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Source: Arctic, Antarctic, and Alpine Research, 46(1) : 191-205

Published By: Institute of Arctic and Alpine Research (INSTAAR), University of Colorado

URL: https://doi.org/10.1657/1938-4246-46.1.191

Arctic, Antarctic, and Alpine Research, Vol. 46, No. 1, 2014, pp. 191–205

Nutrient Uptake and Short-Term Responses of Phytoplankton and Benthic Algal Communities from a Subarctic Pond to Experimental Nutrient Enrichment in Microcosms

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Abstract

Climate warming is anticipated to affect high-latitude regions, including abundant ponds of the Hudson Bay Lowlands (HBL). However, it remains unclear if associated increased frequency of nutrient pulses will be rapidly consumed by aquatic biota and sediment or lead to a rise in pond-water nutrient concentrations. Here, we performed a nutrient-amendment experiment to examine short-term (≤72 h) nutrient uptake and identify the consumers of the added nutrients (planktonic vs. benthic communities). Microcosms (1 L) with and without sediment were experimentally amended with inorganic nitrogen (nitrate, ammonium) with and without phosphate. Amended nitrate and ammonium concentrations remained high in microcosms without sediments, and phytoplankton biomass did not change relative to the un-amended control. However, phosphate concentration declined significantly in microcosms without sediment, resulting in significant increase of phytoplankton biomass after 72 h. In the presence of sediment, amended nutrients were rapidly removed from the water, stimulating benthic algal biomass when phosphate was co-amended with ammonium or nitrate. Phytoplankton biomass was significantly elevated in microcosms with sediment compared to those without sediment, regardless of whether nutrients were amended or not, indicating that sediment and associated benthic biofilm stimulate phytoplankton growth, likely via supply of nutrients to the overlying water column. A key outcome of the experiment is that pulsed nutrients were taken up rapidly and primarily by the benthic community. Findings suggest that shallow ponds in the HBL are capable of rapidly consuming pulsed nutrient supplies, as may occur due to hydroclimatic events, climate warming and other disturbances.

DOI: http://dx.doi.org/10.1657/1938-4246-46.1.191

Introduction

Arctic and subarctic regions are experiencing more rapid climate warming than elsewhere (ACIA, 2004; Walsh et al., 2011). In the western Hudson Bay Lowlands (HBL), 25–40% of the land is covered by shallow water bodies (Duguay and Lafleur, 2003; Macrae et al., 2004). The abundant shallow lakes and ponds (collectively referred to hereafter as ponds) of the HBL play several important roles (Bello and Smith, 1990; Boudreau and Rouse, 1995; Macrae et al., 2004; Petrone et al., 2008; Symons et al., 2012), but concerns continue to grow because we do not know how they have been altered by pronounced climate change that has occurred during the past 50 years (Gagnon and Gough, 2005; Kaufman et al., 2009; Macrae et al., 2014), nor how they will respond in the future. Amongst its many effects, climate warming is expected to stimulate primary productivity of aquatic ecosystems in Arctic and subarctic regions by lengthening the growth season (Rouse et al., 1997; Smol and Douglas, 2007; IPCC, 2007), modifying precipitation patterns (Serreze et al., 2000), and increasing nutrient supply via acceleration of peat decomposition (e.g. Hobbie et al., 1999; Wrona et al., 2006; Chapin et al., 2005; Lougheed et al., 2011) and permafrost thaw (Keuper et al., 2012). Due to their relatively small water volumes, the shallow ponds have been identified as particularly responsive and vulnerable to global warming and changes in nutrient inputs (Rouse et al., 1997; Douglas and Smol, 1999; ACIA, 2004; Schindler and Smol, 2006; Prowse et al., 2006, 2011).

Increased supply of nutrients via the mineralization of peat (e.g. Chapin et al., 1995; Schuur et al., 2008; Nadelhoffer et al., 1991; Lipson et al., 2011) and permafrost thaw (Keuper et al., 2012) is anticipated under warmer conditions, which may increase nutrient transport via runoff (Hobbie et al., 1999; Frey and McClelland, 2009; Harms and Jones, 2012; Buckeridge and Grogan, 2010). Nutrient transport to ponds is expected to occur as episodic pulses during precipitation events, because this is when connectivity between peatlands and ponds occurs (Woo and Guan, 2006; Quinton and Marsh, 1999; Macrae et al., 2004). Nitrogen (N) may also enter ponds in pulses via wet deposition during rainfall events (Alexander and Barsdate, 1974; Holtgrieve et al., 2011). In fact, climate change and long-distance transfer of reactive N via the atmosphere have been associated with increased concentrations of N in lake sediment records in remote areas of the northern hemisphere (Holtgrieve et al., 2011), including ponds in the western HBL (Light, 2011). Yet, it remains unclear if such increases in N supply lead to increases in pond water nutrient concentrations and increases in the productivity of pond biota.

Limnological surveys have shown that ponds in the western HBL are oligotrophic, based on low concentrations of available

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nutrients, low N-to-phosphorus (P) ratios and low algal biomass in the water column (Rautio et al., 2011; Symons et al., 2012; Bos and Pellatt, 2012). Nitrogen limitation (e.g. Jansson et al., 1996; Levine and Whalen, 2001; Keatley et al., 2007) and co-limitation by N and P are common in oligotrophic freshwater systems (e.g., Dodds et al., 1989; Dore and Priscu, 2001). Previous studies have shown that shallow oligotrophic systems are highly responsive to nutrient inputs (e.g. Douglas and Smol, 1999; Hansson, 1988), and bioassay studies have shown that the uptake of added nutrients occurs rapidly (e.g. Hansson, 1988; Symons et al., 2012). But, uncertainty exists as to whether N or P is the main nutrient limiting primary producers in ponds of the western HBL. For example, using a spatial survey of water-column nutrient concentrations at 32 ponds in Wapusk National Park, Bos and Pellatt (2012) concluded that phytoplankton are likely P limited, based on the high molar ratio of total N (TN) to total P (TP) (mean = 133, range = 25–283). They suggested that P limitation of phytoplankton is due to contact of the water column with the sediments, which may serve as a N source. However, nutrient amendment bioassay experiments (7-day duration) by Symons et al. (2012) led to different conclusions for a set of 21 ponds in the same region. Their microcosm experiments utilized only pond water (i.e., sediments were excluded) and determined that phytoplankton growth was limited by P at 26% of the ponds, by co-limitation of N and P at 26% of the ponds, and by N at 13% of the ponds. Interestingly, phytoplankton were not limited by N or P at 38% of the ponds. Because their bioassays did not include addition of pond sediment, we continue to know little about the role of sediments and associated benthic biofilm on pond responses to nutrient additions, despite the fact that pond waters are in relatively close contact with the bottom sediments in the HBL (Alexander et al., 1989). In fact, the presence and absence of sediments in the studies by Bos and Pellatt (2012) and Symons et al. (2012), respectively, may account for their different conclusions about N- versus P-limitation. Also, the absence of sediment in bioassays by Symons et al. (2012) may be one factor accounting for the weak correspondence between nutrient limitation determined by their bioassays versus predicted nutrient limitation based on ratios of nutrients (TN/TP, DIN/TP, NO_3^-/TP) in the pond water (23.8%. 23.8%, 14.3%, respectively, for the three nutrient ratios). Thus, it remains unclear how rapidly pulses of nutrients are taken up by pond biota in the presence of pond sediment and associated benthic biofilm, and what the relative roles of phytoplankton and benthic algal communities are in mediating nutrient uptake.

Due to their shallow depths, aquatic production of Arctic and subarctic ponds is often dominated by benthic biota (Björk-Ramberg, 1985; Björk-Ramberg and Ånell, 1985; Bonilla et al., 2005), and the planktonic community forms a relatively smaller component (Bonilla et al., 2009). In these systems, the benthic community is often dominated by cyanobacteria (Tang et al., 1997; Quesada et al., 1999; Vincent, 2000; Jungblut et al., 2010), which are well adapted to cold, oligotrophic freshwater environments. Some of the cyanobacteria are able to fix atmospheric N, and they often grow along the sediment surface (epipelic) as a benthic biofilm or benthic mat with an associated microbial community (Vézina and Vincent, 1997; Vincent, 2000; Bonilla et al., 2005, 2009). Thus, the benthic producers have access to different sources and ratios of nutrients compared to planktonic producers (Hansson, 1988) and, consequently, their responses to climate-mediated alteration of nutrient supply may differ. For example, Björk-Ramberg and Ånell (1985) and Bonilla et al. (2005) found that phytoplankton in Arctic lakes responded to nutrient additions to the water column, whereas the benthic community did not because the benthic biofilm received its nutrients mainly from the sediments. Therefore, to characterize the response of subarctic ponds to increased nutrient inputs and to identify the drivers of these responses, planktonic and benthic communities should both be investigated. Because one pathway of increase in nutrient delivery to ponds of the HBL likely will occur via short-lived pulses during episodic runoff events from surrounding peatlands into the tundra ponds, it is important to determine how quickly pond communities can regulate terrestrial and atmospheric nutrient inputs after such episodes. This has relevance to sampling surveys, because if nutrients are consumed within a few hours (or one day) after a pulsed transport event, then limnological surveys may not be able to detect these potentially influential nutrient dynamics.

Here, we employ short-term (3 day) nutrient-enrichment microcosm experiments, both with and without surficial pond sediment and associated benthic biofilm (= epipelic algae plus microbial community), to assess if pond organisms and sediments rapidly consume pulses of nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}) added to the pond water, and to identify the consumers of the nutrients via short-term responses of the phytoplankton and epipelic benthic algal biomass. Specifically, the objectives are: (1) to examine whether pond water nutrient concentrations rapidly decline following a pulsed nutrient enrichment; (2) to determine the relative roles of phytoplankton versus sediments and the associated benthic biofilm in mediating nutrient concentrations following enrichment; and, (3) to assess which nutrients (inorganic N and P) have the largest effect on short-term (3 day) biomass responses in each community. We used amendments of NO_3^- (+*N treatment*), NH_4^+ (+*A treatment*), NO_3^- + PO_4^{3-} (+*N+P treatment*), and NH_4^+ + PO_4^{3-} (+*A+P treatment*). We did not include amendment of PO_4^{3-} alone because, at the time we designed our experiment, available evidence suggested that N was likely the limiting nutrient. For example, mid-20th century changes in C/N and $\delta^{15}N$ in sediment cores from several ponds in the western HBL near Churchill suggested algal community responses to an increase in N availability (Light, 2011). Bioassays by Symons et al. (2012) were not available when we conducted our microcosm experiment, but they suggest P limitation of phytoplankton growth is not very common (23.8% of ponds studied).

Study Area

Ponds in the western HBL typically range between 400 and 40,000 m^2 in surface area and mean water depth ranges from ~0.1 to 1 m (Macrae et al., 2004). Left Pond (unofficial name), the source of water and surficial sediment for the microcosm experiment, is a small (~700 m^2), shallow (mean water depth ≈ 0.2 m), oligotrophic, subarctic tundra pond located in the Churchill Wildlife Management Area of the HBL, near the Churchill Northern Studies Centre (CNSC; 58°44′49″N, 93°49′19″W; Fig. 1). It is a closed-drainage pond with no channelized surface inflows or outflows. Left Pond was selected as the source of water and sediment because it is representative of many other subarctic tundra ponds in the Churchill region of western HBL (Fig. 2), and because it lies close to CNSC. Close proximity of the pond to CNSC was important for two main reasons. First, we conducted the microcosm experiment at CNSC to reduce the risk of encounters between polar bears and personnel and equipment. Second, the experiment required transport of a large volume of pond water to the CNSC, and so close proximity to the pond reduced the logistical challenges. Left Pond possesses several limnological characteristics that are similar to a suite of ponds surveyed in the area near the CNSC in July of 2005 (Rautio et al., 2011), 2010 (White et al., 2014), and 2012

FIGURE 1. Photo of Left Pond taken on 17 July 2011 showing surrounding tundra landscape.

(Macrae and Fishback, unpublished data), and in Wapusk National Park in July of 2004 (Bos and Pellatt, 2012) and 2009 (Symons et al., 2012) (Fig. 2). Compared with these spatial surveys, Left Pond is relatively small and shallow, but values of most water chemistry variables are similar to other ponds in the region (Fig. 2). Concentration of NO_3^- in Left Pond falls below the 25th percentile of other ponds near the CNSC, but above the 90th percentile of the ponds surveyed in Wapusk National Park. The bottom of Left Pond consists of soft, unconsolidated, organic-rich sediments (>0.4 m thick and >85% organic content; Light, 2011) that are underlain by permafrost. Macrophytes are very sparse to absent and the bottom is covered by a benthic biofilm. We observed copepods, cladocerans, and tadpoles in the pond. The shores are vegetated with sedges, mosses, and low growth-form shrubs (e.g. *Betula glandulosa*, *Salix candida*, *S. planifolia*).

Methods

Experimental additions of N and P to microcosms were used to assess the rate and timing of nutrient uptake, and the role and short-term (72 hour) responses of natural phytoplankton and benthic communities. The microcosms were designed to be representative of the pond, yet allow nutrient manipulations capable of isolating responses of the phytoplankton from those of the benthic biofilm. For this study, we define the benthic biofilm as the biotic community in the upper ~1 cm of sediment, including epipelic algae and microbes. Each microcosm consisted of a 1000 mL cylindrical clear-plastic, closed-bottom container with a perforated lid that permitted the exchange of gases with the atmosphere. Two categories of microcosms were used: those without sediment, which contained only pond water (-sediment), and those with pond water overlying the upper ~3 cm of surficial pond sediment with intact benthic biofilm (+sediment). Comparisons of nutrient treatments in the +sediment and –sediment categories were used to determine responses of planktonic versus benthic communities. Short sediment cores were collected from Left Pond using a Lucite tube with 7.6-cm internal diameter, and the upper ~3 cm of surficial sediments (with associated benthic biofilm) were carefully removed using a vertical sectioning device (Glew, 1988) and placed in the +sediment microcosms on 17 July 2011. Pond water (700 mL from Left Pond) was then added to each microcosm (+sediment and –sediment). Addition of zooplankton was avoided by filtering the water through numerous pinholes punctured through the plastic lids. For microcosms in the +sediment category, water was added carefully to avoid disturbing the benthic biofilm and sediment. Zooplankton could have entered the +sediment microcosms in the added sediment, but none were observed in any microcosms during the course of the experiment.

The microcosms were placed in a shallow pool (12 cm deep, hereafter referred to as the experimental pool) containing water from Left Pond to mimic the temperature and light environment of tundra ponds. An experimental pool was used instead of Left Pond itself, because we needed to place the experiment near the CNSC to facilitate access during frequent subsampling and to reduce risk of encounter with polar bears. The experimental pool provided a thermal environment that closely mimicked a nearby pond (Strange Pond, 30 cm mean water depth) where water temperatures were monitored at the same time as our experiment (Fig. 3). Water temperatures measured in the experimental pool and Strange Pond closely followed temporal variations in air temperatures. The experimental pool and Strange Pond exhibited similar heating patterns during each day, including comparable peak daily water temperature. However, due to shallower water depth, the experimental pool cooled more rapidly and to lower temperatures during night. Water temperature in the experimental pool was not higher than occurred in Strange Pond, indicating that rates of biological activity should not have been elevated in the experimental microcosms compared to other shallow ponds. Unfortunately, direct measurements of temperatures in Left Pond are not available due to equipment malfunction, but based on the close proximity between Left and Strange ponds (<2 km) and comparable water depth (20 versus 30 cm mean depth, respectively) water temperatures were likely similar.

Individual microcosms in the +sediment and –sediment categories were randomly assigned to one of five different nutrient treatments: added NO_3^- (*+N treatment*), added NH_4^+ (*+A treatment*), added NO_3^- and PO_4^{3-} (*+N+P treatment*), added NH_4^+ and PO_4^{3-} (*+A+P treatment*), and an un-enriched control (termed experimental control) for the 72 h experiment (Table 1). A randomized factorial experimental design was adopted given the potential synergistic effects of combined nutrients (*sensu* Elser et al., 1990; Nydick et al., 2004; Bonilla et al., 2005). The 30 individual microcosms (3 independent replicates for each of the 10 treatment combinations [i.e., 2 sediment categories × 4 nutrient amendment treatments, and 2 sediment categories × the experimental control]) were randomly assigned to locations within the experimental pool. Before nutrients were added, the microcosms were allowed to acclimate for 32 h in the experimental pool.

We determined the amount of nutrients (NO_3^-, NH_4^+, and PO_4^{3-}) that were added to the microcosms, the duration of the experiment, and the timing of subsampling based on a set of preliminary nutrient uptake experiments (duration = 72 h) we performed four weeks before the experiment reported here. The preliminary experiments

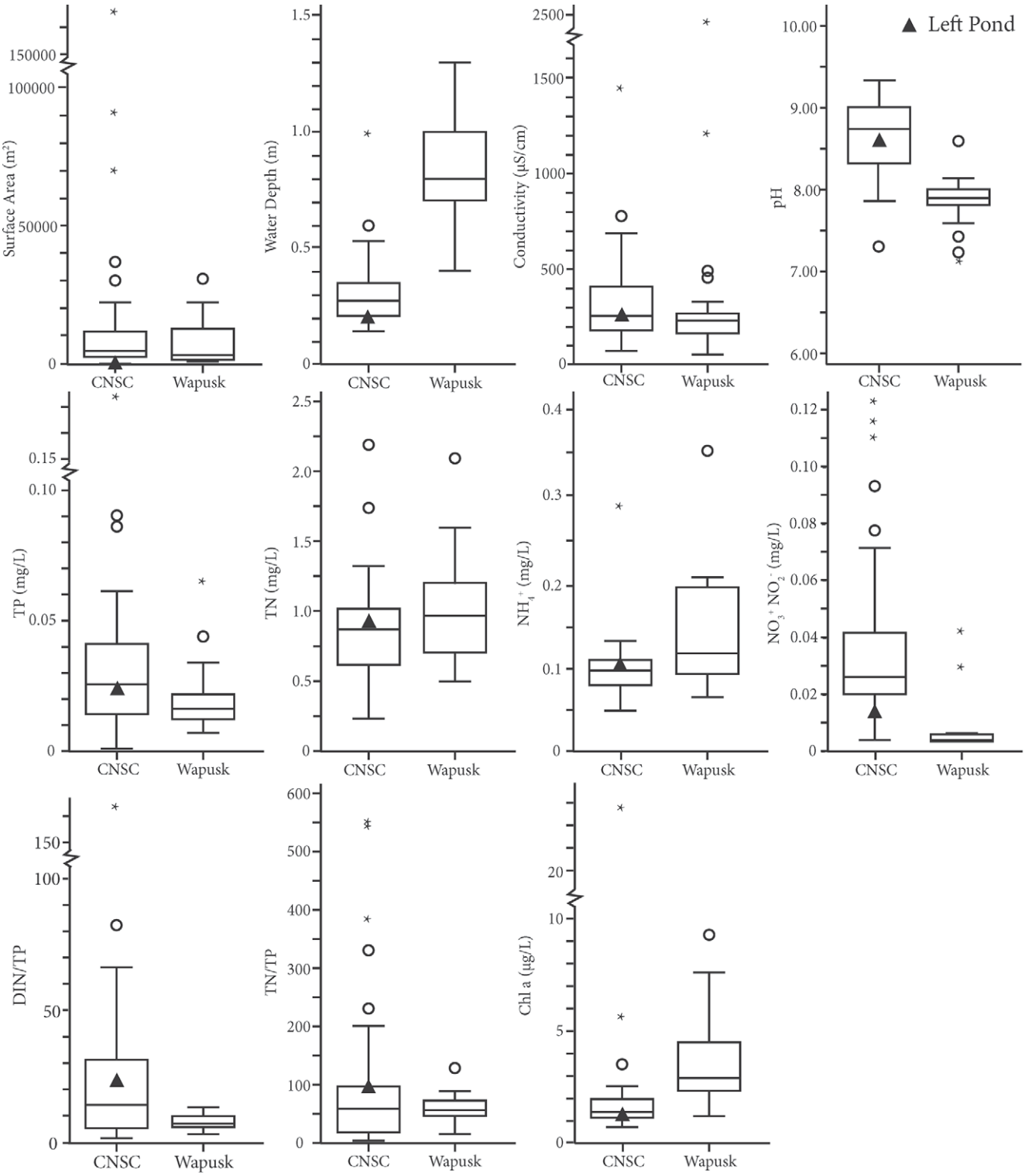


FIGURE 2. Box plots showing physical and limnological characteristics of ponds located in the Churchill region (near the Churchill Northern Studies Centre [labeled CNSC]) and in Wapusk National Park [labeled Wapusk]). The boxes show the 25th, 50th (median), and 75th percentiles, and the whiskers represent the 10th and 90th percentiles. The study pond (Left Pond) is shown as a solid triangle with the CNSC data. Data for box plots were pooled from Bos and Pellatt (2012) (24 ponds sampled in July 2004; 21 classified as Wapusk, 3 classified as CNSC), Rautio et al., (2012) (averages of 3 dates in July 2005 for 3 ponds; classified as CNSC), Symons et al. (2012) (12 ponds sampled in July 2009; classified as Wapusk), Macrae et al. (2004) (20 ponds measured in July of 1995 and 1997; classified as CNSC), White et al. (2014) (22 ponds, sampled once in July 2010; classified as CNSC), and Macrae and Fishback (unpublished data) (averages of 4 sampling dates in July 2011; classified as CNSC). Data for Left Pond are a mean of data in July of 2010 collected by White et al. (2014) and 2012 by Macrae and Fishback (unpublished data).

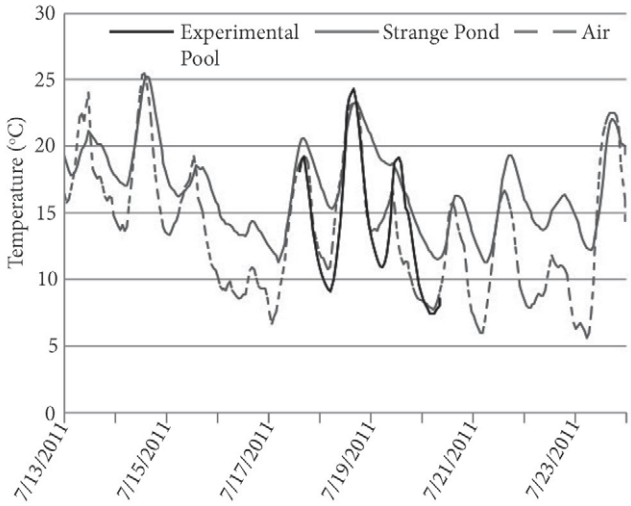


FIGURE 3. Air temperatures and water temperatures recorded in the experimental pool where the microcosms were incubated and a nearby pond (Strange Pond) during the experiment. Dates are m/dd/yyyy.

measured the decline in nutrient concentrations after they were supplied at three different concentrations for each nutrient (10×, 20×, and 50× the highest concentrations measured in Left Pond during the prior summer of 2010 [PO_4^{3-}-P: 0.002 mg L^{-1}; NH_4^+-N: 0.164 mg L^{-1}; NO_3^--N: 0.4 mg L^{-1}; White et al., 2014), which resulted in the following final concentrations in the amended microcosms, respectively, for PO_4^{3-}-P: 0.02 mg L^{-1}, 0.04 mg L^{-1}, and 0.1 mg L^{-1}; NH_4^+-N: 1.64 mg L^{-1}, 3.27 mg L^{-1}, and 8.2 mg L^{-1}; NO_3^--N: 0.4 mg L^{-1}, 0.8 mg L^{-1}, and 2.0 mg L^{-1}. The results of the preliminary nutrient-uptake experiments indicated the 20× additions of NO_3^- (2.14 mL of 0.8 mg NO_3^--N L^{-1}added to microcosms) and NH_4^+ (2.14 mL of 3.27 NH_4^+-N mg L^{-1}, added to microcosms) provided sufficient analytical precision to detect differences in the uptake of the nutrients between the treatments and the control by the end of the experiment. Because the natural concentration of soluble reactive P is very low in Left Pond, and ponds in this region in general (Rautio et al., 2011; Bos and Pellatt, 2012; Symons et al., 2012), we amended a higher concentration (50×, 5.36 mL of 0.1 mg PO_4^{3-}-P L^{-1} added to microcosms) that allowed us to detect differences in PO_4^{3-} between treatment and control concentrations (Table 1). The preliminary experiments also showed that nutrient uptake occurred rapidly. Nutrient levels declined to those in the experimental control by 48 h for the 20× and 10× NO_3^- treatments (50× NO_3^- concentration remained elevated at 72 h, when the experiment ended), by 48 h for the 10× NH_4^+ treatment, and by 72 h for the 20× NH_4^+ treatment (50× NH_4^+ concentration remained elevated at 72 h). Hansson (1988) also observed that added PO_4^{3-} declined in the water column to the pre-enrichment level over the course of three days when benthic algae covered the sediment. The final experiment was run for 72 h because the preliminary experiment demonstrated that 72 h exceeded the potential duration of nutrient removal to control levels and allowed us to effectively track nutrient uptake following the added nutrient pulse.

The nutrient amendments were initiated on 19 July 2011 at 08:30. For analysis of changes in NH_4^+, NO_3^-, and PO_4^{3-} concentrations, a 50 mL syringe was used to collect depth-integrated water samples from the microcosms at 0, 4.5, 12.5, 28, and 72 h after nutrient additions. Immediately following water sampling, the remaining water in each microcosm was mixed gently and aerated using a small syringe to avoid development of anoxic conditions. During the mixing and aeration, care was taken to avoid disturbing the sediment and benthic biofilm in the +sediment microcosms. Subsamples of water for analysis of NH_4^+, NO_3^-, and PO_4^{3-} concentrations were filtered immediately through a 0.45-µm cellulose acetate filter and refrigerated until analysis. Analysis for PO_4^{3-} concentration was performed within one week of sample collection. Nutrient analyses were performed at the Biogeochemistry Lab, University of Waterloo, using standard colorimetric techniques (Bran Luebbe AA3, Seal Analytical, Seattle, U.S.A., Methods G-189-097 [TKN], G-102-93 [NH_4^+-N], G-109-94 [NO_3^-N], G-103-93 [PO_4^{3-}P]).

At the end of the experiment (72 h after the nutrient amendment), water from all microcosms was sampled to determine phytoplankton chlorophyll *a* (Chl a) concentration. For this, a measured volume of water from each microcosm (150–328 mL) was filtered through a glass fiber filter (Whatman GF/F, pore size 0.7 µm) and frozen until analysis. Extraction and quantification of Chl a concentrations were performed on these samples at the Aquatic Ecology Group Analytical Laboratory at the University of Waterloo using a standard fluorescence technique described by Stainton et al. (1977).

Samples of surficial sediment (upper 1 cm) were obtained from all the +sediment microcosms at the end of the experiment (i.e., 72 h after the nutrient amendment) for analysis of photosynthetic pigment concentrations in the benthic biofilm

TABLE 1

Final concentration of the amended nutrients in water of the microcosms for each of the treatment groups (four different nutrient amendments and an experimental control without nutrient addition) and the chemical formulae of the salts that were added. Water in the microcosms came from Left Pond. Samples of the upper 3 cm of sediments from Left Pond, with associated benthic biofilm, were added to half of the microcosms and half of the microcosms did not receive sediment. The experiment included three independent replicate microcosms for each of the 10 treatment groups (5 treatment groups without sediment + 5 treatment groups with sediment).

Treatment	Final concentration (salt added)
Experimental Control	No addition
+NO_3^- (+N)	0.80 mg NO_3^--N/L (KNO_3)
+NH_4^+ (+A)	3.27 mg NH_4^+-N/L (NH_4Cl)
+NO_3^- + PO_4^{3-} (+N+P)	0.80 mg NO_3^--N/L (KNO_3) and 0.10 mg PO_4^{3-}-P/L (Na_2HPO_4)
+NH_4^+ + PO_4^{3-} (+A+P)	3.27 mg NH_4^+-N/L (NH_4Cl) and 0.10 mg PO_4^{3-}-P/L (Na_2HPO_4)

(including Chl a content). The sediment samples were obtained using an open-ended 5 mL syringe and plunger (surface area of 1.13 cm^2, sediment volume range: 1.07–2.32 cm^3), and were filtered immediately after collection onto Whatman GF/F glass-fiber filters, and frozen until analysis. For analysis by High Performance Liquid Chromatography (HPLC), pigments from each sample were extracted into a solution of Acetone:Methanol:Water (80:15:5 by volume) for 24 h at –20 °C. Samples were then filtered through 0.22 µm polytetrafluoroethylene (PTFE) syringe filters to remove large particles and other impurities. The filtered samples were dried under inert gas (N_2) and re-eluted in 500 µL of injection solution (Acetone:Ion Pairing Reagent:Methanol; 70:25:5 by volume). Samples were then analyzed using a Waters 2695 HPLC, reverse-phase system with a Symmetry C18 column (3.5 µm) following the procedure described by Mantoura and Llewellyn (1983) and modified by Leavitt et al. (1989). A gradient delivery of 2 mobile phases was used to separate the pigments. Mobile phase A consisted of Methanol:IPR (90:10 by volume), whereas phase B consisted of Methanol:Acetone (73:27 by volume). Sudan II was used as an external standard at either end of the run, as well as an internal standard in each sample to account for dilution and injection errors. Geranium samples were used at the beginning and end of each run to account for shifts in retention time during each run. Pigments were identified using a Waters 2998 PDA detector and a Waters 2475 Fluorescence detector. Pigment identification was based on chromatographic mobility (Leavitt et al., 1989) and spectral characteristics following standards from Jeffrey et al. (1997). Our system quantified the content of pigments from all algae and plants (Chl a, β-carotene), chlorophytes (Chl b), total cyanobacteria (echinenone, canthaxanthin), N-fixing cyanobacteria (aphanizophyll), and siliceous algae and some dinoflagellates (fucoxanthin, Chl c2). Because zeaxanthin (cyanobacteria) and lutein (Chlorophyta) did not separate on our HPLC system, they are reported here together. Concentrations of pigments were expressed in molar units as aerial concentrations (nMoles m^{-2}) for both water and sediment samples.

NUMERICAL AND STATISTICAL ANALYSES

To assess nutrient uptake during the experiment, one-way analysis of variance (ANOVA) tests were used to determine if the molar concentrations of dissolved nutrients in water of the microcosms (NH_4^+-N, NO_3^--N, and PO_4^{3-}-P) differed among treatments (nutrient amendments and experimental control with sediment, nutrient amendments and experimental control without sediment). The tests were performed on data obtained for 0, 28, and 72 h after the nutrient amendments to explore patterns of nutrient uptake over time during the experiment. For all tests that identified a significant difference among treatments, subsequent Tukey's post-hoc tests were run to determine which pairs of treatment groups differed. For all statistical tests, alpha was set at 0.1.

To assess the influence of nutrient amendments on phytoplankton biomass, a two-way ANOVA test was used to determine if there was an interactive effect of the nutrient amendments and the presence/absence of sediment in the microcosms on mean phytoplankton Chl a concentration in water of the microcosms at the end of the experiment (i.e., after 72 h of incubation following nutrient amendments). Since no interactive effect occurred ($P > 0.1$), we performed two separate one-way ANOVA tests to determine if phytoplankton Chl a concentration differs among nutrient amendments and the experimental controls in the presence of sediment (+sediment category) and again in the absence of sediment (–sediment category). Finally, a randomized block ANOVA test was used to determine if phytoplankton Chl a concentration differed after 72 h of incubation due to the presence versus absence of sediment, after controlling for effects of the nutrient amendments. Tukey's post-hoc tests were run to determine pair-wise differences for all the above tests that produced significant results.

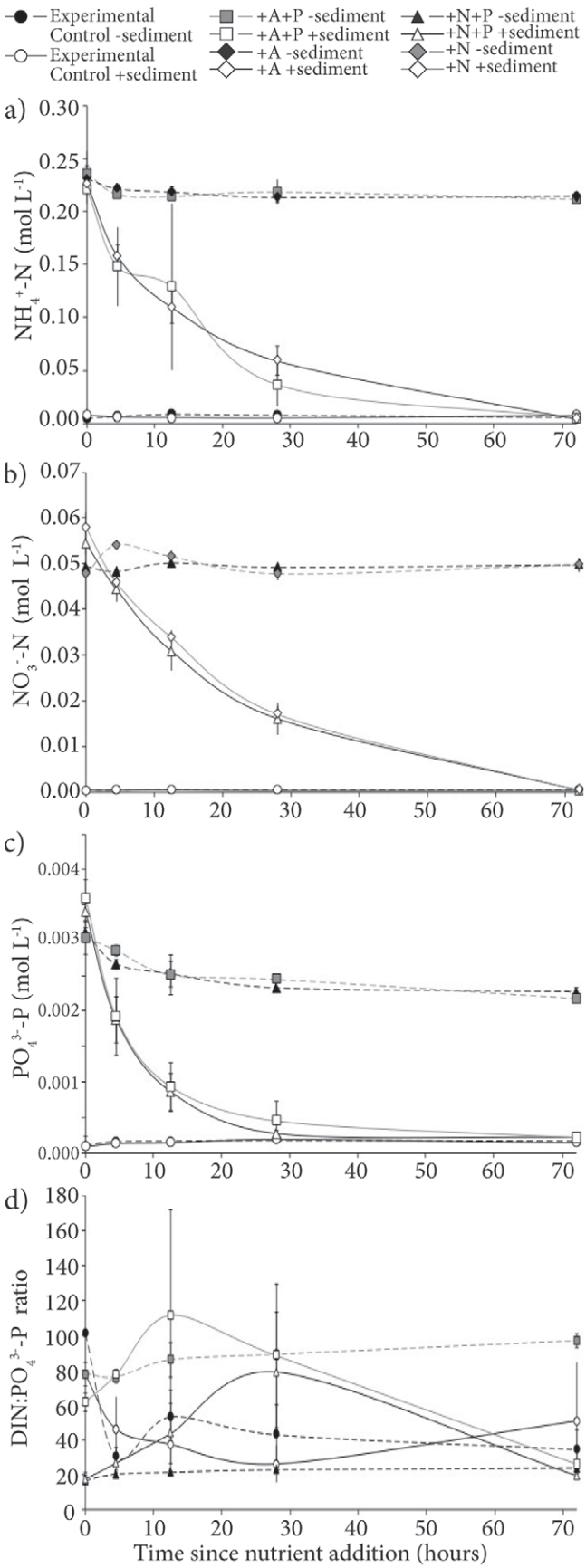
The responses of the epipelic algae after 72 h of incubation with the nutrient amendments and the experimental control were assessed using a series of numerical and statistical methods. First, multivariate principal components analysis (PCA) was performed to explore differences among nutrient amendments on patterns of the abundance and composition of algal pigments in the surficial sediment samples (i.e., the benthic biofilm). Then, an analysis of similarities (ANOSIM) test was performed to determine if the abundance and composition of the photosynthetic pigments differed among the nutrient amendments. Prior to running the PCA ordination and the ANOSIM test, pigment concentrations were log(x+1)-transformed. Since the ANOSIM test identified that the abundance and composition of benthic algal pigments differed among nutrient amendments, a series of one-way ANOVA tests were performed to identify which of the pigments differed among the nutrient amendments after 72 h of incubation. For the one-way ANOVA tests with significant outcomes, Tukey`s post-hoc tests were used for subsequent pair-wise comparisons.

All ANOVA tests were performed using the software SPSS (v.19). PCA was performed using the software CANOCO version 4.5 (ter Braak and Šmilauer, 2002). ANOSIM tests were performed using the software PRIMER version 6 (Clarke and Gorley, 2006).

Results

NUTRIENT CONCENTRATIONS FOLLOWING NUTRIENT AMENDMENTS

In microcosms that served as experimental controls (i.e., they received no nutrient amendments), concentrations of NH_4^+-N, NO_3^--N, and PO_4^{3-}-P remained low and relatively constant throughout the 72-h incubations, and concentrations of these chemical species did not differ significantly in microcosms with sediment versus those without sediment (Fig. 4, parts a–c; Table 2). In contrast, amendments with NH_4^+, NO_3^-, and PO_4^{3-} resulted in significant initial elevation of their concentrations in all microcosms (+sediment and –sediment) relative to experimental controls that did not receive nutrient amendments. However, uptake of the dissolved N and P species differed tremendously depending on whether sediment was present or absent in the microcosms. After the nutrient amendments, concentrations of NH_4^+-N, NO_3^--N, and PO_4^{3-}-P in the water declined rapidly (within the first 4.5 h) in microcosms with sediment present (+sediment category), but not in those without sediments (–sediment category; Fig. 4). In the +sediment microcosms, patterns of decline of NH_4^+-N and NO_3^--N concentrations were similar, and they were not altered markedly by the co-addition of PO_4^{3-} (Fig. 4, parts a and b; Table 2). By 72 h of incubation, concentrations of NH_4^+-N and NO_3^--N in +sediment microcosms were reduced to values indistinguishable from those of the experimental controls that received no nutrient amendment. Concentrations of PO_4^{3-}-P in the +sediment microcosms became equivalent to those of the experimental controls after 28 h of incubation, and patterns of decline in PO_4^{3-}-P concentration did not differ if PO_4^{3-} was



amended with NH_4^+ or NO_3^- (Fig. 4, part c; Table 2). In contrast, concentrations of NH_4^+-N and NO_3^--N in the microcosms without sediment (–sediment category) remained significantly elevated compared to the experimental controls, and remained similar throughout the duration of the experiment at near-initial values (Fig. 4, parts a and b; Table 2). PO_4^{3-}-P concentrations declined in microcosms without sediment, but at a much slower rate than was observed in the microcosms with sediment (Fig. 4). After 72 h, PO_4^{3-}-P concentrations remained significantly elevated in the microcosms without sediment compared to the experimental controls, as also occurred for NH_4^+-N and NO_3^--N.

Examination of molar ratios of NH_4^+ and NO_3^- to PO_4^{3-} (i.e., DIN:PO_4^{3-}-P ratios) demonstrated that PO_4^{3-}-P concentrations declined much more rapidly in water of the +sediment microcosms than did concentrations of NH_4^+-N and NO_3^--N (Fig. 4d). This occurred most rapidly for the +sediment microcosms amended with NH_4^+ and PO_4^{3-} after 12.5 h (i.e., *+A+P +sediment treatment*), and after 28 h for microcosms amended with NO_3^- and PO_4^{3-} (i.e., *+N+P +sediment treatment*). For the microcosms with sediment, DIN:PO_4^{3-}-P ratios became similar to those in the experimental controls after 72 h (Fig. 4, part d). Ratios of DIN:PO_4^{3-}-P were relatively constant throughout the duration of the experiment in the nutrient-amended microcosms without sediment compared to those with sediment. Interestingly, DIN:PO_4^{3-}-P ratios declined markedly between 0 and 4.5 h of incubation in the control microcosms (i.e., no nutrient amendment) with and without sediment (Fig. 4, part d), which appears to have been driven mainly by a decrease in NH_4^+-N concentrations, and to a lesser extent by a small increase in PO_4^{3-}-P concentrations.

PHYTOPLANKTON BIOMASS

The response of phytoplankton biomass (as Chl a concentration) to the nutrient amendments was examined at the conclusion of the experiment, 72 h after the nutrients were added, and compared to the experimental controls that did not receive nutrient amendments (Fig. 5). The most marked differences in Chl a concentration occurred between microcosms with sediment versus those without sediment. Phytoplankton Chl a concentrations were typically at least twofold higher in microcosms with sediment compared to those without sediment. Indeed, a randomized block ANOVA test showed that mean phytoplankton Chl a concentration was significantly higher in microcosms with sediment versus those without sediments, after controlling for the effects of the nutrient amendments ($F_{1,4} = 14.399$, $P = 0.001$).

FIGURE 4. Concentration in water of (a) NH_4^+ (moles N L^{-1}), (b) NO_3^- (moles N L^{-1}), (c) PO_4^{3-} moles P L^{-1}), and (d) the ratio of DIN:PO_4^{3-}-P in the microcosms (mean ± 1 SD) at 0, 4.5, 12.5, 28, and 72 h after nutrients were added to microcosms with pond water (–sediment) and to microcosms with pond water plus surficial pond sediment and associated benthic biofilm (+sediment). The experiment included experimental controls (with and without sediment), and four different nutrient treatments: +A treatment, +A+P treatment, +N treatment, and +N+P treatment (with and without sediment). Three independent replicates were run for each nutrient amendment and control. Microcosms without sediment are represented by the dotted lines, while microcosms with sediment are represented by solid lines.

TABLE 2

Results of one-way ANOVA tests and subsequent post-hoc tests to determine differences in (a) NH_4^+, (b) NO_3^-, and (c) PO_4^{3-} concentrations in the water of experimental microcosms at 0, 28, and 72 h after nutrient amendments to microcosms with pond water only (–sed.) and with pond water plus sediment and associated benthic biofilm (+sed.). The ANOVA tests compared mean concentrations of the nutrients among the experimental control (Cont.) and the amendments with NH_4^+ alone (+A), NO_3^- alone (+N), NH_4^+ + PO_4^{3-} (+A+P), and NO_3^- + PO_4^{3-} (+N+P), as specified in Table 1. Three independent replicate microcosms were run for each level of the treatment groups. Bolded P values are significant at $\alpha = 0.10$. For the one-way ANOVA tests, the degrees of freedom are 5, 12.

(a) NH_4^+-N concentration

Hour	1-way ANOVA	Tukey post-hoc test	Cont. +sed.	+A+P –sed.	+A –sed.	Tukey post-hoc test	+ A+P +sed.	+ A +sed.
0	$F = 278.5$, ***P* < 0.001**	Cont. –sed.	$P = 1.00$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	***P* < 0.001**	***P* < 0.001**
28	$F = 227.3$, ***P* < 0.001**	Cont. –sed.	$P = 1.00$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	***P* = 0.0743**	***P* = 0.001**
72	$F = 783.0$, ***P* < 0.001**	Cont. –sed.	$P = 0.972$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	$P = 0.999$	$P = 1.00$

(b) NO_3^--N concentration

Hour	1-way ANOVA	Tukey post-hoc test	Cont. +sed.	+N+P –sed.	+N –sed.	Tukey post-hoc test	+ N+P +sed.	+N +sed.
0	$F = 168.1$, ***P* < 0.001**	Cont. –sed.	$P = 1.00$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	***P* < 0.001**	***P* < 0.001**
28	$F = 753.5$, ***P* < 0.001**	Cont. –sed.	$P = 1.00$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	***P* < 0.001**	***P* < 0.001**
72	$F = 783.0$, ***P* < 0.001**	Cont. –sed.	$P = 1.00$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	$P = 1.00$	$P = 1.00$

(c) PO_4^{3-}-P concentration

Hour	1-way ANOVA	Tukey post-hoc test	Cont. +sed.	+ N+P –sed.	+A+P –sed.	Tukey post-hoc test	+N+P +sed.	+A+P +sed.
0	$F = 229.5$, ***P* < 0.001**	Cont. –sed.	$P = 1.00$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	***P* < 0.001**	***P* < 0.001**
28	$F = 251.4$, ***P* < 0.001**	Cont. –sed.	$P = 1.00$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	$P = 0.944$	$P = 0.135$
72	$F = 743.2$, ***P* < 0.001**	Cont. –sed.	$P = 1.00$	***P* < 0.001**	***P* < 0.001**	Cont. +sed.	$P = 0.826$	$P = 0.826$

A two-way ANOVA test identified no significant interaction among the factors presence/absence of sediment and the nutrient amendments (including the experimental control) on phytoplankton Chl a concentration after 72 h of incubation ($F_{1,4} = 1.983$, $P = 0.136$). Consequently, the effects of nutrient amendments on phytoplankton biomass were examined using one-way ANOVA tests run separately using microcosms with and without sediment added.

Mean phytoplankton Chl a concentration did not differ among nutrient amendments in the one-way ANOVA test of the +sediment microcosms ($F_{4,10} = 2.216$, $P = 0.140$; Fig. 5). However, mean Chl a concentrations differed significantly among nutrient amendments in microcosms without sediment ($F_{4,10} = 7.567$, $P = 0.004$). Tukey post-hoc tests identified Chl a concentration as being significantly higher in the *+N+P treatment* without sediment ([mean ± 1 standard deviation] 87.3 ± 17.4 nmol Chl a m^{-2}) than the experimental control without sediment (39.2 ± 9.04 nmol Chl a m^{-2}; $P = 0.016$). However, Chl a concentrations did not differ between the experimental control and the other possible comparisons among treatment levels (*+N treatment* = 48.6 ± 12.3 nmol Chl a m^{-2}, $P = 0.974$; *+A treatment* = 35.0 ± 7.16 nmol Chl a m^{-2}, $P = 0.991$; *+A+P treatment* = 72.3 ± 22.8 nmol Chl a m^{-2} $P = 0.138$).

BENTHIC ALGAL BIOMASS AND COMMUNITY COMPOSITION

Principal components analysis (PCA) was performed to explore patterns of differences among treatments in abundance and composition of epipelic algal pigments in the upper 1 cm of the sediment (i.e., the benthic biofilm) after 72 h of incubation since nutrient amendment (Fig. 6). Eigenvalues for the first and second PCA axes were 0.548 and 0.241, respectively, which explains 78.9% of the variation within the data set. The main gradient of variation among treatments (axis 1) separated microcosms that received PO_4^{3-} co-additions (*+N+P treatment*, *+A+P treatment*; positioned on the right side along axis 1), from those that did not receive PO_4^{3-} additions (experimental control, *+N treatment*, *+A treatment*; positioned to the left along axis 1). PO_4^{3-} additions were associated with elevated concentrations of all algal pigments. Axis 2 separated the samples mainly according to the species of N added, with microcosms that received NH_4^+ additions generally positioned above axis 1, and microcosms that received NO_3^- additions positioned below axis 1. Sample scores for the experimental controls spanned across axis 2. The microcosms that received the NH_4^+ and PO_4^{3-} amendments elicited the most consistent response as indicated by the relatively tight clustering of replicate sample scores, and were associated with high pigment concentrations. Overall, the various nutrient amendments appeared to cause greater differences in concentrations of pigments rather than in the composition of pigments, as illustrated by the strong correlation among the pigment vectors (Fig. 6). However, relatively distinct orientation of the vectors for aphanizophyll and Chl b towards the lower right quadrant suggest that growth of potentially N-fixing cyanobacteria and green algae responded most strongly to co-addition of NO_3^- and PO_4^{3-} to the microcosms.

Results from an ANOSIM test confirmed that the composition and abundance of benthic algae differed significantly among the nutrient amendments (Global $R = 0.239$, $P = 0.042$), consistent with patterns observed from the PCA ordination. Pairwise tests showed that there were significant differences between the experimental control and the *+N+P treatment* ($R = 0.667$, $P = 0.1$), the experimental control and the *+A+P treatment* ($R = 0.63$, $P = 0.1$), the *+A treatment* and *+N+P treatment* ($R = 0.481$, $P = 0.1$), and the *+A treatment* and *+A+P treatment* ($R = 0.481$, $P = 0.1$).

Concentrations of individual pigments were examined further by one-way ANOVA tests to determine which of the benthic algal pigments differed significantly among the nutrient amendments (Fig. 7; Table 3). Concentrations of pigments found in all algae (Chl a, Chl a′, chlorophyllide a, β-carotene) tended to be highest in microcosms that received co-amendment of NH_4^+ or NO_3^- with PO_4^{3-} (*+A+P treatment* and *+N+P treatment*; Fig. 7). However, only concentrations of Chl a and Chl a′ differed significantly among treatments (Table 3). Chl a concentration was significantly higher in the *+N+P treatment* ([mean ± 1 standard deviation] 35,159 ± 7109 nmol Chl a m^{-2}) compared to the *+N treatment* (17,507 ± 10,176 nmol Chl a m^{-2}) and the experimental control (16,444 ± 1454 nmol Chl a m^{-2}) (Table 3, part a). The one-way ANOVA test identified that Chl a′ concentration differs significantly among treatments, but Tukey post-hoc tests did not identify any significant pairwise differences (Table 3, part a).

Of the pigments produced mainly by cyanobacteria (canthaxanthin [colonial cyanobacteria], aphanizophyll [potentially N-fixing cyanobacteria], and echinenone [total cyanobacteria]), only the sedimentary concentration of echinenone differed significantly among the nutrient amendments. However, Tukey post-hoc tests did not identify any significant pairwise differences (Table 3, part b).

Sedimentary concentrations of two other pigments increased when PO_4^{3-} was added relative to the experimental control or amendments without PO_4^{3-} (Table 3, part c). Concentration of Chl b (Chlorophyta and Euglenophyta) was significantly higher in the *+N+P treatment* ([mean ± 1 standard deviation] 2888 ± 1764 nmol Chl b m^{-2}) than the *+A treatment* (649 ± 317 nmol Chl b m^{-2}) or experimental control (693 ± 475 nmol Chl b m^{-2}). Concentration of fucoxanthin (diatoms, chrysophytes, and dinoflagellates) was significantly higher in the *+N+P treatment* (4184 ± 1641 nmol fucoxanthin m^{-2}) and *+A+P treatment* (4506 ± 1481 nmol fucoxanthin m^{-2}) than the *+N treatment* (1475 ± 578 nmol fucoxanthin m^{-2}) (Table 3, part c).

Discussion

NUTRIENT UPTAKE IN HBL PONDS

Supply of dissolved forms of N and P to tundra ponds is expected to increase as continued climate warming modifies precipitation patterns, increases temperature, and increases rates of permafrost thaw and peat decomposition (Hobbie et al., 1999; Wrona et al., 2006; Smol and Douglas, 2007; IPCC, 2007; Prowse and Brown, 2010; Lougheed et al., 2011; Keuper et al., 2012). Based on results of the microcosm experiment, short-lived processes that increase supply of dissolved forms of N or N plus P, such as summer storm events in regions of decomposing peat and thawing permafrost, likely will result in rapid uptake from the water in shallow ponds of the HBL. Although nutrient amendments in this experiment elevated concentration in water of the microcosms well above natural concentrations (20× higher NO_3^--N and NH_4^+-N, and 50× higher PO_4^{3-}-P), the sediments and biota were able to consume the amended nutrients and return them to levels found in the un-amended experimental control. Interestingly, rapid consumption only occurred when sediment was present, indicating that the sediment and biota in the associated benthic biofilm play an important role in nutrient cycling in shallow tundra ponds of the HBL. This finding suggests that the ponds will be able to consume higher nutrient concentrations that may occur due to climate change or elevated atmospheric deposition. The rapid nutrient uptake by sediments and associated biota may account for continued low nutrient concentrations in tundra ponds observed elsewhere, despite increased supply of N by long-distance transport for several decades (Bergström et al., 2005; Bergström and Jansson, 2006; Elser et al., 2009; Holtgrieve et al., 2011).

IMPORTANCE OF POND SEDIMENTS AND BENTHIC BIOTA IN NUTRIENT CYCLING

This study has demonstrated that the highly organic sediments found in many of the ponds of the Churchill region of the HBL, and their associated epipelic community, play an important role in the uptake of nutrients. Nutrient concentrations in the amended microcosms decreased from the water more rapidly when sediments were present than when they were absent, indicating that biological uptake by organisms in the benthic biofilm and physico-chemical uptake by sediments play a more active role than nutrient uptake by phytoplankton (Fig. 4). Indeed, concentrations of NH_4^+,

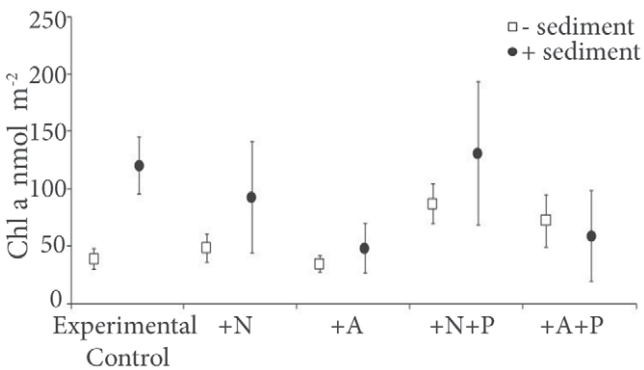


FIGURE 5. Chlorophyll a areal concentrations (nmoles m^{-2}; mean ± 1 SD) of the phytoplankton sampled at 72 h after nutrient additions for the experimental control and the four nutrient treatments: +A treatment, +A+P treatment, +N treatment, and +N+P treatment. Open squares represent the microcosms without sediment (–sediment category), solid black circles represent the microcosms with surficial sediment and associated benthic biofilm from Left Pond (+sediment category). Three independent replicates were run for each nutrient amendment and control.

TABLE 3

Results of one-way ANOVA tests and subsequent post-hoc tests to determine differences among treatment levels in concentrations of benthic algal pigments in the upper 1 cm of sediment from experimental microcosms. Microcosms received nutrient additions (+A, +N, +A+P, +N+P) or served as an experimental control, which received no nutrient amendment. Three independent replicate microcosms were run for each level of the treatment groups. Results are shown for pigments representing (a) total benthic algal biomass, (b) benthic cyanobacterial biomass, and (c) biomass of other benthic algal groups. Bolded P values are significant at $\alpha = 0.10$. Degrees of freedom for all one- way ANOVA tests are 4, 10.

Pigment	One-way ANOVA	Tukey post-hoc
(a) Total benthic algal indicators		
Chlorophyll *a*	$F = 4.2$, $\mathbf{P = 0.029}$	+N+P > control (**$P = 0.065$**)
		+N+P > +N (**$P = 0.086$**)
Chlorophyll *a'*	$F = 3.2$, $\mathbf{P = 0.057}$	No significant pairwise differences
Chlorophyllide *a*	$F = 2.0$, $P = 0.177$	No significant pairwise differences
-carotene	$F = 1.5$, $P = 0.285$	No significant pairwise differences
(b) Cyanobacteria indicators		
Echinenone	$F = 3.0$, $\mathbf{P = 0.069}$	No significant pairwise differences
Canthaxanthin	$F = 2.3$, $P = 0.133$	No significant pairwise differences
Aphanizophyll	$F = 1.1$, $P = 0.418$	No significant pairwise differences
(c) Specific algal indicators		
Chlorophyll b (Chlorophyta)	$F = 3.3$, $\mathbf{P = 0.056}$	+N+P > control (**$P = 0.065$**)
		+N+P > +A (**$P = 0.059$**)
Lutein/Zeaxanthin (Chlorophyta/ Cyanobacteria)	$F = 1.3$, $P = 0.319$	No significant pairwise differences
Fucoxanthin (Chromophytes)	$F = 3.2$, $\mathbf{P = 0.061}$	+A+P > +N (**$P = 0.057$**)
Chlorophyll c2 (Chromophytes)	$F = 1.2$, $P = 0.355$	No significant pairwise differences

NO_3^-, and PO_4^{3-} did not decline substantially from the water over the 72-h amendment experiment when sediments were absent (Fig. 4). As most northern freshwater systems are dominated by benthic organisms rather than phytoplankton (Björk-Ramberg, 1985; Björk-Ramberg and Ånell, 1985; Bonilla et al., 2005), which was also found in the microcosms (benthic Chl a levels were several orders of magnitude greater than phytoplankton Chl a), this key finding is likely to be applicable to Arctic and subarctic ponds in regions beyond the HBL. Indeed, Hansson (1988) also showed rapid uptake of experimentally added PO_4^{3-} from the water column, with greater uptake when periphytic algae covered the sediment.

Nutrient cycling between the sediments, their associated benthic biofilm, and the water column may be an important source of nutrients to phytoplankton. If sediments supply micronutrients or PO_4^{3-} to the water column, as Søndergaard et al. (1992) proposed occurs during wind events that suspend sediment in the water column, a common occurrence in shallow ponds (Luettich et al., 1990), nutrients would be made available to the phytoplankton. Alexander et al. (1989) suggested that N is released from the sediment during wind-mixing events and stimulates phytoplankton activity in shallow tundra ponds. Using labeled PO_4^{3-} additions, Hansson (1988) showed that planktonic algae incorporate PO_4^{3-} released from the sediment. Indeed, nutrient supply from sediment to the water column may explain why phytoplankton biomass was consistently higher in the +sediment microcosms compared to the microcosms without sediment, and why differences were not observed in response to the nutrient amendments in the +sediment microcosms but were observed in microcosms without sediment (Fig. 5). Given these findings, it is plausible that the experimental nutrient additions to the +sediment microcosms were not large enough to offset the supply of nutrients from the sediment. However, if sediments did indeed supply NO_3^-, NH_4^+, or PO_4^{3-} to the water column, the uptake of these additional nutrients must have been very rapid because nutrient concentrations were not elevated in the water column of the experimental controls of the +sediment microcosms (Fig. 4). Alternatively, the sediments may have supplied micronutrients that were not measured, but which may play an important role in limiting growth of phytoplankton at high latitudes (Bonilla et al., 2005).

VARIABLE SHORT-TERM RESPONSES BY PLANKTONIC AND BENTHIC ORGANISMS TO NUTRIENT INPUTS

In consideration of cool temperatures, which support slow growth rates, nutrient-limitation experiments conducted at high latitudes typically include a 7-day-long incubation (or longer) to allow for detection of biomass responses of primary producer communities (Symons et al., 2012). Thus, our use of a 3-day incubation to assess short-term nutrient uptake likely does not allow accurate assessment of nutrient limitation of the algal growth

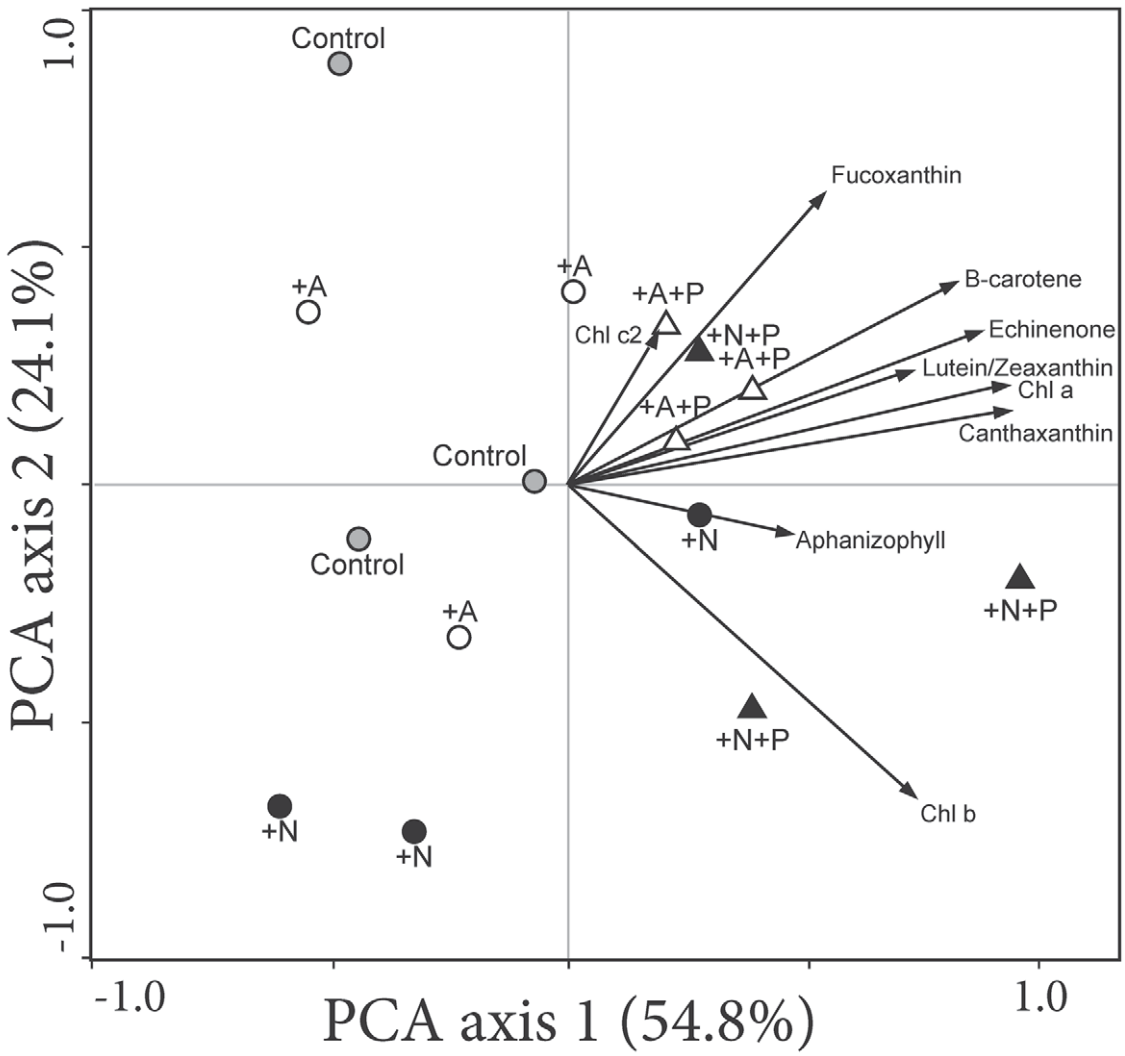


FIGURE 6. Principal components analysis (PCA) showing patterns of variation in concentrations of photosynthetic pigments in the benthic biofilm (upper 1 cm of sediment) of the microcosms 72 h after receiving one of four different nutrient treatments (+A treatment, +A+P treatment, +N treatment, and +N+P treatment) or an experimental control (no nutrient amendment). Three independent replicates were run for each nutrient amendment and control.

response, because the growth response may play out over a longer period of time. Instead, we employed measurements of algal biomass (as concentration of Chl a and other photosynthetic pigments) to assess the short-term growth responses as a way to determine the relative roles of phytoplankton versus epipelon in mediating nutrient uptake. In microcosms without sediment and associated benthic biofilm, phytoplankton biomass was significantly elevated above the experimental control when NO_3^- and PO_4^{3-} were added together, with mean Chl a concentration double that in the control at the end of the experiment. But, this growth response was not associated with marked nutrient uptake. Instead, marked nutrient uptake from the water occurred only when sediments were included in the microcosms, and the co-additions of NH_4^+ + PO_4^{3-} (*+A+P treatment)*, and NO_3^- + PO_4^{3-} (*+N+P treatment*) significantly elevated benthic algal biomass after 72 h compared to treatments without nutrient amendment (experimental control), NH_4^+ alone, or NO_3^- alone. The *+A+P* and *+N+P treatments* resulted in almost tripling of Chl a concentration in the surface sediments compared to the experimental control (no nutrients added), indicating that the epipelic algae are able to convert co-addition of inorganic N and P into short-term growth. Given that the biomass response of the epipelic algae in the microcosms is several orders of magnitude greater than for the phytoplankton, the epipelic algae appear to be the more important drivers of the nutrient uptake that occurred.

The marked uptake of added inorganic N and P and short-term stimulation of epipelic algal biomass in the *+N+P treatment* observed in our experiment appear to contradict conclusions based on nutrient-limitation experiments conducted at other Arctic ponds, which have determined that the benthic algal community is often nutrient sufficient (Blumenshine et al., 1997; Bonilla et al., 2005). Bonilla et al. (2005) suggested the benthic algal biomass response to nutrient amendment can be muted because the benthic community obtains sufficient nutrients from the sediments, or because other factors, such as ice cover, light, and temperature, exert strong control on benthic algal biomass. Light can exert stronger control of algal biomass responses in deeper basins due to

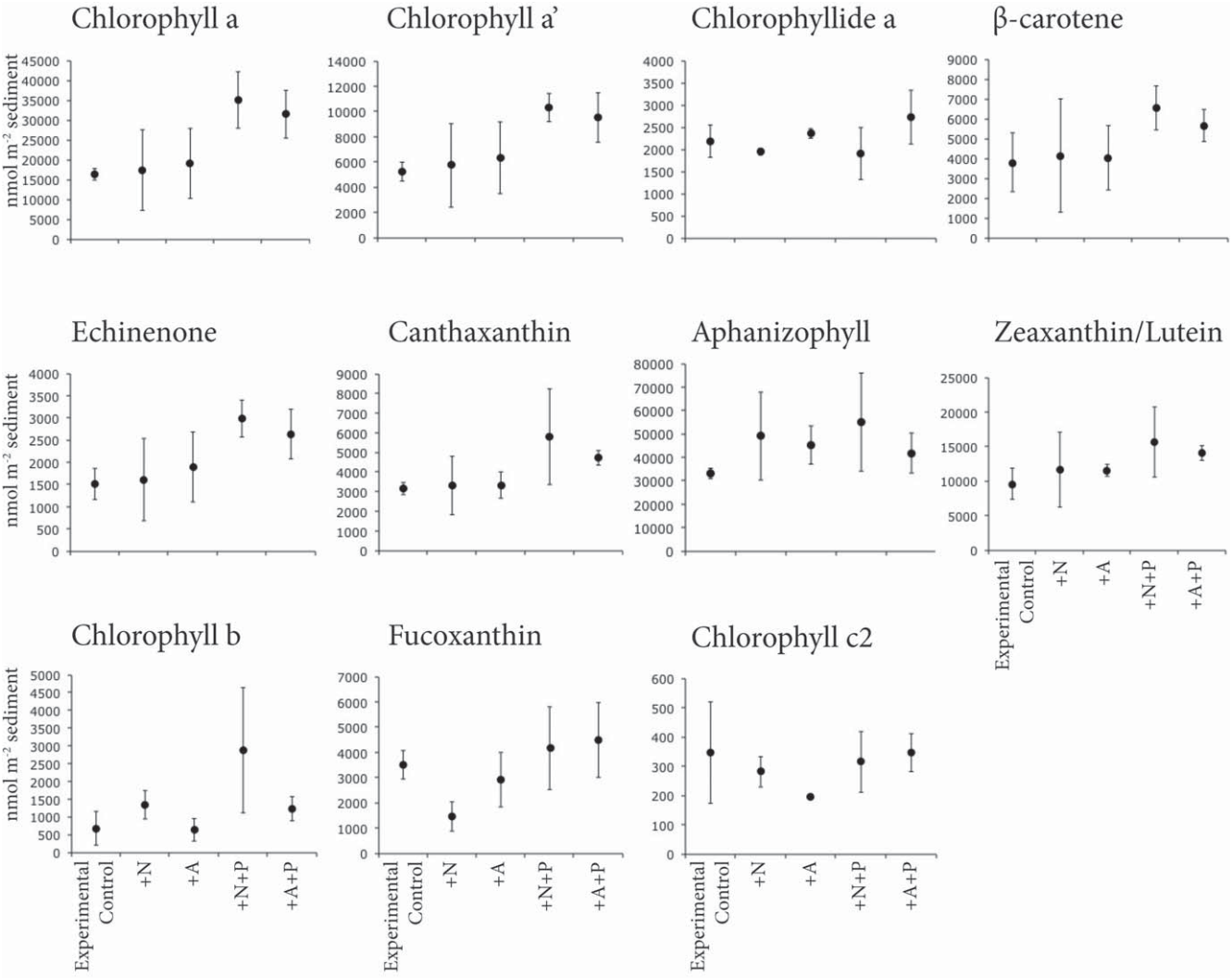


FIGURE 7. Areal concentrations of photosynthetic pigments (nmol pigment m^{-2} of sediment; mean ± 1 SD) in the benthic biofilm (upper 1 cm of sediment) of the microcosms 72 h after receiving one of four different nutrient treatments (+A treatment, +A+P treatment, +N treatment, and +N+P treatment) or an experimental control (no nutrient amendment). Three independent replicates were run for each nutrient amendment and control.

attenuation of photosynthetically active radiation (Björk-Ramberg and Ånell, 1985), but likely does not limit primary producers in our shallow experimental pool and microcosms or the nearby shallow tundra ponds. Alternatively, differences between findings from those studies and this study may be due to the more extreme environments found in the Arctic compared to subarctic conditions at the HBL near Churchill, Manitoba.

Examination of pigments in surficial sediments of the microcosms revealed that the benthic algal community is dominated by potentially N-fixing cyanobacteria, as indicated by markedly higher concentrations of aphanizophyll than other pigments. Nitrogen-fixing cyanobacteria tend to dominate ecosystems with relatively little biologically available N in the water column and abundant light supply, such as occurs in many subarctic and Arctic ponds (Tang et al., 1997; Vincent, 2000; Bonilla et al., 2005). Under these conditions, there is a competitive advantage for the ability to fix atmospheric N (Tilman et al., 1986). Despite substantial inorganic N amendments, concentrations of aphanizophyll remained high. Therefore, the potentially N-fixing cyanobacteria did not appear to lose their competitive advantage compared to other algal groups when bioavailable inorganic N was amended. It is possible that the inorganic N was removed more quickly by heterotrophic microbes in the benthic biofilm than by the cyanobacteria. But, PO_4^{3-} was removed more rapidly on a molar basis than dissolved inorganic N (NH_4^+ and NO_3^-) during the first 12.5–28 h after amendment with NH_4^+ and NH_4^+ + PO_4^{3-} in microcosms with sediment (Fig. 4), suggesting higher short-term demand for PO_4^{3-} than NH_4^+ and NO_3^-. Collectively, these findings do not support existence of short-term N limitation, despite dominance of the epipelic algal community by potentially N-fixing cyanobacteria. However, without more knowledge of the metabolic and growth responses of heterotrophic microbes in the benthic biofilm (not measured in our study) we cannot determine their importance in mediating the nutrient uptake, or whether they outcompete the algae for inorganic N and P. Despite strong net uptake of inorganic N and P from the water in the *+A+P treatment* and *+N+P treatment* microcosms with sediment and significant stimulation of benthic algal growth compared to the experimental

control, phytoplankton biomass also increased significantly under these conditions—a finding that suggests activities of heterotrophic microbes in the benthic biofilm influence phytoplankton biomass. Thus, we suggest that future studies include measurements of the heterotrophic microbial community.

For this study, we employed a field-based microcosm experimental approach to assess nutrient uptake and short-term (72 h) algal growth responses to nutrient amendments. This approach provides one of the few logistically manageable methods to manipulate nutrient additions while holding other possible confounding factors relatively constant. However, microcosms have potential limitations. For example, container effects may accrue over time, which can confound the effects of nutrient amendments. Thus, the 1-L microcosms may only capture processes that play out over relatively short time periods, such as the 72 h duration of this study. Second, we incubated the microcosms in an experimental pool rather than *in situ* in Left Pond. This was required for logistical and safety reasons. We took care to maintain conditions similar to Left Pond. For example, the microcosms were incubated in water obtained from Left Pond and the water depth in the experimental pool was sufficient to ensure water temperature in the microcosms was not elevated above that of a nearby tundra pond (Fig. 3). Nevertheless, we cannot preclude the possibility that conditions in the microcosms varied from those in Left Pond. Due to logistical constraints, samples were only collected from Left Pond rather than a suite of ponds, and so it remains unsure how representative results based on materials from this pond are of tundra ponds in the Churchill region of the HBL. But, comparisons with spatial survey data from nearby ponds identify that water chemistry at Left Pond is not unusual. Finally, this experiment was conducted only once and consequently, our findings may only represent what is experienced by Left Pond in mid-summer. Further study is required to assess the extent to which results from this microcosm experiment can be extrapolated to pond ecosystems in the western HBL and to other times during the ice-free season.

Conclusions

This study, based on controlled nutrient amendments to microcosms and 72-h incubation with and without surficial sediments and associated benthic biofilm, demonstrated that shallow subarctic ponds of the western HBL are capable of rapidly consuming a pulsed, elevated supply of dissolved inorganic forms of N (NH_4^+, NO_3^-) and P (PO_4^{3-}), as may occur as climate continues to warm in the western HBL. However, amended nutrient concentrations remained high and elevated above values in the experimental control (no nutrients added) in microcosms without sediments, and planktonic communities did not appear to use the amended N. Instead, rapid, marked nutrient uptake only occurred when sediment and biota in the associated benthic biofilm were present, indicating they play an important role in nutrient cycling in the shallow tundra ponds. In the microcosms with sediment, concentration of NH_4^+-N and NO_3^--N declined to values in the un-amended control within 72 h, whereas PO_4^{3-}-P concentration declined to values in the un-amended control within 28 h. Phytoplankton biomass (as Chl a concentration) was elevated in microcosms co-amended with NO_3^- and PO_4^{3-} relative to the un-amended control, but the relatively small amount of phytoplankton growth cannot account for all of the nutrient uptake. In contrast, in the presence of sediment, both amended N and PO_4^{3-} were rapidly removed from the water column, stimulating benthic algal biomass in PO_4^{3-} and inorganic N co-additions. It was also observed that in the presence of sediment, phytoplankton biomass (inferred from Chl a concentration) increased both with and without nutrient additions, indicating that sediment and associated biota may be a supply of nutrients to phytoplankton. The findings of this study illustrate that shallow tundra ponds are able to respond rapidly to increased supply of inorganic nutrients from external sources. And, it demonstrates the importance of the sediments and associated benthic communities in nutrient uptake. We suggest that analysis of the heterotrophic components (bacteria, fungi, etc.) of the benthic biofilm is an important future research direction because our results indirectly suggest they play an important role in nutrient uptake (see also Jansson et al., 1996).

Acknowledgments

Funding for this study was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) via Discovery Grant/Northern Research Supplement Programs, Aboriginal Affairs and Northern Development Canada's Northern Scientific Training Program, and the Churchill Studies Centre Northern Research Fund. We thank Kat Jansen, Carley Basler, Jessica Mendoza, and Emma Henderson for their logistical help in conducting the fieldwork, and Gillian Merritt, Don Allin, Katie Thomas, Lauren MacDonald, Ann Balasubramaniam, and Monica Tudorancae for help with lab work and analysis involved in this research.

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MS accepted July 2013