and preserved in the field with buffered formalin. In the laboratory, bacterial cells were detached from each disk by probe ultrasonication for 1 min on ice. Sample aliquots were stained with 4',6-diamino-2-phenylindole (DAPI) (Porter and Feig 1980) and vacuum-filtered onto a 0.2  $\mu\text{m}$  pore-size black filter (OSMONIC Inc., Livermore, California, USA). A minimum of 300 cells or 25 fields were counted per filter at 1000× magnification, and linear dimensions were measured with an ocular micrometer using a Leica DM 4000 microscope with fluorescence (Leica Microsystems, Wetzlar, Germany). Mean cell

**Table 1.** Measures of algal chlorophyll *a* (Chl *a*) concentration (mg·m<sup>-2</sup>), cell abundance (10<sup>5</sup> cells·cm<sup>-2</sup>), gross primary productivity (mg C·cm<sup>-2</sup>·h<sup>-1</sup>), and N fixation (μg ethylene·m<sup>-2</sup>·h<sup>-1</sup>) and bacterial biomass (μg C·cm<sup>-2</sup>) on nutrient diffusing substrates among nutrient treatments in ambient and warmed temperature conditions (mean ± 1 SE, *n* = 3).

	Treatment							
	Control		N		P		NP	
	Ambient	Warmed	Ambient	Warmed	Ambient	Warmed	Ambient	Warmed
<b>Algae</b>								
Chl <i>a</i>	7.37±2.06a	4.48±1.47a	22.6±0.53b	39.2±8.28c	9.48±5.18a	7.37±3.04a	47.4±11.1c	78.6±12.2d
Cell abundance	8.8±0.7a	5.2±0.7a	14.3±1.1b	21.6±1.2c	7.08±0.4a	10.90±0.6a	39.8±6.2d	55.6±1.7e
Gross productivity	6.78±0.71a	8.22±1.09a	14.8±3.04b	18.9±1.00c	6.7±1.8a	8.3±3.7a	44.4±3.75c	139.9±20.9d
N fixation	12.5±1.50a	16.5±0.13a	0.57±0.09b	0.98±0.49b	11.1±2.25a	14.3±5.04a	0.24±0.04b	0.54±0.94b
<b>Bacteria</b>								
Biomass	13.4±0.78a	14.3±1.43a	14.3±1.09a	17.7±1.30b	12.7±0.69a	14.1±0.84a	16.6±1.41b	20.7±0.60c

Note: Different letters indicate significant differences among treatments ( $p < 0.05$ ).

volume (μm<sup>3</sup>) was calculated by applying the relationship  $V = (w^2 \times \pi/4) \times (l - w) + (\pi \times w^3/6)$ , where *V* is volume and *w* and *l* are the cell width and length, respectively. Bacterial biomass was calculated as carbon content (CC) (fg of C per cell) from bacterial abundance and mean cell volume (*V*), using the allometric conversion factor  $CC = 435 \times V^{0.86}$  according to Loferer-Krösbacher et al. (1998).

### Data analyses

Differences in mean water temperature, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, water depth, DO, PAR, and pH among treatments were evaluated with analysis of variance (ANOVA) models. A three-way ANOVA was used to determine the independent and interactive effects of N (with and without), P (with and without), and temperature (warmed and ambient) on algal parameters and bacterial biomass. In instances when ANOVA indicated significant differences among treatments, Tukey's post hoc comparison of means tests were used to discriminate between different factor levels. A multivariate ANOVA was used to evaluate the independent and interactive effects of nutrients and warming on algal taxonomic composition. Algal community data were log(*x* + 1)-transformed to correct for non-normal distribution and unequal variances among treatments prior to analysis. All statistical analyses were performed with SYSTAT (version 11; SYSTAT Software Inc., Point Richmond, California, USA).

### Results

Mean water depth (19 ± 0.25 cm) and pH (6.52 ± 0.09) were relatively constant inside mesocosms and in the open wetland over time and were similar among treatments ( $p > 0.05$ ). Mean DO (mg·L<sup>-1</sup>) was similar between the ambient treatment (8.0 ± 1.1) and the open wetland (8.4 ± 1.1) ( $p = 0.38$ ), and DO was slightly elevated in the NP<sub>(W)</sub> treatment (9.4 ± 1.2), but differences were not statistically significant ( $p = 0.11$ ). Mean PAR (μmol·m<sup>-2</sup>·s<sup>-1</sup>) at 15 cm below the water surface was similar between the ambient (205.8 ± 63.1) and warmed (213.0 ± 87.5) mesocosms and between treatment mesocosms (ambient and warmed) and the open wetland (251.2 ± 50.7) ( $p = 0.87$ ). After 16 days, mean water column NO<sub>3</sub><sup>-</sup> concentrations (μg·L<sup>-1</sup>; ambient and warmed) were significantly elevated in the N (676.3 ± 42.5 and 647.7 ± 28.3) and NP (635.3 ± 24.8 and 621.7 ± 38.8) treatments compared with the P (39.0 ± 6.5 and 37.0 ± 3.1) and control (34.7 ± 3.8 and 34.2 ± 4.7) treatments and the open wetland (40.6 ± 4.1) ( $F_{[1,16]} = 826.3, p < 0.001$ ). Mean PO<sub>4</sub><sup>3-</sup> concentrations (μg·L<sup>-1</sup>; ambient and warmed) were significantly elevated in P (420.3 ± 17.1 and 438.7 ± 17.3) and NP (401.7 ± 36.25 and 369.7 ± 36.9) treatments compared with the N (26.6 ± 5.5 and 28.6 ± 4.2) and control (26.0 ± 4.8 and 24.7 ± 5.6) treatments and the open wetland (23.3 ± 3.8) ( $F_{[1,16]} = 461.9, p < 0.001$ ). Patterns of temperature variation were similar between ambient and warmed treatments (Fig. 2), and the mean temperature separation between the ambient and warmed treatments was 3.13 ± 0.14 °C throughout the experiment ( $F_{[1,16]} = 7.75, p = 0.006$ ). Water temperature was similar

between the ambient treatment (22.4 ± 0.37 °C) and the open wetland (21.9 ± 0.33 °C) throughout the study ( $p > 0.05$ ).

Nitrogen enrichment had a significant effect on algal biomass and productivity with and without P, and the effects of N enrichment were enhanced by warming. There was no effect of warming on algal parameters in the absence of nutrient enrichment ( $p > 0.05$ ). Measures of algal biomass and productivity were elevated by nutrient enrichment (Chl *a*:  $F_{[1,16]} = 1309.2, p = 0.008$ ; cell abundance:  $F_{[1,16]} = 1158.4, p < 0.001$ ; GPP:  $F_{[1,16]} = 0.85, p < 0.001$ ) and significantly greater in the NP<sub>(A)</sub> treatment compared with the N<sub>(A)</sub> treatment ( $p \leq 0.02$ ); algal parameters were significantly greater in the N<sub>(A)</sub> and NP<sub>(A)</sub> treatments compared with the P<sub>(A)</sub> and control<sub>(A)</sub> treatments ( $p \leq 0.04$ ; Table 1). Warming significantly enhanced the effect of N enrichment (N and NP) on algal biomass and productivity compared with ambient temperatures (Chl *a*:  $F_{[1,16]} = 7.27, p = 0.02$ ; cell abundance:  $F_{[1,16]} = 11.06, p = 0.004$ ; GPP:  $F_{[1,16]} = 18.0, p \leq 0.001$ ), and there was no effect of warming on algal parameters in the P and control treatments ( $p > 0.05$ ). Averaged across nutrient treatments, exudate production by algae was 16% (0.16 ± 0.04 mg C·h<sup>-1</sup>·mg<sup>-1</sup> fixed) of GPP.

Green algae (Chlorophyta) represented the most abundant taxonomic group (≥44%) among all nutrient treatments in warmed and ambient conditions (Table 2) and were significantly elevated in the N<sub>(W)</sub> and NP<sub>(W)</sub> treatments compared with the control ( $F_{[1,16]} = 6.67, p < 0.0001$ ). *Chlamydomonas* was the most abundant green algae in all treatments and was significantly greater in treatments with N enrichment (N, NP) compared with treatments without N enrichment ( $F_{[1,16]} = 12.8, p = 0.005$ ; Table 2). The green alga *Gloeochaete* was significantly enhanced by warming in the absence of nutrient enrichment ( $F_{[1,16]} = 18.4, p = 0.001$ ), and *Gloeocystis* was significantly greater in ambient treatments without N enrichment compared with ambient temperature treatments with N enrichment ( $F_{[1,16]} = 13.3, p = 0.004$ ; Table 2). The relative abundance of *Gloeocystis* was lower in the control<sub>(W)</sub> and N<sub>(W)</sub> treatments compared with control<sub>(A)</sub> and N<sub>(A)</sub> treatments ( $p < 0.05$ ) and greater in the NP<sub>(W)</sub> treatment compared with the NP<sub>(A)</sub> treatment (Table 2). *Oedogonium*, a filamentous green algae, was significantly reduced in warmed treatments compared with ambient treatments, independent of nutrients ( $F_{[1,16]} = 5.91, p = 0.03$ ). Euglenoids (mainly *Trachelomonas*) represented between 16% and 34% of cell abundance among all nutrient treatments in warmed and ambient conditions and were not significantly different among treatments (Table 2). Diatoms and chrysophytes composed only a small proportion (<2%) of the algal community among nutrient and temperature treatments and were not significantly different among treatments (Table 2).

Rates of N fixation were elevated in the presence of N-fixing cyanobacteria, which were most abundant in treatments without N enrichment. *Anabaena*, a N-fixing taxon, was significantly greater in treatments without N enrichment (P, control) compared with treatments with N enrichment (N, NP), independent of warming ( $F_{[1,16]} = 5.95, p = 0.03$ ; Table 2). Warming significantly increased the relative abundance of *Anabaena* in P<sub>(W)</sub> and control<sub>(W)</sub> treat-

**Table 2.** Mean relative abundance of algal taxonomic groups and genera among nutrient treatments in ambient and warmed conditions.

Algal taxa and taxonomic groups	Relative abundance							
	Control		N		P		NP	
	Ambient	Warmed	Ambient	Warmed	Ambient	Warmed	Ambient	Warmed
Chlorophyta	61.1a	43.9a	75.1ab	79.3b	65.2ab	50.2a	64.1ab	79.4b
<i>Chlamydomonas</i>	18.9a	31.8ab	55.0b	72.0b	22.7a	34.9ab	54.9b	63.8b
<i>Gloeochaete</i>	0.91a	8.58b	2.82a	4.41a	4.70a	1.86a	2.55a	3.20a
<i>Gloeocystis</i>	9.62a	0.61b	2.82c	0.82b	7.52a	6.27a	0.92b	2.44c
<i>Oedogonium</i>	10.9a	1.23b	1.51b	0.79b	12.7a	5.13c	4.77c	2.95b
Chrysophyta	1.24a	0.53a	0.26a	0.33a	0.19a	0.08a	0.06a	0.11a
Cyanobacteria	17.8a	20.7a	4.35b	1.72b	10.2a	18.2a	10.7a	1.52b
<i>Anabaena</i>	11.4a	18.2b	2.75c	0.83c	8.73a	14.6b	0.43c	0.41c
<i>Leptolyngbya</i>	2.42a	1.75a	0.84a	0.72a	0.43a	0.66a	9.55b	0.62a
Diatoms	1.63a	1.17a	1.25a	0.25a	1.35a	1.00a	0.61a	2.00a
Euglenoid	13.4a	34.5a	19.0a	19.3a	22.5a	30.6a	16.3a	17.4a
<i>Trachelomonas</i>	13.1a	32.5a	17.1a	18.3a	21.2a	29.4a	16.3a	17.4a

Note: Different letters indicate significant differences among treatments ( $p < 0.05$ ). Table includes only genera with  $>5\%$  relative abundance in any single treatment.

ments compared with the control<sub>(A)</sub> and P<sub>(A)</sub> treatments ( $p \leq 0.016$ ). *Leptolyngbya*, a non-N-fixing cyanobacteria, was significantly greater in the NP<sub>(A)</sub> treatment compared with all other treatments ( $F_{[1,16]} = 7.68$ ,  $p = 0.02$ ; Table 2). Rates of N fixation ( $\mu\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) were most elevated in treatments without N enrichment, which were significantly greater compared with treatments with N enrichment, independent of warming ( $F_{[1,16]} = 80.2$ ,  $p < 0.001$ ; Table 1).

Bacterial biomass ( $\mu\text{g C}\cdot\text{cm}^{-2}$ ) showed a similar response to nutrients and temperature as that observed in the algae. Bacterial biomass increased with nutrient enrichment ( $F_{[1,16]} = 24.3$ ,  $p < 0.001$ ) and was significantly greater in the NP<sub>(A)</sub> treatment compared with the N<sub>(A)</sub> treatment ( $p = 0.05$ ; Table 1); bacterial biomass was significantly greater in the NP<sub>(A)</sub> and N<sub>(A)</sub> treatments compared with the P<sub>(A)</sub> and control<sub>(A)</sub> treatments ( $p \leq 0.039$ ; Table 1). Warming significantly enhanced the effect of nutrients on bacterial biomass compared with ambient temperatures ( $F_{[1,16]} = 10.4$ ,  $p = 0.005$ ). Bacterial biomass was significantly greater in the N<sub>(W)</sub> and NP<sub>(W)</sub> treatments compared with the N<sub>(A)</sub> and NP<sub>(A)</sub> treatments ( $p \leq 0.015$ ; Table 1), and there was no effect of warming on bacterial biomass in the P<sub>(W)</sub> and control<sub>(W)</sub> treatments ( $p > 0.05$ ).

## Discussion

Aquatic ecosystems in northern boreal regions are often characterized by low energy, low temperatures, and a short growing season (Rouse et al. 1997; Duff et al. 1999). Nutrient levels also tend to be low in northern ecosystems, especially N, due in large part to slow rates of nutrient mineralization (Wrona et al. 2006; Sorensen et al. 2012). Low temperatures exacerbate the effect of minimum nutrient levels on metabolic processes by decreasing enzymatic activity, which reduces the efficiency of nutrients for energy production (Markager et al. 1999). Processes associated with ongoing climate change, including more variable hydroperiods and permafrost degradation (Schuur et al. 2013), are anticipated to promote nutrient availability in aquatic ecosystems at high latitudes (Bridgman et al. 1998; Flanagan et al. 2003), including peatlands (Kane et al. 2010). Given the current energetic restrictions on ecosystem metabolism, boreal ecosystems may be poised to respond rapidly to increased nutrient availability associated with a warmer climate. Although temperature is an important factor influencing the metabolic activity of aquatic organisms, most studies evaluating algae and heterotrophic responses to enhanced nutrient levels have been conducted in the absence of elevated temperature (Rosa et al. 2013).

Our results indicate that warmer water temperatures and nutrient enrichment will elevate algal metabolism in northern

peatlands, and the magnitude of increase will depend on the combination of nutrients released with accelerated nutrient cycling. Following Liebig's Law, we would expect for primary production to be restricted by a single limiting nutrient (Liebig 1855). In some cases, a second nutrient can almost simultaneously become limiting (i.e., co-limiting) after the primary nutrient constraint is lifted (Borchardt 1996). The role of N as the initial limiting nutrient was indicated by greater stimulation of algal production in treatments enriched with N alone compared with treatments enriched with P alone. This finding is interesting and may signal the importance of processes in northern wetlands, including denitrification, which has the potential to reduce N supply without a corresponding loss mechanism for P. Simultaneous or co-limitation was indicated by significantly greater algal production in treatments enriched with a combination of N and P compared with the addition of either N or P alone. Similar results have been reported in aquatic ecosystems across northern latitudes (Elser et al. 2007), where a combination of N and P tends to enhance algal production more so than single nutrient applications. In contrast, wetlands occurring in lower latitudes are typically limited by P owing to elevated background levels of N (McCormick and Stevenson 1998), and enrichment with P often decreases the abundance of native algae (i.e., cyanobacteria mats) in favor of filamentous forms (Gaiser et al. 2005, 2006). The results of our current study demonstrate the potential for nutrients (especially N) to stimulate algal production in a boreal poor fen and offer support for the hypothesis that nutrients are responsible for promoting algal growth when they are made available following the rewetting of previously dried sediments as observed previously in northern peatlands (Wyatt et al. 2012; Rober et al. 2013, 2014).

In contrast with ecosystems at lower latitudes where nutrient enrichment is often a consequence of direct inputs from human activities (e.g., urban and agricultural runoff), accelerated nutrient cycling in northern ecosystems is likely to be caused by, and confound with, variables related to climate change, including warmer temperatures (Rouse et al. 1997; Gudmundsdottir et al. 2011). We accounted for the predicted temperature increases by evaluating algal responses inside warming chambers simulating the range of temperatures expected for the boreal region (IPCC 2007). In contrast with our expectations, warming alone did not enhance algal metabolism. Reports for other aquatic ecosystems (e.g., streams, lakes) in the region have demonstrated that increases in temperature tend to promote algal photosynthesis (Baulch et al. 2005; Demars et al. 2011). Disparities in responses may be due in part

to differences in background levels of nutrients that occur among ecosystems, with peatlands typically among the most nutrient deplete (Wieder 2006). Additionally, the magnitude of warming in studies reporting an increase in algal metabolism was slightly more elevated than temperatures observed in the present study (e.g., Baulch et al. 2005; Demars et al. 2011), while studies evaluating a similar temperature increase found only small changes in algal production (Werner and Matthiessen 2013). A combination of warming and nutrient enrichment did, however, have a synergistic effect on algal metabolism, indicating that even minimum warming will further accelerate the effects of nutrient enrichment on algal production.

Overall, the taxonomic composition of algae described in this study was diverse and representative of an early-season community that commonly appears following the spring thaw in northern peatlands (Rober et al. 2014). Previous studies have found that filamentous green algae were the dominant taxonomic group in boreal peatlands, particularly during periods of elevated nutrient availability (Rober et al. 2013, 2014). However, in the present study, filamentous green algae did not represent a substantial portion of the algal community in enriched treatments, likely due to the timing of the study (following the spring thaw) and the short time frame allowed for in community development. Instead, the community was composed of small coccoid and colonial taxa (*Chlamydomonas*, *Gloeocystis*) and euglenoids (*Trachelomonas*), many of which are motile and able to readily colonize new substrates (Graham et al. 2009). These taxa, which are often considered pioneer species (Stevenson et al. 1996), are commonly inferior competitors for light and nutrients, and we expect that larger filamentous taxa would out-compete this group for space if given more time for community development.

Within the early-season community, nutrient dynamics had a strong effect on taxonomic composition with potential consequences for ecosystem function. A significant proportion of the algal community in low N conditions was composed of N-fixing cyanobacteria, which are among the few specialized organisms that can convert atmospheric N<sub>2</sub> to biologically available forms (Graham et al. 2009). This finding was expected, as N fixation by cyanobacteria are considered among the most important source of N to high-latitude ecosystems under low nutrient conditions (Solheim et al. 2006; Larmola et al. 2014). Conditions of elevated N availability are predicted to suppress the metabolically costly process of N fixation and result in lower overall N inputs from cyanobacteria (Howarth et al. 1988; Marcarelli and Wurtsbaugh 2006). A reduced abundance of cyanobacteria in N enriched treatments support this hypothesis, indicating that cyanobacteria will probably contribute less to ecosystem N inputs if N is made available through mineralization of organic matter. However, N-fixing cyanobacteria will likely remain a major contributor to the N pool under current conditions of low nutrient availability (Rober et al. 2013, 2014), and warmer soil and surface water temperatures will likely enhance the contribution of N-fixing cyanobacteria to ecosystem N inputs. The observed increase in N-fixing cyanobacteria and N fixation rates with warming is consistent with studies demonstrating that warming increases the physiologic ability to fix N<sub>2</sub> (Marcarelli and Wurtsbaugh 2006; Rosa et al. 2013) and provides support for a growing literature evaluating the release of metabolic constraints by warmer temperatures on nutrient uptake and cycling in aquatic ecosystems (Yvon-Durocher et al. 2010; Gudmundsdottir et al. 2011).

Measures of heterotrophic metabolism increased in step with algal production. Algae and heterotrophic bacteria grow in close association (Battin et al. 2007), and similar responses to nutrients and warming may not have been independent but instead linked to an exchange of resources within the biofilm (Kuehn et al. 2014). In a concurrent study, we demonstrated that nutrient enrichment alone had no effect on bacterial growth without the simultaneous addition of a carbon source (K.H. Wyatt and M.R. Turetsky, in review). Thus, patterns of heterotrophic bacteria in the present study were likely in response to increased algal production with nutrient enrichment

and warming. Our current estimates of exudates release indicate that algae were contributing up to 22.3 mg C·h<sup>-1</sup>·mg<sup>-1</sup> in N-enriched treatments compared with less than 1.5 mg C·h<sup>-1</sup>·mg<sup>-1</sup> in treatments without N, and a substantial portion (approximately 25%) of exudates was probably composed of simple carbohydrates (Wyatt et al. 2012). Studies have demonstrated that algae can promote heterotrophic metabolism (e.g., Rodríguez et al. 2013; Kuehn et al. 2014), and they may have been an important driver of heterotrophic metabolism during periods of elevated production in this current study. However, it is difficult to evaluate this hypothesis directly because of the absence of experimental conditions with nutrients and warming in the absence of algae.

The independent effects of nutrients and temperature have been studied extensively in response to eutrophication and thermal pollution at low latitudes (Borchardt 1996; DeNicola 1996) and climate change in lakes and rivers occurring at high latitudes (Baulch et al. 2005; Gudmundsdottir et al. 2011; O'Gorman et al. 2012). Less attention has been paid to examining the combined effects of warming and nutrients in wetland ecosystems, particularly those occurring in high latitudes, despite the potential direct and indirect effects of nutrients and warming on primary production. Our results indicate that warmer temperatures and nutrient enrichment will elevate algal and heterotrophic metabolism in northern peatlands, and the magnitude of increase will depend on the combination of nutrients available during periods of inundation. Algae grow in association with heterotrophic microorganisms on the peat surface, and the relevance of algal exudates (amino acids, simple carbohydrates) in the microbial loop places algae in a key position to enhance energy flow through detrital pathways in northern peatlands where decomposition is often limited by the availability of labile organic matter (Moore and Basiliko 2006). These results should be considered when addressing questions related to ecosystem metabolism in response to nutrient enrichment in a warmer climate. Studies that aim to evaluate algal responses to nutrient enrichment may be underestimating the magnitude of future algal production by as much as 70% if conducted in the absence of expected warming.

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