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The importance of nutrient co-limitation in regulating algal community composition, productivity and algal-derived DOC in an oligotrophic marsh in interior Alaska

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SUMMARY

1. Compared to lakes and streams, we know relatively little about the factors that regulate algae in freshwater wetlands. This discrepancy is particularly acute in boreal regions, where wetlands are abundant and processes related to climate change (i.e. increased permafrost collapse and soil weathering) are expected to increase nutrient inputs into aquatic systems. To investigate how accelerated nutrient inputs might affect algal structure and function in northern boreal wetlands, we added nitrogen, phosphorus and silica to mesocosms in an oligotrophic marsh in interior Alaska.

2. We conducted two *in situ* mesocosm enrichment experiments during consecutive summer growing seasons, each lasting 24 days. In 2007, we investigated the effects of +N, +P, +Si and +N+P+Si enrichment on benthic algal biomass (chlorophyll-*a*, ash-free dry mass, biovolume), chemistry (N : P ratio) and community composition. In 2008, we expanded our first experiment to investigate the effects +N+P, +N+Si, +P+Si and +N+P+Si on the same algal parameters as well as productivity (mg C m^{-2} h^{-1}).

3. In both experiments, we measured water-column dissolved organic carbon (DOC) inside treatment enclosures and related changes in DOC to standing algal biomass.

4. Benthic algal accrual did not increase following 24 days of enrichment with any nutrient alone or with P and Si together (+P+Si), but increased significantly with the addition of N in any combination with P and Si (+N+P, +N+Si, +N+P+Si).

5. Algal productivity (20 mg C m^{-2} h^{-1}) increased between three- and seven-fold (57–127 mg C m^{-2} h^{-1}) with the addition of N in combination with any other nutrient (+N+P, +N+Si, +N+P+Si). Water-column DOC concentration was significantly higher inside N-combination treatments compared to the control during each season, and DOC increased linearly with benthic algal biomass in 2007 ($r^2 = 0.89$, $P < 0.0001$) and 2008 ($r^2 = 0.74$, $P < 0.0001$).

6. Taxonomic composition of the wetland algal community responded most strongly to N-combination treatments in both seasons. In 2007, there was a significant shift from *Euglena* and *Mougeotia* in the control treatment to *Chroococcus* and *Gloeocystis* with +N+P+Si enrichment, and in 2008, a *Mougeotia*-dominated community was replaced by *Gloeocystis* in the +N+P treatment and by *Nitzschia* in +N+Si and +N+P+Si treatments.

7. Together, these data provide several lines of evidence for co-limitation, and the central importance of N as a co-limiting nutrient for the wetland algal community. Changes in

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algal dynamics with increased nutrient concentrations could have important implications for wetland food webs and suggest that algae may provide a functional link between increasing nutrient inputs and altered wetland carbon cycling in this region.

Keywords: Alaska, algae, climate change, dissolved organic carbon, nutrients, productivity, wetland

Introduction

Wetlands are widely distributed freshwater habitats with important ecosystem functions (Mitsch & Gosselink, 2006). Algal communities in wetlands are often taxonomically unique and important drivers of ecosystem function (Vymazal, 1995; Goldsborough & Robinson, 1996). In shallow wetlands, where sufficient light reaches the bottom, benthic algae can account for a significant amount of total primary production (Robinson, Gurney & Goldsborough, 1997b; McCormick *et al*., 2001), increase nutrient transformation and retention (Wetzel, 1996; Inglett, Reddy & McCormick, 2004) and are habitat and food for a variety of organisms (Campeau, Murkin & Titman, 1994; Liston, Newman & Trexler, 2008). Despite their importance, the major factors controlling algal communities in freshwater wetlands continue to be poorly understood relative to other aquatic habitats such as lakes and rivers (Stevenson, Bothwell & Lowe, 1996). This discrepancy is particularly acute in northern boreal regions, where wetlands are abundant and processes related to ongoing climate warming are expected to increase nutrient inputs into aquatic systems (Rouse *et al*., 1997).

Benthic algae are sensitive to changes in water quality, and nutrients are often the single most important factor regulating communities in freshwater habitats (Borchardt, 1996). A review of the literature allows us to make broad generalisations as to the importance of nitrogen (N) and phosphorus (P) limitation of benthic algae in lakes (Fairchild, Lowe & Richardson, 1985; Rodusky *et al*., 2001) and streams (Francoeur, 2001; Tank & Dodds, 2003), but there are too few data to make such generalisations about them in freshwater wetlands. Of the studies reviewed by Goldsborough & Robinson (1996), most investigations of N and P limitation of wetland algae have been limited to temperate and subtropical climes. Other potentially limiting nutrients, such as silica (Si), have received little attention in wetlands (but see Hooper-Reid & Robinson, 1978) even though diatoms frequently dominate benthic habitats.

Research on the effects of nutrient enrichment on wetland algae has been driven primarily by the need for management strategies to mitigate human impairment of wetlands (see review in McCormick & Stevenson, 1998). In the Florida Everglades, for example, there is an ongoing effort to develop algal-nutrient relationships to manage functional and structural changes in the native periphyton assemblage associated with agricultural and urban land use (Gaiser *et al*., 2004, 2006). In other regions, such as the northern boreal forest, algal-nutrient relationships in wetlands have been less studied, perhaps because it has been less directly impacted by human development. However, even in relatively remote areas of the boreal biome, such as the interior region of Alaska, anthropogenic sources of nutrient enrichment are apparent, as nitrate and ammonium concentrations in precipitation are enriched by six orders of magnitude relative to seawater (Hinzman *et al*., 2006). Boreal regions also are experiencing rapid climate change, which has led to a longer growing season with rising temperature (Chapin *et al*., 2006). Changes in thermal regime are expected to increase the extent of seasonal ice thaw, which will probably promote N and P mineralisation in the expanded active soil layer (Bridgham *et al*., 1995), as well as chemical weathering of parent rock material (Rouse *et al*., 1997). While regional variability of nutrient inputs may be significant, these changes are expected to have widespread impacts on nutrient concentrations of aquatic systems throughout the boreal forest (Rouse *et al*., 1997).

Wetlands are a dominant feature on the boreal landscape and may comprise the largest freshwater habitat directly affected by nutrient enrichment. In Alaska alone, wetlands make up more than 43% of the land surface, equivalent to approximately 60% of the total wetland area of the United States (Hall, Frayer & Wilen, 1994). Wetlands provide a number of

ecosystem services for boreal regions, including important summer nursery and stopover habitat for migrating waterfowl (Sedinger, 1997). Boreal wetlands also serve as an important global carbon reservoir (Bridgham *et al*., 2006), and there is an ongoing effort to identify processes that may alter carbon cycling in the region (Wickland, Neff & Aiken, 2007). A better understanding of the effects of nutrient enrichment on algal structure and function in boreal wetlands may help to identify and forecast changes in primary production and biogeochemical cycling associated with climate warming and increased N deposition throughout the region.

In this study, we manipulated water-column concentrations of N, P and Si in a completely crossed experimental design using mesocosms in an Alaskan marsh. We tested the hypothesis that nutrients are an important factor limiting algal biomass and constraining community structure in northern boreal wetlands and that increases in algal biomass would be driven by taxa requiring high nutrient conditions. Additionally, since algae can release significant amounts of carbon fixed during photosynthesis into the water column as dissolved organic carbon (DOC) (Myklestad, 1995), we hypothesised that water-column DOC concentrations would be related to algal accrual, and increase with algal biomass following nutrient enrichment.

Methods

Site description

We conducted this study in a freshwater marsh located on the floodplain of the Tanana River (latitude 64°42′ N, longitude 148°18′ W) just outside the Bonanza Creek Experimental Forest, approximately 35 km southwest of Fairbanks, Alaska, U.S.A. This region within interior Alaska experiences a relatively short growing season (135 days or less) with more than 21 h of light per day in June. The Tanana River floodplain is located within an intermontane plateau characterised by wide alluvium-covered lowlands and underlain by discontinuous permafrost (Begét, Stone & Verbyla, 2006). Oxbows and thaw ponds dominate the floodplain landscape, and fluvial deposition and erosion are annual disturbance events. The study site is characteristic of other marsh habitats that occur in oxbows along the flood plain, which are shallow with dense stands of beaked sedge (*Carex utriculata* Boott) and swamp horsetail (*Equisetum fluviatile* Linnaeus) surrounding open water pools with sparse (approximately 10% cover) emergent vegetation. The wetland supports a diverse grazer fauna, including wood frog tadpoles (*Rana sylvatica* LeConte) in early spring and high densities of the common pond snail (*Lymnaea spp*.) throughout the summer growing season. Background concentrations of inorganic nutrients were generally low during the study and within the range of other wetlands and lakes in the region (see Table 1). Phytoplankton biomass (measured as chlorophyll-*a*) was <0.28 μg L^{-1} throughout the growing season.

Nutrient enrichment

We manipulated nitrate, phosphate and silicate in a completely crossed design and *in situ* using mesocosms modified from the design described by Greenwood & Lowe (2006). A raised boardwalk was constructed prior to the beginning of the study to prevent the disturbance of wetland sediments during experimental set-up and regular sampling. We constructed 20 mesocosm enclosures by rolling-welded wire mesh into a cylinder (40 cm in diameter) and enclosing each cylinder with a layer of 0.1-mm thick clear window vinyl. Enclosures were evenly spaced throughout an area of the wetland with open canopy and pushed into the sediments so that approximately 15 cm extended above the water surface. This design allowed water inside enclosures to be in contact with sediments and also kept natural vegetation intact to simulate natural wetland conditions more effectively. We deployed *Equisetum fluviatile* stems, cut into 10-cm segments from live plants, as a standard substratum for sampling benthic algae inside treatment enclosures. We suspended stems attached to paper clips that could be repositioned to maintain a consistent depth of 5 cm below the water surface inside each enclosure.

We added nutrients from a stock solution to achieve concentrations for nitrogen (+N) of 1000 μg L^{-1} $NaNO_3$, phosphorus (+P) of 100 μg L^{-1} $NaPO_4$ and silica (+Si) of 20 mg L^{-1} Na_2O_3Si following each addition. We assumed these concentrations would saturate algal growth rates because they exceeded those reported to be limiting for benthic algae in studies reviewed by Borchardt (1996). Our

Table 1 Comparison of nutrient data from freshwater habitats within the boreal region of western North America

Habitat type	Country Region/ province	*n*	DIN μg L^{-1}	TN μg L^{-1}	SRP μg L^{-1}	TP μg L^{-1}	SiO_2 mg L^{-1}	Reference
Fen (riverine)	Alberta, Canada	1	16.2 ± 2.3		14.9 ± 3.9	102.4 ± 21.0		Bayley & Mewhort (2004)
Fen (floating)	Alberta, Canada	1	22.0 ± 5.2		6.0 ± 1.0	95.0 ± 13.1		Bayley & Mewhort (2004)
Marsh (lacustrine)	Alberta, Canada	1	265.7 ± 90.9		69.7 ± 26.2	354.7 ± 51.9		Bayley & Mewhort (2004)
Marsh (riverine)	Alberta, Canada	1	95.8 ± 41.3		9.1 ± 2.9	157.8 ± 16.9		Bayley & Mewhort (2004)
Wetland Lake	Alberta, Canada	148	17.9 (0.2–218.9)		35.2 (0.0–617.6)	123.2 (15.7–726.6)		Bayley & Prather (2003)
Lake (boreal-forest)	Yukon & Northwest Territories, Canada	17	21.5 (BD–220.0)	722.4 (259.0–1585.0)	1.9 (BD–7.5)	14.3 (4.3–35.4)	4.46 (0.17–12.47)	Pienitz *et al.* (1997a)
Lake (boreal-forest)	Northwest Territories, Canada	4	18.0 (2.0–29.0)	373.5 (247–478)	0.7 (0.6–0.8)	8.1 (3.9–9.6)	0.68 (0.92–0.41)	Pienitz *et al.* (1997b)
Fen (moderate-rich)	Interior Alaska, U.S.A	2	11.9 (7.8–15.9)	1795.0 (1090.0–2500.0)	2.8 (2.5–3.24)	35.0 (30.0–40.0)	3.22 (3.11–3.32)	K.H. Wyatt, unpubl. data
Marsh (riverine)	Interior Alaska, U.S.A	4	17.38 (5.0–33.0)	2000.0 (1120.0–3510.0)	2.54 (1.9–3.3)	32.2 (20.0–50.0)	6.23 (1.93–9.23)	K.H. Wyatt, unpubl. data
Marsh (riverine)	Interior Alaska, U.S.A (This Study)	1	6.6 (5.3–21.2)	1232.0 (960.1–1412.2)	9.1 (2.91–20.4)	23.3 (18.4–63.4)	9.4 (2.23–14.47)	This study
Lake (northern-southern forest)	Alaska, U.S.A	26	30.6 (16.0–385.0)	835.5 (208.0–2833.0)		37.2 (3.3–475.8)	2.69 (0.08–10.80)	Gregory-Eaves *et al.* (2000)

DIN, dissolved inorganic N; TDN, total dissolved N; TN, total N; SRP, soluble reactive P; TP, total P; BD, below detection; *n*, number of sites; mean and ±SE or range are given based on available information.

enrichments began after the seasonal thaw to simulate nutrient inputs from groundwater or surface water runoff (McDougal, Goldsborough & Hann, 1997). Our goals with enrichments were to ensure determination of which nutrient could be limiting and the potential magnitude of responses in an appropriate seasonal context.

Because of constraints on the area of the wetland that was suitable for experiments, we conducted half of the experiment during 2007 and half during 2008. Our first objective was to determine if the wetland algal community was nutrient limited and, if so, whether it was limited by a single nutrient or some combination of nutrients. In 2007, we randomly assigned each enclosure to one of three single-nutrient treatments (+N, +P or +Si) or a combination treatment (+N+P+Si), with four replicates each. We added nutrient amendments to enclosures every 4 days for 20 days beginning on 29 June 2007. The second phase of the experiment was conducted in June 2008 to determine which combination of nutrients was co-limiting. We deployed fresh *Equisetum* stems and randomly assigned each enclosure to one of three pair-wise nutrient treatments (+N+P, +N+Si, +P+Si) or +N+P+Si, with four replicates each. We added nutrient amendments to enclosures every 4 days for 20 days beginning on 17 June 2008. During each experiment (2007 and 2008), we used four enclosures without nutrients as a control treatment and, to evaluate container effects, designated four sampling sites within the wetland without enclosures or nutrient additions (open wetland).

In both experiments, we monitored changes in water depth inside each enclosure as well as in open wetland sites with a metre stick, and measured conductivity, temperature and pH every 4 days using a calibrated model 556 YSI® Multi-Probe (YSI Incorporated, Yellow Springs, OH, U.S.A.). We collected and filtered water for dissolved nutrient analysis immediately following each nutrient addition (every 4 days for 20 days) using a 0.45-μm Millex®-HA syringe-driven filter unit (Millipore Corporation, Bedford, MA, U.S.A.). We later analysed concentrations of dissolved inorganic N (DIN) as NO_3 + O_2 in water samples following the cadmium reduction method, of silicate (SiO_2) following the molybdate method using a Skalar® auto-analyser (Skalar Analytical, Breda, the Netherlands), and of soluble reactive P (SRP) using the ascorbic acid colorimetric method on a Genesys™ 2 UV-Vis spectrophotometer (Spectronic Analytical Instruments, Garforth, U.K.) (APHA, 1998). A portion of the filtered sample collected on day 24 was acidified and placed on ice in the field for later DOC analysis using a Shimadzu TOC-V carbon analyser (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.).

Collection and processing of benthic algae

In both experiments, we allowed algae to colonise *Equisetum* stems inside treatment enclosures for 24 days. We assumed this length of colonisation period allowed us to observe the algal response to nutrient inputs following the spring thaw, while minimising container effects. We removed algae from stems with a soft toothbrush and homogenised the resulting algal slurry from each treatment in 100 mL of filtered water for subsequent analyses. We filtered a known volume of each homogenate onto a GF/F glass fibre filter (Whatman, Springfield Mill, U.K.) and stored filters frozen in the dark for chlorophyll-*a* analysis. We later measured chlorophyll-*a* using a TD-700 fluorometer (Turner Designs, Sunnyvale, CA, U.S.A.) after extraction with 90% ethanol and corrected for phaeophytin (APHA, 1998). We preserved a separate aliquot with 2.5% formalin for algal compositional analysis and ash-free dry mass (AFDM) and placed a known volume on ice for algal chemistry analysis. We determined AFDM following standard methods (APHA, 1998). We dried samples at 105 °C for 48–72 h and then ashed them at 500 °C for 1 h in pre-weighed aluminium pans to measure dry mass and ashed mass, respectively. We analysed algal chemistry for total P (TP) and total N (TN) by oxidising particulate matter with persulphate and then analysing SRP following the ascorbic acid method and NO_3 following the second-derivative UV spectroscopy method (APHA, 1998). The proportion of N and P in samples was calculated by dividing the mass of N and P by AFDM, and nutrient content was reported per unit dry mass.

We homogenised preserved algal samples and identified and counted at least 300 cells per sample to genus using a Palmer-Maloney nanoplankton counter chamber (Wildlife Supply Company, Buffalo, NY, U.S.A.) at 400 magnification with a Leica model DM LB light microscope (Leica Microsystems,

Wetzler, Germany). Cell volume (μm^3 cm^{-2}) for each genus was determined by inserting average dimensions into geometric formulae from Hillebrand *et al.* (1999) and Wetzel & Likens (2000). We calculated the cell density (cells cm^{-2}) for each genus following Lowe & Laliberte (2006), and then calculated total biovolume by multiplying cell density by estimated cell volume.

During the 2008 experiment, we split a portion of each homogenised sample into two separate biological oxygen demand (BOD) bottles to measure benthic algal productivity (mg C m^{-2} h^{-1}) following McCormick *et al.* (1998). We filled each BOD bottle with filtered water from the wetland and recorded initial DO using a Hach HQ 40d luminescent DO probe (Hach Company, Loveland, CO, U.S.A.). We wrapped one bottle from each set with aluminium foil for incubation in the dark and determined production by measuring oxygen changes produced by algal samples incubated *in situ* in light and dark bottles. Light and dark bottles were used to measure net primary productivity (NPP) and respiration, respectively. We calculated gross primary productivity (GPP) following Wetzel & Likens (2000) and converted GPP values into units carbon based on a C : O molar ratio of 0.375 and a photosynthetic quotient of 1.2 (Wetzel & Likens, 2000).

Data analyses

Our analyses focused on variables indicative of algal structure and function, including chlorophyll-*a*, ash-free dry mass, total cell biovolume, productivity, N : P ratio, DOC concentration and percent of total biovolume of common genera. The distributions of variables were log (x + 1) transformed if necessary to correct for non-normal distribution and unequal variances among treatments prior to analysis.

Largely, because of space constraints within our experimental study area, our nutrient manipulations were conducted across two separate study years. We analysed the 2007 and 2008 experimental treatments separately using ANOVA models for two reasons. First, treatments were confounded with study year. Second, *t*-tests revealed differences in water-table between 2007 control data and 2008 control data, probably because of interannual variability in climate. *Post hoc* comparisons of means were performed using Tukey's Honestly Significant Difference (HSD) tests. All analyses were performed using SYSTAT (version 11.0; SYSTAT, Evanston, IL, U.S.A.).

In addition to the approach outlined above, we also evaluated differences in algal assemblages among treatments with an Analysis of Similarities (ANOSIM) using PRIMER for Windows (version 5.2.9; PRIMER-E Ltd., Plymouth, U.K.). ANOSIM operates directly on a dissimilarity matrix and tests whether there is a significant difference between two or more groups of sampling units. We used Bonferroni corrections for the algal assemblage analyses to preserve the experiment-wise Type I error rate of $P = 0.05$ (Zar, 1999). Finally, we used linear regression analysis to examine the relationship between algal biomass and water-column DOC following nutrient enrichment.

Results

Physical conditions and nutrient concentrations

Standing water ranged from 44–49 cm (mean 46 ± 1.60 cm) between June–July 2007 and from 14–28 cm (mean 22 ± 0.76 cm) between June–July 2008, and differences between seasons were statistically significant ($t = -13.54$, $P < 0.0001$). Background concentrations of inorganic nutrients in the control treatment were similar to the open wetland during each season (*t*-test, $P > 0.05$; Table 2). Conductivity, temperature and pH varied over time during each experiment but did not differ significantly among treatments (ANOVA, $P > 0.05$; data not shown).

In 2007, DIN in the +N+P+Si treatment increased to target concentrations following each N addition over 20 days whereas, in the +N treatment, DIN began to accumulate following enrichment on day 12,

Table 2 Mean (±SE) dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP) and silicate concentrations measured at open wetland sites and the control treatment (mesocosms without nutrient enrichment) measured every 4 days between June and July during each experiment, 2007 and 2008

	n	DIN (μg L^{-1})	SRP (μg L^{-1})	Silicate (mg L^{-1})
2007				
Open wetland	28	8.02 ± 1.28	8.69 ± 1.28	12.09 ± 0.49
Control treatment	28	13.85 ± 1.63	8.37 ± 1.77	7.53 ± 0.55
2008				
Open wetland	28	5.25 ± 1.25	9.98 ± 0.96	4.10 ± 0.37
Control treatment	28	9.75 ± 1.75	13.07 ± 1.98	3.09 ± 0.57

increasing to a mean of 4984.40 ± 459.90 μg N L^{-1} on day 20 (Fig. 1). Following the second P addition, mean SRP concentrations increased to 184.98 ± 31.45 μg P L^{-1} and 132.78 ± 25.26 μg P L^{-1} in +P and +N+P+Si treatments, respectively, but then increased to near target values throughout the remainder of the study (Fig. 1). Silicate concentrations in +Si and +N+P+Si treatments met or exceeded target values following each addition over 20 days (Fig. 1).

In 2008, DIN increased to near target concentrations following each N addition over 20 days (Fig. 2). SRP exceeded target concentrations with P enrichment, especially in the +P+Si treatment where SRP began to accumulate following enrichment on day 4, reaching 680.44 ± 74.76 μg P L^{-1} on day 20 (Fig. 2). Silicate concentrations met or exceeded target values following each addition over 20 days (Fig. 2).

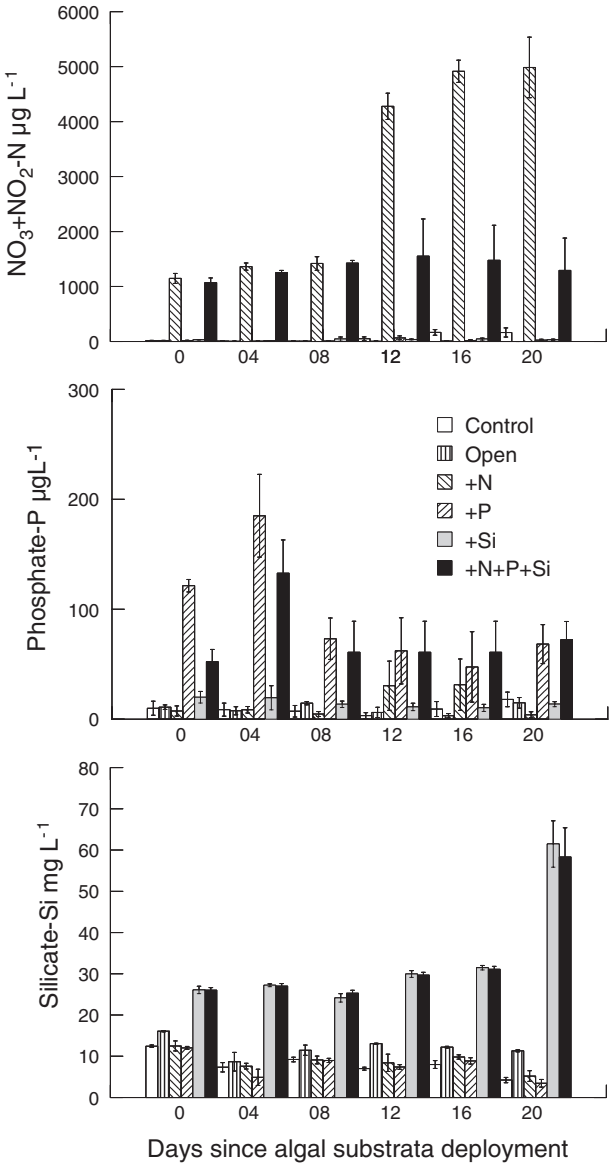

Fig. 1 Dissolved inorganic N (NO_3 + NO_2 − N), phosphate-P and silicate-Si concentrations among treatment enclosures following each nutrient addition during the 2007 experiment. Points are means of four replicates ± SE.

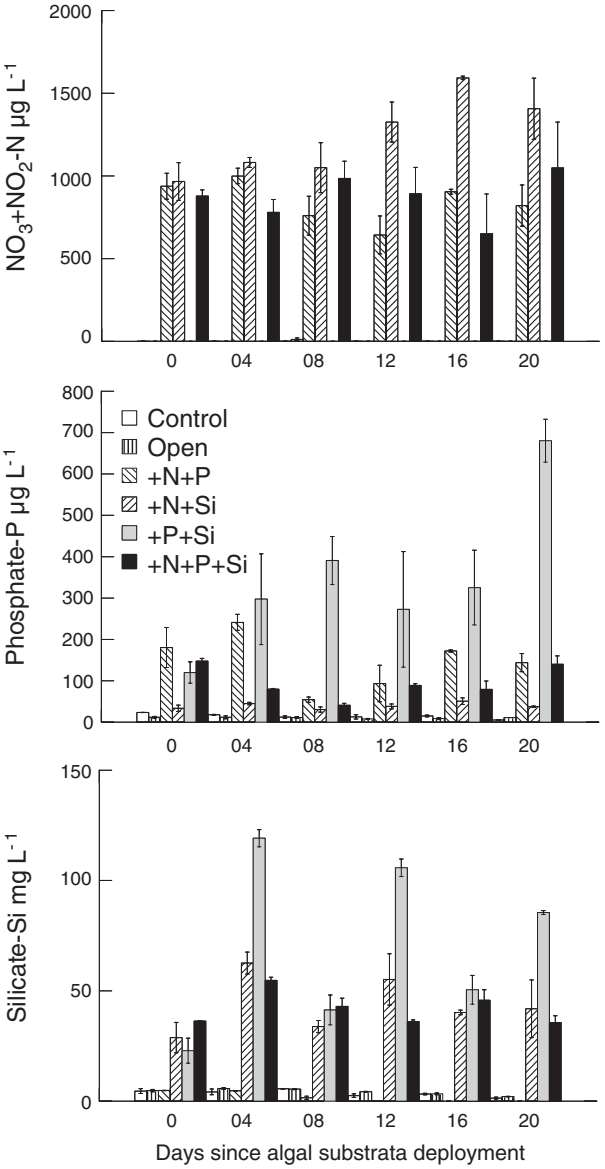

Fig. 2 Dissolved inorganic N (NO_3 + NO_2 − N), phosphate-P and silicate-Si concentrations among treatment enclosures following each nutrient addition during the 2008 experiment. Points are means of four replicates ±SE.

Benthic algal biomass, stoichiometry and productivity

Benthic algal biomass (chlorophyll-*a* concentration, g AFDM and total biovolume) was similar between the open wetland and the control treatment during each experiment (2007 and 2008) ($P > 0.05$) (Figs 3 & 4). In 2007, there was no increase in algal biomass with either nutrient alone ($P > 0.05$), but there was a significant increase in chlorophyll-*a* concentration ($F_{5,18} = 29.29$, $P < 0.0001$), g AFDM ($F_{5,18} = 32.68$, $P < 0.0001$) and total biovolume ($F_{5,18} = 6.76$, $P = 0.0010$) in the +N+P+Si treatment compared to the control treatment (Figs 3 & 4).

In 2008, chlorophyll-*a* concentration ($F_{5,18} = 26.12$, $P < 0.0001$) and total biovolume ($F_{5,18} = 14.76$, $P < 0.0001$) were significantly greater in +N+P, +N+Si and +N+P+Si treatments compared to +P+Si and control treatments (Figs 3 & 4). Ash-free dry mass was also significantly higher in +N+P and +N+P+Si treatments compared to +P+Si and control treatments ($F_{5,18} = 16.90$, $P < 0.0001$) (Fig. 3). Although mean AFDM was higher in the +N+Si treatment than in the control treatment, differences were not statistically significant ($P = 0.145$). All measures of algal biomass (chlorophyll-*a*, g AFDM, total biovolume) were similar between the +P+Si treatment and the control treatment ($P > 0.05$) (Figs 3 & 4).

In 2007, algal N : P ratios in the +N (21.59 : 1 ± 3.68) and +N+P+Si (14.48 : 1 ± 0.97) treatments were not significantly different on a mass basis, but both were higher compared to treatments without N addition (<5 : 1) ($F_{5,18} = 21.29$, $P < 0.0001$) (Fig. 5). In 2008, algal N : P ratios were significantly higher in the +N+Si treatment (31.27 : 1 ± 0.23) and lower in the +P+Si treatment (7.15 : 1 ± 0.47) compared to the control treatment ($F_{5,18} = 34.71$, $P < 0.0001$); and they were similar among +N+P (15.18 : 1 ± 2.05) and +N+P+Si (16.91 : 1 ± 1.14) treatments (Fig. 5).

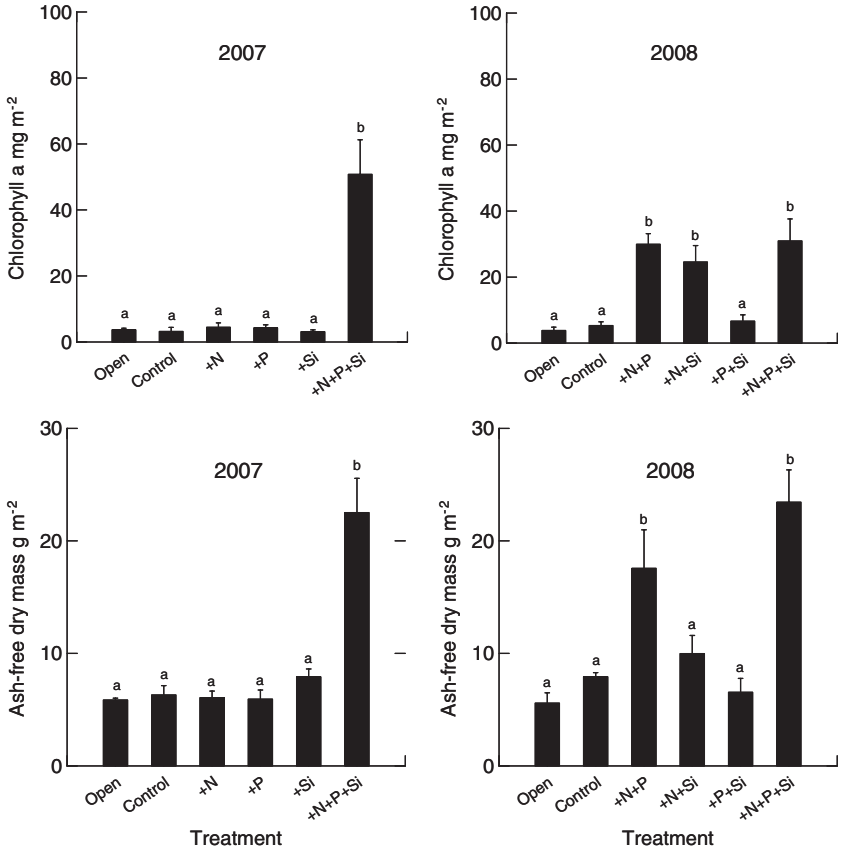

Fig. 3 Comparison of mean chlorophyll-*a* concentration and g ash-free dry mass among treatment enclosures and the open wetland in 2007 and 2008. Bars are means of four replicates ± SE. Significant difference indicated by different letters above bars (ANOVA, $P < 0.05$, Tukey's test $P < 0.05$).

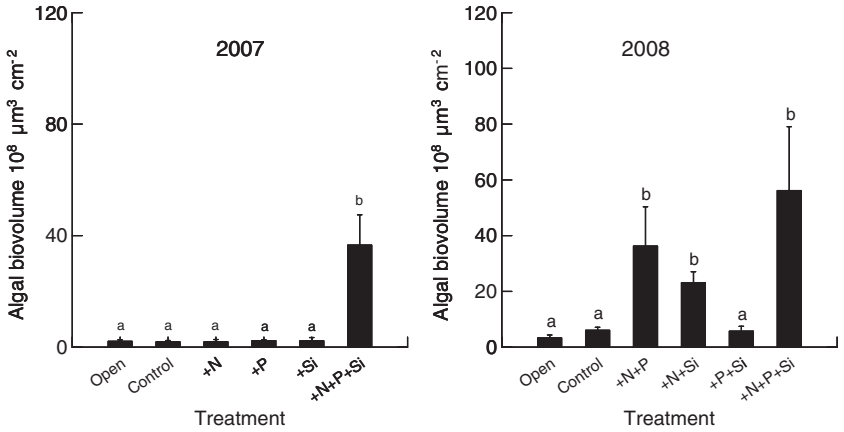

Fig. 4 Comparison of mean total biovolume μm^3 cm^{-2} among treatment enclosures and the open wetland in 2007 and 2008. Bars are means of four replicates ± SE. Significant difference indicated by different letters above bars (ANOVA, $P < 0.05$, Tukey's test $P < 0.05$).

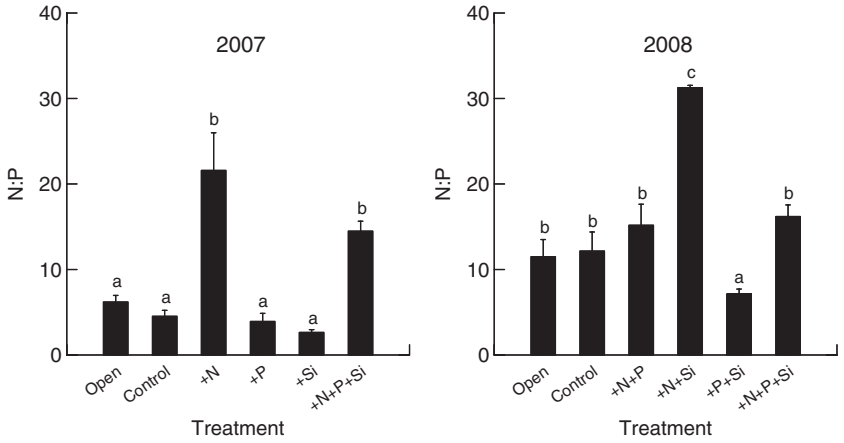

Fig. 5 Comparison of algal N : P ratios among treatment enclosures and the open wetland in 2007 and 2008. Bars are means of four replicates ± SE. Significant difference indicated by different letters above bars (ANOVA, $P < 0.05$, Tukey's test $P < 0.05$).

In 2008, benthic algal productivity (mg C m^{-2} h^{-1}) in the +N+P+Si treatment (127.98 ± 22.32) was significantly greater compared to +N+P and +N+Si treatments (73.59 ± 10.43 and 57.84 ± 9.53, respectively), and productivity rates in all N treatments were significantly higher compared to +P+Si (27.53 ± 5.16) and control (20.55 ± 5.32) treatments ($F_{5,18} = 13.82$, $P < 0.0001$) (Fig. 6). Algal productivity was similar among the +P+Si treatment, control treatment and the open wetland ($P > 0.05$).

In 2007, water-column DOC concentration (mg L^{-1}) was similar among individual nutrient treatments and the control treatment ($P > 0.05$), but DOC concentration was significantly higher in the +N+P+Si treatment (43.96 ± 1.50) than in the control treatment (20.72 ± 0.31) ($F_{5,18} = 400.98$, $P < 0.0001$) (Fig. 7). In 2008, DOC concentration was significantly greater in +N+P (49.61 ± 2.58) and +N+P+Si (47.49 ± 1.63) treatments than in the +N+Si treatment (39.56 ± 1.72), and DOC concentrations in all N-combination treatments (+N+P, +N+Si, +N+P+Si) were significantly greater compared to +P+Si (25.94 ± 1.06) and control (28.62 ± 0.80) treatments ($F_{5,18} = 31.89$, $P < 0.0001$) (Fig. 7). There was no difference in DOC concentration among the +P+Si treatment, control treatment and the open wetland ($P > 0.05$). Water-column DOC increased linearly with increasing standing algal biomass in 2007 ($r^2 = 0.89$, $P < 0.0001$) and 2008 ($r^2 = 0.74$, $P < 0.0001$) (Fig. 8).

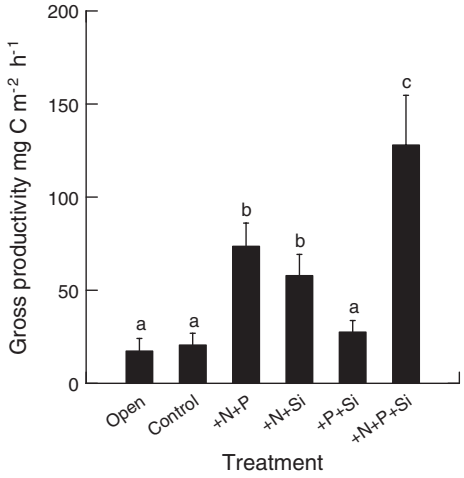

Fig. 6 Comparison of algal primary productivity mg C m^{-2} h^{-1} among treatment enclosures and the open wetland in 2008. Bars are means of four replicates ± SE. Significant difference indicated by different letters above bars (ANOVA, $P < 0.05$, Tukey's test $P < 0.05$).

Benthic algal community response to nutrient enrichment

Of the 43 genera identified in 2007 and 2008, seven comprised >80% of the total biovolume in both seasons (Fig. 9). Multivariate analysis (ANOSIM) indicated differences in the composition of benthic algal assemblages occurring in different nutrient treatments in 2007 (Global R = 0.510, P < 0.001) and 2008 (Global R = 0.311, P < 0.001). In 2007, the algal community in the open wetland was comprised primarily of *Mougeotia* (Chlorophyta), *Euglena* (Euglenophyta), *Anabaena* (Cyanophyta) and *Gloeocystis* (Chlorophyta), which made up approximately 39%, 25%, 13% and 12% of the total biovolume, respectively (Fig. 9). ANOVA indicated that all taxa represented a similar percent of total biovolume in the control treatment compared to the open wetland, except that *Mougeotia* ($F_{5,18} = 9.77$, $P < 0.0001$) was significantly lower, and *Euglena* ($F_{5,18} = 43.27$, $P < 0.0001$) significantly greater, in the control treatment than the open wetland. All taxa occurred at similar percent of total biovolume among individual nutrient treatments (+N, +P, +Si) and the control treatment (ANOVA, $P > 0.05$). In the +N+P+Si treatment, the percent of total biovolume of *Gloeocystis* ($F_{5,18} = 12.76$, $P < 0.0001$) and *Chroococcus* ($F_{5,18} = 7.66$, $P < 0.0001$) were significantly greater, and *Euglena* ($F_{5,18} = 43.27$, $P < 0.0001$) and *Mougeotia* ($F_{5,18} = 9.77$, $P < 0.0001$) significantly lower, than in the control treatment (Fig. 9).

In 2008, the percent of total biovolume of all taxa was similar between the open wetland and the control treatment (ANOVA, $P > 0.05$) and comprised primarily of *Mougeotia* (70% and 67%, respectively), *Euglena* (7% and 6%) and *Nitzschia* (Bacillariophyceae) (7% and 8%) (Fig. 9). *Nitzschia* increased to 56% and 71% of the total biovolume in +N+Si and +N+P+Si treatments, respectively, which were significantly greater than the control treatment ($F_{5,18} = 20.74$, $P < 0.0001$). The percent of total biovolume of *Gloeocystis* was significantly greater in the +N+P treatment compared

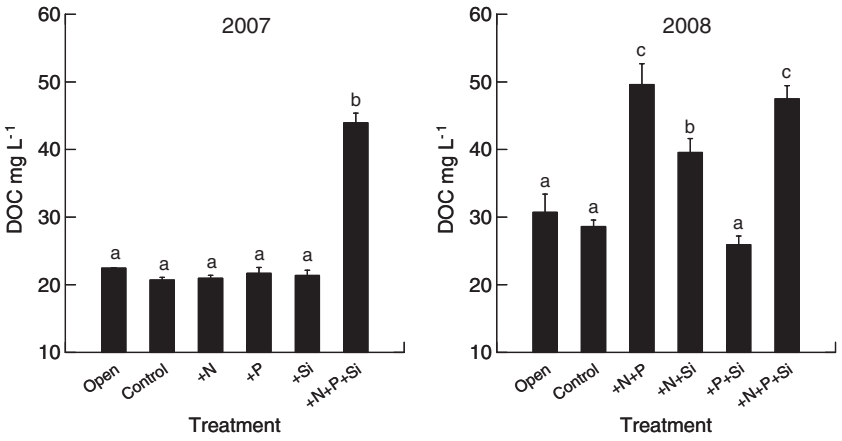

Fig. 7 Comparison of water-column dissolved organic carbon (DOC) mg L^{-1} among treatment enclosures and the open wetland in 2007 and 2008. Bars are means of four replicates ± SE. Significant difference indicated by different letters above bars (ANOVA, $P < 0.05$, Tukey's test $P < 0.05$).

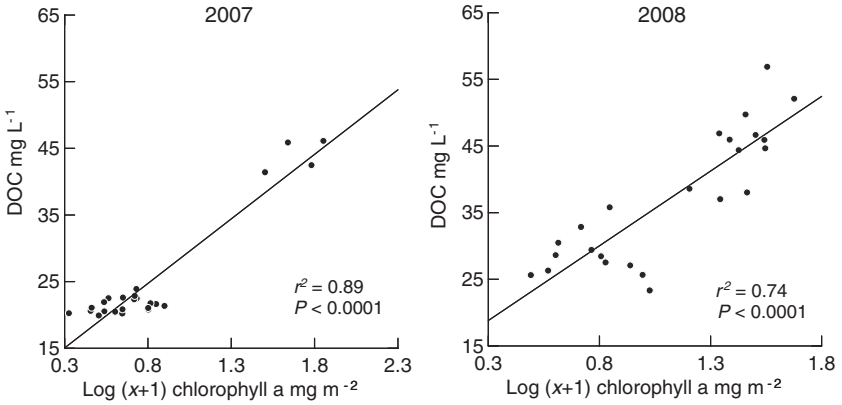

Fig. 8 Linear regression analysis between log (x + 1) Chlorophyll-*a* mg m^{-2} and water-column dissolved organic carbon (DOC) mg L^{-1} across all treatment enclosures and the open wetland in 2007 and 2008.

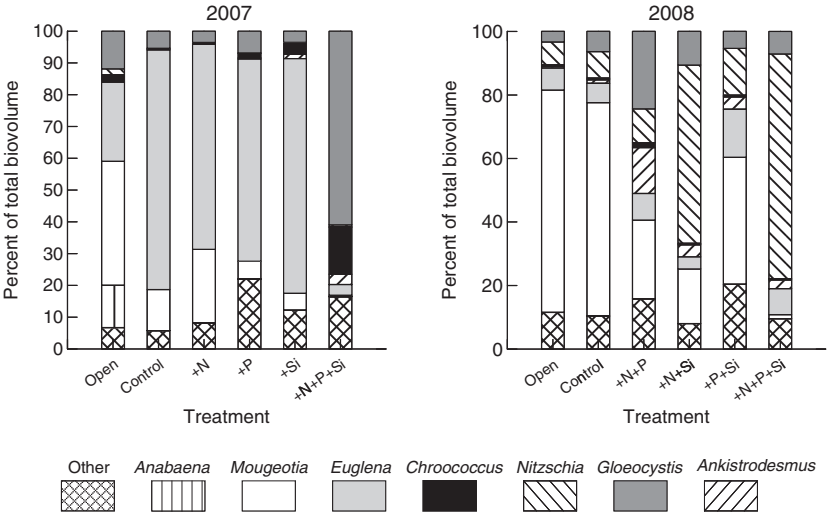

Fig. 9 Percent of total biovolume of dominant algal genera in the open wetland and treatment enclosures following 24 days of enrichment in 2007 and 2008.

to the control treatment (ANOVA, $F_{5,18} = 77.27$, $P < 0.0001$), and *Mougeotia* was significantly lower in all N-addition treatments (+N+P, +N+Si, +N+P+Si) than in the control treatment (ANOVA, $F_{5,18} = 8.19$, $P = 0.0004$) (Fig. 9). There were no differences in the percent of total biovolume of any taxa between the +P+Si and control treatments (ANOVA, $P > 0.05$).

Discussion

Our results provide several lines of evidence for nutrient co-limitation and the central importance of N limitation for regulating algal production and taxonomic composition in the wetlands of interior Alaska. Nutrient co-limitation was indicated by low background DIN and SRP concentrations in wetland water during each summer growing season and the lack of treatments responses to any nutrient alone. Additionally, algal N : P ratios increased to approximately 16 : 1 with the addition of N and P together but were symptomatic of N limitation with the addition of P without N, and P limitation with the addition of N without P. It was only in treatments with the highest N : P ratio (N treatments without P) that a pool of DIN remained unexploited and in treatments with the lowest N : P ratio (P treatments without N) that PO_4 began to accumulate in the water column. The central importance of N limitation was indicated by

significant increases in algal biomass and productivity with the addition of N in any combination with P and Si, but no biomass and productivity responses were observed in the +P+Si treatment.

Following Liebig's Law of the Minimum, we would expect algal growth to be regulated by the scarcest available resource, or a single limiting nutrient (Liebig, 1855). Simultaneous limitation by multiple nutrients, i.e. co-limitation (Borchardt, 1996), has been observed in freshwater systems occurring across high latitude regions, where combined N and P enrichment results in a larger increase in algal accrual than enrichment with either nutrient alone (Elser *et al*., 2007). More specific to wetland studies, similar results have been reported from Delta Marsh in southern Manitoba, Canada (see review in Robinson, Gurney & Goldsborough, 2000) and in the southeast United States (Scott, Doyle & Filstrup, 2005), where enrichment with either N or P alone induces limitation by the alternative nutrient. In contrast, our findings are markedly different from those for the Florida Everglades, which are naturally P limited, and enrichment results in the decrease in algal biomass as a result of the loss of the native cyanobacterial mat (see review in McCormick & Stevenson, 1998; Gaiser *et al*., 2005, 2006; Richardson, 2009).

We observed a significant shift in community composition in response to nutrient enrichment, which reflects the interaction between nutrient limitation and resource competition. In 2007, nutrient effects on the algal community were strongest in the +N+P+Si treatment, where *Euglena* was almost completely displaced by a combination of *Chroococcus* and *Gloeocystis*. Although similar shifts in response to nutrient enrichment have not been widely reported from other wetland studies, high abundances of *Chroococcus* have been reported in shallow lakes with high nutrient concentrations across North America (Komárek & Anagnostidis, 1998). The increase in *Gloeocystis* with nutrient enrichment is interesting, as it has been argued that mucilaginous taxa are good competitors for nutrients in shallow oligotrophic lakes and wetlands (McCormick *et al*., 1996). Their increase in relative biovolume in our study may reflect a high latitude community adapted to sequester available nutrients rapidly during the short summer growing season.

In 2008, the increase in diatom taxa following enrichment with Si along with N or N and P was surprising, since background concentrations of Si were an order of magnitude higher than those known to be growth saturating for phytoplankton (Hecky & Kilham, 1988). Diatom growth was constrained in the control and +N+P treatments, in which the filamentous green alga *Mougeotia* was abundant. The increase of *Nitzschia* with the addition of Si with N and/or P does offer support for early culturing experiments, which show that some benthic diatoms grow best when Si concentrations are greater than 30 mg L^{-1} (Chu, 1942). Higher Si concentrations may be needed to satisfy demand of benthic versus planktonic algae because a) densities of algae are higher on substrata than suspended in water and b) high algal density on substrata severely constrains nutrient supply because nutrient uptake rates exceed diffusion and mixing rates (Stevenson & Glover, 1993).

Although functional responses of the algal community as a whole were quite similar among years, seasonal shifts in community composition demonstrate the importance of temporal variability in shaping algal responses to nutrient inputs in the region. Shifts in community composition may reflect interannual variability in timing and concurrence of antecedent seasonal conditions, such as changes in the water-table resulting from seasonal drying and rewetting from seasonal flood pulses (*sensu* Junk, Bayley & Sparks, 1989). The water-table at our study site varied between the two study years and was on average 50% lower during the 2008 study. In particular, shallow wetlands such as our study site tend to be highly variable. Shallow conditions during the second year may have favoured filamentous taxa over euglenoid flagellates in the control treatment (i.e. Robinson, Gurney & Goldsborough, 1997a) and aided the resuspension of diatom cells from the sediments.

Ambient rates of algal productivity in the wetland (20 mg C m^{-2} h^{-1}) were similar to values reported from marshes in temperate climates (see review in Goldsborough & Robinson, 1996). Following enrichment with N in any combination with P and Si, productivity increased significantly and became more similar to daily values reported from oligotrophic subtropical wetlands (McCormick *et al*., 1998; Ewe *et al*., 2006). Assuming that peak macrophyte biomass at our site (47.23 g C m^{-2}; unpubl. data) is equivalent to annual net productivity (g m^{-2} year), our measured values of 52–341 g C m^{-2} $year^{-1}$ for benthic algae (based on 135 day ice-free period) are notably higher.

These results offer evidence in support of the hypothesis that algal productivity in wetlands can be as significant as that of macrophytes (Robinson *et al*., 2000), and as such, may support significant proportions of the secondary production in boreal wetlands.

The strong positive relationship between increasing algal biomass and water-column DOC concentration suggests that a significant portion of the carbon fixed by algae during photosynthesis was released into the water column as carbon exudates. It is widely accepted that phytoplankton lose significant amounts (5–35%) of photoassimilated carbon as organic compounds, much of which (80–90%) is often carbohydrates (Myklestad, 1995). However, there have been discussions in the literature as to whether this is a normal process performed by healthy cells or an overflow mechanism in response to low nutrient conditions (Sharp, 1977). We observed a significant increase in DOC concentration with high algal biomass resulting from nutrient enrichment (N : P ratio of approximately 16 : 1), suggesting that algae in high nutrient conditions may release significant amounts of DOC in boreal wetlands.

Many areas of the boreal biome such as western North America have undergone rapid climate warming in recent years, and climate models predict that temperatures will continue to increase with human activity (Serreze *et al*., 2000). There is uncertainty with respect to how some aspects of climate change will affect aquatic systems, but there is consensus that processes such as increased organic matter mineralisation and mineral weathering will lead to increased nutrient cycling and nutrient inputs into aquatic ecosystems (Carpenter *et al*., 1992; Rouse *et al*., 1997). Our findings suggest that an increase in N and P availability will probably increase benthic algal biomass and productivity and alter their community structure in northern boreal wetlands. Although the quantitative significance of algae as a food source has not been established for wetlands in this region, its potential importance is evident from the gut contents of animals from other wetland ecosystems (Browder, Gleason & Swift, 1994). From a management standpoint, alteration of the proportions and biomass of algal assemblages may be important because algal groups differ in their relative utilisation by consumers (Lamberti & Moore, 1984). Shifts in taxonomic composition, especially an increase in diatom abundance, may have important implications for secondary production in the wetland food web.

The results of this study are limited in scope as they only show algal response to nutrient enrichment in a single wetland complex within interior Alaska. Although background concentrations of inorganic nutrients at our study site are within the range of other wetlands and shallow lakes in the region, wetlands across the boreal biome will almost certainly respond to nutrients in different ways. Future research should include additional wetland sites that may vary in geology, food-web structure, energy and nutrient inputs. Also, research is needed to understand more completely the consequences of altered algal community dynamics for wetland secondary production (Sedinger, 1997), as well as the role of algal-derived DOC in wetland biogeochemistry (Reddy & DeLaune, 2008). This, coupled with a better understanding of permafrost degradation effects on water-table position and nutrient cycling, will help predict the consequences of climate change for the structure and function of wetlands, which are the most common freshwater ecosystem in this region.

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