



Determining Critical Nitrogen Loads to Boreal Lake Ecosystems

The Response of Phytoplankton at Acadia and Isle Royale National Parks

Natural Resource Technical Report NPS/ACAD/NRTR—2014/862



ON THE COVER

Bubble Lake, Arcadia National Park.

Photograph by: Kristin Strock

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Natural Resource Technical Report NPS/ACAD/NRTR—2014/862

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April 2014

U.S. Department of the Interior
National Park Service
Natural Resource Stewardship and Science
Fort Collins, Colorado

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Please cite this publication as:

Saros, J. E. 2014. Determining critical nitrogen loads to boreal lake ecosystems: The response of phytoplankton at Acadia and Isle Royale National Parks. Natural Resource Technical Report NPS/ACAD/NRTR—2014/862. National Park Service, Fort Collins, Colorado.

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Executive Summary

In the northeast and upper Midwest, boreal lake ecosystems are common, particularly in national parks such as Acadia (ACAD) and Isle Royale (ISRO). Atmospheric deposition is of concern in these boreal lakes, as many are situated in relatively close proximity to industrial areas and along pollution dispersal trajectories. Only slight declines in nitrogenous compounds have been observed over the last decade in these areas, and enhanced atmospheric nitrogen (N) deposition can affect aquatic ecosystems by lowering pH as well as by providing a biologically-available form of N for algal growth. Furthermore, the reduction in sulfur deposition, along with changes in climate, are affecting the delivery of dissolved organic carbon (DOC) to boreal lake ecosystems, with increasing DOC concentrations observed in surface waters in both ACAD and ISRO in recent decades. The effects of increasing DOC on phytoplankton production can vary, as DOC can add nutrients to lakes but also reduce light availability in the water column.

The response of algae in lakes in ACAD and ISRO to these N and DOC changes was assessed using experiments and lake sediment cores. Experiments were used to assess which nutrients (N, phosphorus (P), or N and P) are limiting in Jordan Pond and Echo Lake in ACAD and in Sargent Lake and Richie Lake in ISRO. A second set of experiments was established in one lake in each park (Jordan Pond and Sargent Lake) for two reasons: to assess the response of algae along a N gradient, and to assess the response to DOC manipulations. To determine the type of effect that DOC has on algae (light versus nutrient effects), two types of DOC manipulations were used in this experiment: DOC shaded (no direct contact with algae), and DOC added (direct contact). The response of algae to environmental change over the past century was determined in two lakes in ACAD (Jordan Pond and Bubble Pond) and one lake in ISRO (Siskiwit Lake). This response was measured using diatom fossils and algal pigments, as well as the carbon and nitrogen content of sedimentary organic material.

Algal growth in the two ACAD lakes was co-limited by N and P, whereas it was limited by N alone in the two ISRO lakes. There was thus no response to the N gradient experiment in ACAD, whereas algal biomass increased with N additions in Sargent Lake, a N-limited ISRO lake. The first positive response to N additions in Sargent Lake occurred at $10 \mu\text{g L}^{-1} \text{NO}_3^- \text{N}$, resulting in a 10% increase in biomass. The DOC shaded treatments had no effect in any case, whereas the DOC added treatments stimulated algal growth in both cases (in Jordan Pond and in Sargent Lake), indicating that DOC additions added nutrients. Overall, the effects of N additions were dependent on nutrient limitation status (i.e., effect observed in N-limited lakes), whereas regardless of the nutrient limitation pattern, the DOC additions always stimulated algal growth. While the nitrogen isotopes in sediment cores reflect increasing N deposition since 1950 in all three lakes, there was generally little change in diatom fossils or algal pigments over the 20th century. The exception to this was the diatom fossil record of Siskiwit Lake in ISRO, in which changes in diatom community structure in this large, deep lake suggested climate-driven changes in this lake over the past century.

Overall, both atmospheric deposition and climate change have important implications for the base of lake food webs in both of these parks. Considering the environmental factors investigated here, the

results of this study suggest that the greatest threat to lakes in ACAD is changes in DOC loading (which appear to be a consequence of both declining sulfur deposition and climate change), whereas the lakes of ISRO are vulnerable to both N deposition and climate change.

Introduction

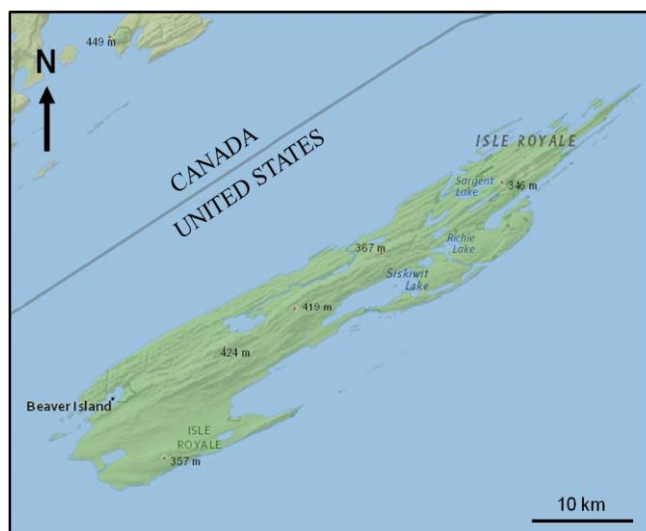
Problem Statement

In the northeast and upper Midwest, boreal lake ecosystems are common, particularly in national parks such as Acadia (ACAD) and Isle Royale (ISRO; Fig. 1). Atmospheric deposition is of concern in these boreal lakes, as many are situated in relatively close proximity to industrial areas and along pollution dispersal trajectories. While sulfur oxide emissions have declined in recent decades, only slight declines in nitrogenous compounds have been observed over the last decade in these areas. Increased atmospheric nitrogen (N) deposition can affect aquatic ecosystems by lowering pH as well as by providing a biologically-available form of N for algal growth.

Furthermore, the reduction in sulfur deposition, along with changes in climate, are affecting the delivery of dissolved organic carbon (DOC) to boreal lake ecosystems, with increasing DOC concentrations observed in surface waters in both ACAD and ISRO in recent decades. During the course of this project, an important trend in both ACAD and ISRO of declining water clarity and increasing concentrations of dissolved organic carbon (DOC) in surface waters were pointed out by National Park Service staff (Alan Ellsworth, Water Resource Liaison, Natural Resource Stewardship and Science, National Park Service, personal communication) and a long-term data report (Toczydlowski and Stottlemeyer 2009). The effects of increasing DOC on phytoplankton production can vary, as DOC can add nutrients to lakes but also reduce light availability in the water column.



A.



B.

Figure 1. Maps of A) Acadia National Park; B) Isle Royale National Park.

Background

Aside from paleolimnological work that uses diatoms to infer pH trends, there is little work on which factors influence phytoplankton communities in these boreal lakes, making it difficult to use changes in phytoplankton species as an indicator of N deposition. Staff at ACAD and ISRO conduct routine monitoring of a set of lakes in their respective parks for physical and chemical variables. These data provide background information for biological investigations that can elucidate effects resulting from environmental change. While some phytoplankton data are available for inland lakes in these parks (e.g., Toczydlowski et al. 1978, Whitman et al. 2000), there has been no attempt to relate species composition to relevant environmental variables. Although few significant trends in total N deposition have been noted for National Atmospheric Deposition (NADP) sites near ACAD and ISRO (<http://nadp.sws.uiuc.edu/>), park managers are concerned about N deposition because even minor changes in N deposition trends in western US alpine lakes have resulted in biological change (Saros et al. 2005b), and significant increases (over natural background levels) in N deposition may have occurred prior to deposition sampling. Furthermore, future increases in N emissions near ISRO are anticipated, due to recent proposals for a new power plant, a copper processing facility, and a combined taconite processing-steel making facility in northern Minnesota. Nutrient ratios (dissolved inorganic nitrogen:total phosphorus) for lakes at ISRO suggest that algae are co-limited by both N and P (see data in Kallemeyn 2000), and that future increases in N deposition could rapidly affect algal communities.

Several recent park and regional documents indicate that atmospheric deposition and climate change are significant concerns for these boreal waters. The ACAD Water Resources Management Plan listed effects of atmospheric deposition and climate change as the first of its top five management issues (Kahl et al. 2000). This concern is underscored by the proposed critical loads of 3.5 to 6.0 kg N ha⁻¹ yr⁻¹ for nutrient enrichment effects in Northeastern lakes (Baron et al. 2011), as deposition rates in ACAD have met the low end of this range over the past few decades (<http://nadp.sws.uiuc.edu/>). The ISRO Water Resources Management Plan also listed atmospheric deposition as a high priority (Crane et al. 2006). Similarly, in a recent synthesis of aquatic studies in Great Lakes area parks, Lafrancois and Glase (2005) noted the need for multi-park investigations and monitoring related to nitrogen deposition in northern lakes. Finally, the Midwest Region Science Strategy placed atmospheric deposition (particularly at Class I parks like ISRO and ACAD) among its top 13 management issues (NPS 2006).

In addition to changes in N deposition, widespread changes in DOC are occurring across lakes situated in northern forests (Monteith et al. 2007; Weyhenmeyer and Karlsson 2009), due to a combination of reduced sulfur emissions as well as increasing air temperatures and changing hydrology. While trends in DOC concentrations have been documented, the implications of these changes for the biota of lakes situated in northern forests remain unclear. In particular, DOC may affect the abundance of algae by providing organic forms of nutrients (Klug 2002). At present, it remains unclear how widespread and severe the biological effects of DOC additions are.

Objectives

The objectives of this project were to:

1. determine nutrient limitation patterns in a sub-set of lakes in both ACAD and ISRO, to evaluate the potential sensitivity of these lakes to N deposition;
2. determine the response of phytoplankton growth along a gradient of N concentrations, to identify specific nitrogen concentrations that elicit increased phytoplankton growth;
3. determine whether increasing DOC concentrations will affect phytoplankton growth;
4. use the response along the N gradient to define a critical N load for boreal lake ecosystems in each park;
5. investigate past changes in algal communities in at least one lake in each park, to assess whether N deposition has elicited changes in these communities over time.

Methods

Nutrient Limitation Experiments

These methods address Objective 1. A suite of nutrient enrichment assays was conducted during June and August of 2009 to determine the key limiting nutrients in two lakes from each area: Jordan Pond and Echo Lake in ACAD, and Sargent Lake and Richie Lake in ISRO (Table 1).

Water for the experiments was collected with a van Dorn sampler from the chlorophyll a maximum as determined using a Hydrolab5 mini Sonde. The water was filtered through 150- μ m mesh to remove large zooplankton. The filtered water was placed in 500-mL, UV-transparent Bitran S Series bags. There were four treatments with three replicates each: N addition (8 μ M N as NaNO₃), P addition (1 μ M P as NaH₂PO₄-), N&P addition (8 μ M N and 1 μ M P) and the control (no additions). The bags were incubated in submerged PVC racks. Based on light profiles from a Biospherical Instruments profiling radiometer, bags were incubated below the 1% attenuation depth for ultraviolet-B radiation (UVR) (measured at 320 nm) but above the 10% attenuation depth for photosynthetically active radiation (PAR) (5 m in Jordan Pond, 2 m in Echo Lake, 1.5 m in Richie Lake, and 2 m in Sargent Lake). Incubations lasted for 7 days in ACAD and 5 days in ISRO. The incubation time in ISRO was shorter to account for temperature differences that were 4°C higher in ISRO than ACAD.

Table 1. Acadia and Isle Royale National Park water chemistry data (Vaux et al. 2008; Elias 2009). (ACAD=Acadia National Park, ISRO=Isle Royale National Park, Chl a =Chlorophyll a, TP=Total phosphorus, TN=Total nitrogen, DOC=Dissolved organic carbon).

Region	Lake	Max.Depth (m)	Area (ha)	Chl a (μ g/L)	TP (μ g/L)	TN (μ g/L)	DOC (mg/L)	NO ₃ (μ g/L)
ACAD	Echo	20	95	3	8	128	3	40
ACAD	Jordan	46	75	4	4	115	2	130
ISRO	Sargent	13	143	2	14	431	10	<1
ISRO	Richie	11	216	6	25	585	11	<1

At the end of the experiment, samples for chlorophyll a analyses were collected in duplicate from each bag on Whatman GF/F filters and processed using standard methods (APHA 2000) with modifications as outlined in Saros et al. (2005a). Phytoplankton taxa were determined to phylum level by counting samples on an inverted microscope. Data analyses were performed using one-way ANOVA with Tukey's post-hoc test and Levene's test for equal variances on chlorophyll a data from the nutrient limitation assays to determine the limiting nutrients in each of the study lakes.

Nitrogen Gradient and DOC Experiments

These methods address Objectives 2 & 3. A factorial experiment combining a nitrogen gradient with two different types of DOC treatments (Fig. 2) was carried out in June of 2010 in ACAD and August 2010 in ISRO. One lake in ACAD, Jordan Pond, and one in ISRO, Sargent Lake, were used for this experiment. Lake water was collected from the midpoint of the epilimnion, which was 6 m in Jordan Pond and 2 m in Sargent Lake. The water was then filtered using 150- μ m mesh to remove large grazers and placed in 1-L UV transparent Bitran S series bags made of polyethylene (reduction of 6% PAR (400-700 nm) and 14% UVR (295-399 nm)). The nitrogen enrichment treatments consisted of 0, 5, 10, 20, or 40 μ g NO₃⁻ N L⁻¹. Three sets of this N gradient were created in triplicate (i.e., three sets of 5 N x 3 replicates). One set of N gradient triplicates was not combined with any DOC treatment (i.e., these were controls for the DOC additions, and are referred to as N alone). A second set was shaded by DOC (as prepared below) by adding DOC to a second bag of lake water and attaching this bag on top of the treatment bag with waterproof tape. This allowed for shading to occur without changing the chemical composition of the treatment bag containing phytoplankton. These are referred to as “shaded” treatment. The third set was directly spiked with the DOC (referred to as “DOC added”). To simulate DOC changes of a realistic magnitude, additions were chosen that reflect DOC increases that have already occurred in each area (Vaux et al. 2008; Toczydlowski and Stottlemeyer 2009). Thus, DOC additions were 1 mg L⁻¹ to Jordan Pond (ambient DOC of 2 mg L⁻¹) in ACAD and 4 mg L⁻¹ to Sargent Lake (ambient DOC of 10 mg L⁻¹) in ISRO.

0	5	10	20	40	N alone
0	5	10	20	40	Shaded
0	5	10	20	40	DOC added

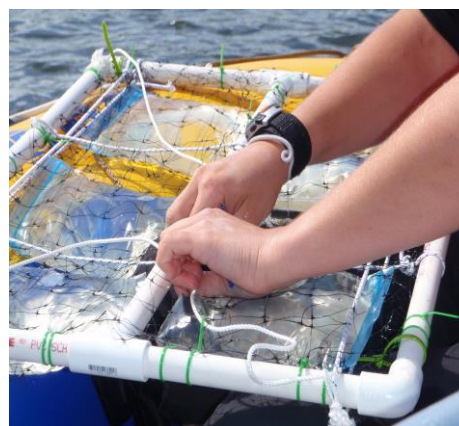


Figure 2. Experimental design, with the same nitrate gradient (0, 5, 10, 20, and 40 μ g L⁻¹ NO₃⁻-N) used in all series. Photo depicts incubation system for the experiment.

Dissolved organic carbon spikes for each location were prepared by placing 10 grams of finely ground organic matter (fallen leaves) from each of the corresponding watersheds and allowing it to steep for three days in 1 L of deionized water. While steeping, the samples were sterilized by tyndallization at 70° C for 8 hours and then allowed to cool, with this process repeated three times to sterilize the leachate. Samples were filtered using Whatman GF/F filters, then diluted and analyzed for DOC concentration and refrigerated prior to experimental use.

The bags were placed in PVC racks with light reducing window mesh and plastic to prevent UV and PAR inhibition. The mesh and plastic used reduced UVA (320-399) by 55-58%, UVB (295-310) by 55%-70%, and PAR (400-700 nm) by 40-45%. The racks were incubated at the surface of each lake for seven days. Samples were collected for chlorophyll *a*, as described above. Phytoplankton taxa were determined to phylum level by counting samples on an inverted microscope. Chlorophyll *a* was compared using a two-way ANOVA with Tukey's post-hoc tests and Levene's test for equal variances, with the two factors being N (0 or 40 µg L⁻¹) and DOC (None (N alone), Shaded, or DOC added). Regression analyses were also conducted across the N gradient. All statistical analyses were conducted using Sigma Plot. Relative responses (RR) of algal biomass to the highest N treatment, Shaded, and DOC added treatments were calculated for each lake by using the following equation:

$$RR = [(Chlorophyll\ a\ in\ the\ treatment/Chlorophyll\ a\ in\ the\ Control)-1]$$

Determination of Critical N Load

These methods address Objective 4. To determine surface water nitrate concentrations that stimulated algal biomass, a relationship was determined between the relative response of chlorophyll *a* and the nitrate gradient. Using this relationship, a percent change can be selected for use in setting a critical load. Ultimately, the goal is to relate this surface water concentration to an atmospheric deposition load, but this requires further data synthesis and investigation as described below.

Lakebed Sediment Cores

These methods address Objective 5. Three lake sediment cores, two from ACAD (Jordan Pond and Bubble Pond) and one from ISRO (Siskiwit Lake), were analyzed as well in this project. Jordan and Siskiwit were selected as representative deep lakes that would experience relatively less watershed influence and so potentially reflect an atmospheric signal. Bubble is a shallower lake; it was selected to assess whether benthic species of diatoms might also shed insight into changes in N loading to these lakes.

The Jordan Pond and Siskiwit Lake cores were already collected and dated, the Bubble Pond core was a new collection and was dated using 210Pb analysis at the University of Maine Physics Lab. Upon closer examination of the archived core from Siskiwit Lake, however, there was not enough material, so Saros collected a new core, which was dated by Dr. Dan Engstrom at the St. Croix Watershed Research Station, MN.

Diatom assemblages were identified after treating sediment with 10% HCl and 30% H₂O₂ to digest carbonate and organic matter. The processed samples were settled onto coverslips and mounted onto slides with Naphrax. A minimum of 300 valves per slide were counted on an Olympus BX-51 microscope with differential interference contrast under oil immersion at 1000× magnification. Diatom taxonomy was based primarily on Krammer and Lange-Bertalot (1986–1991) and Camburn and Charles (2000).

Fossil algal pigments were used to provide a record of lake productivity and algal community structure. This provides an expanded view of algal changes over what can be determined from diatom fossils alone, because pigments from multiple types of algae can be preserved in sediments.

While all algae contain chlorophyll a, a specific algal taxon contains pigments unique to that taxon. Pigments were isolated from sediment samples using high performance liquid chromatographic separations of chlorophylls, carotenoids and their derivatives (Leavitt & Findlay 1994). Pigments isolated from sediments were identified by spectral characteristics and chromatographic mobility by comparison with those from unialgal cultures (Chen et al. 2001).

To further assess whether N deposition has affected these lakes, the carbon (C) and N content of organic material in sediments, as well as its isotopic signature, was measured. Nitrogen isotope values that become lighter (closer to zero and/or more negative in value) over time suggest a source of more recently fixed N and are often inferred as a sign of enhanced N deposition.

Results and Interpretation

Nutrient Limitation Experiments

Based on measured changes in chlorophyll *a* concentrations across treatments, ***algal growth in both ACAD lakes was co-limited*** by N&P ($p < 0.01$) (Fig. 3). In the ***ISRO lakes N alone was enough to stimulate algal biomass*** ($p < 0.01$) (Fig. 3). After alleviating N limitation, the growth of algae in Sargent Lake was further stimulated by P. The ISRO lakes were dominated by Cyanobacteria while the ACAD communities consisted primarily of chrysophytes and chlorophytes (see examples in Fig. 4). In Jordan Pond in ACAD, N or P enrichment led to an increase in the relative proportion of chlorophyte algae and decrease in chrysophytes (Fig. 4A). In Sargent Lake in ISRO, the relative abundances of algal phyla did not change across treatments (Fig. 4B).

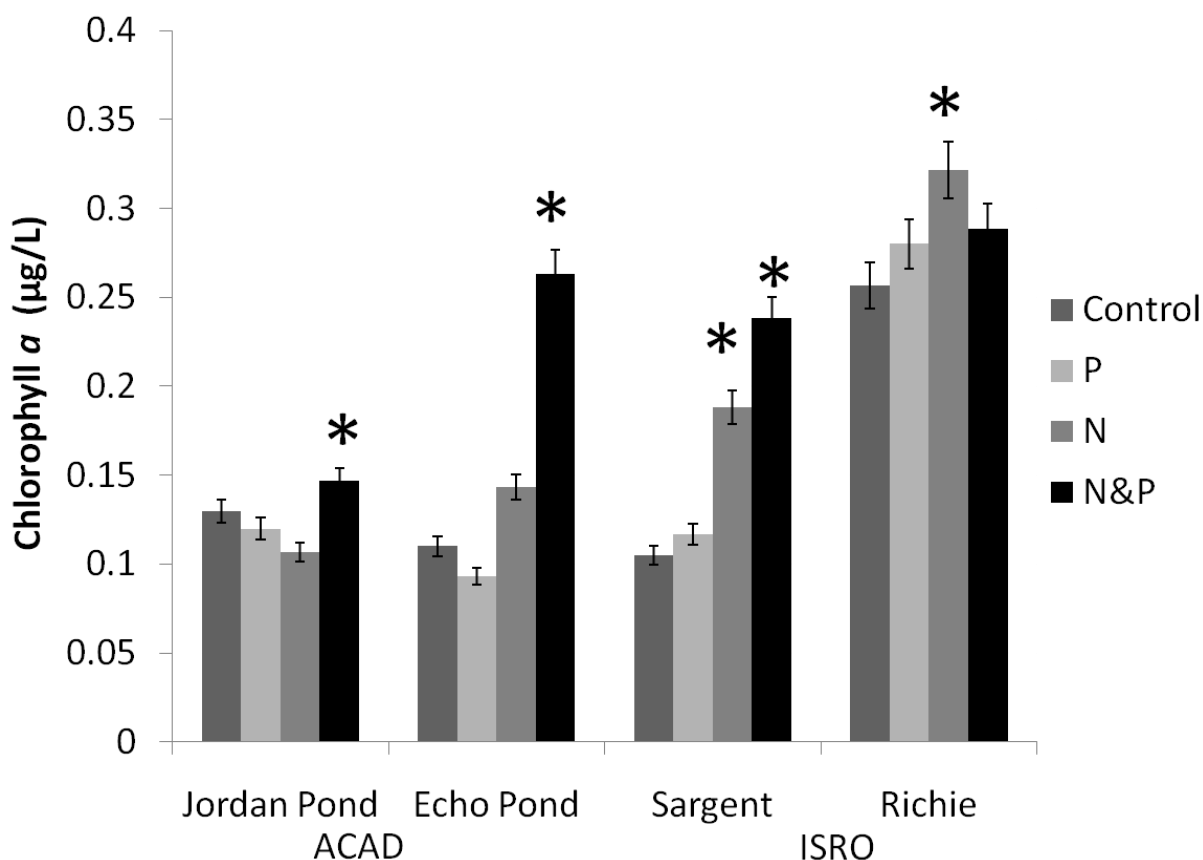


Figure 3. Results of nutrient enrichment experiments, with significant responses indicated by an asterisk.

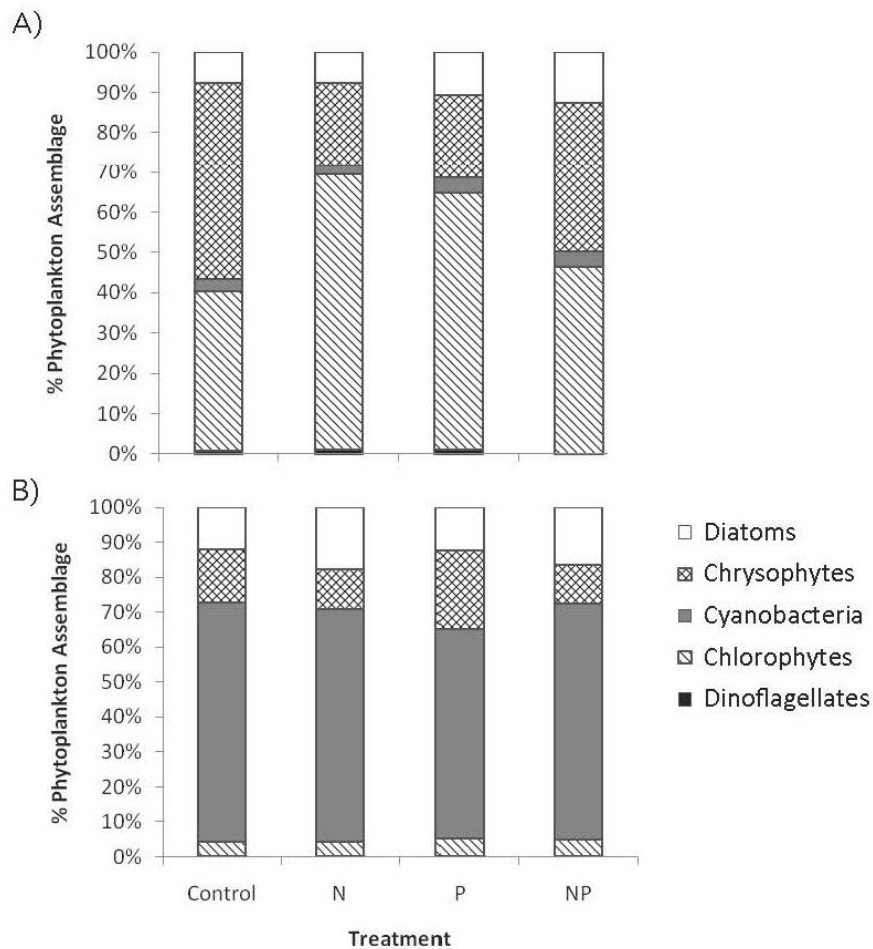
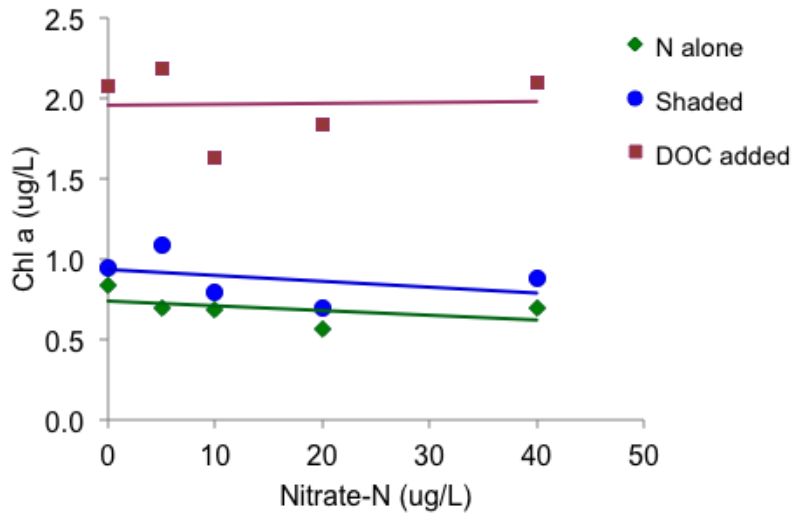


Figure 4. Relative abundance of algal phyla across a sub-set of the nutrient enrichment experiments: A) Jordan Pond in ACAD, B) Sargent Lake in ISRO.

Nitrogen Gradient and DOC Experiments

Effects of the nitrogen additions on algal biomass differed in the two parks. In Jordan Pond in ACAD, identified as co-limited by N and P, chlorophyll *a* did not change along the N gradient in any of the treatments (N alone ($p=0.425$), DOC shaded treatment ($p=0.516$), DOC addition ($p=0.948$); (Fig. 5)), further confirming that ***N additions alone do not stimulate algal growth in this co-N&P limited lake in ACAD.*** The treatments receiving the DOC addition had algal growth that was twice as high as those not receiving the DOC addition, indicating that ***the DOC addition contained nutrients that stimulate algal growth,*** and likely contain both N and P given the positive response here.

A)



B)

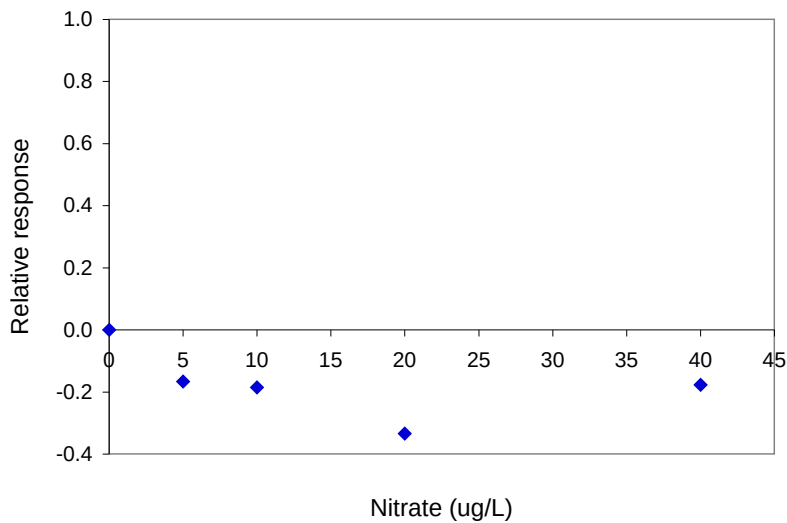
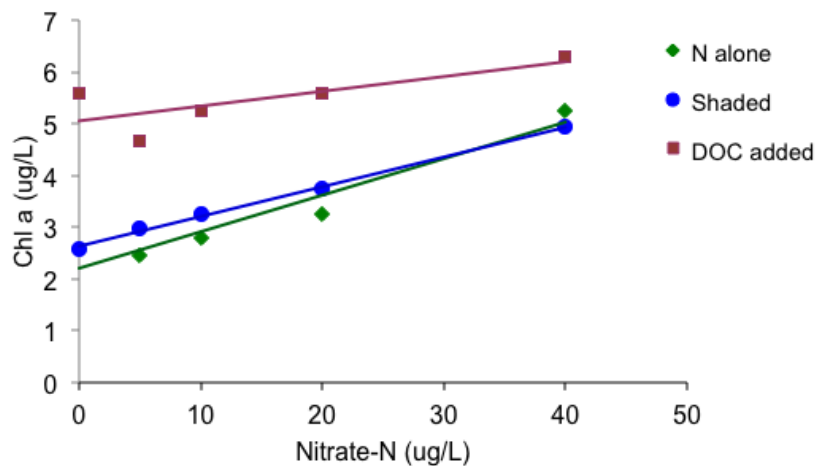


Figure 5. Results of nitrate gradient and DOC addition experiments in 2010 for Jordan Pond, ACAD: A) changes in chlorophyll concentrations across the N gradient and with different DOC amendments; B) the percent change (i.e., change in chlorophyll relative to control) along the nitrate gradient.

In Sargent Lake, a N-limited ISRO lake, regression analysis revealed that **algal biomass increased with N additions** (indicated by “N alone” in Fig. 6A; $p=0.006$). This was also the case in the DOC shading treatment ($p=0.001$). In contrast, N additions had no effect in conjunction with the DOC addition series ($p=0.136$; Fig. 6A), but algal biomass was higher in all bags to which DOC was added, indicating that **DOC additions provide nutrients that stimulate algal growth**.

With N additions alone, the first positive response to N additions in Sargent Lake occurred at 10 $\mu\text{g L}^{-1}$ NO_3^- -N, resulting in a 10% increase in biomass (Fig. 6B). This is similar to the 7 $\mu\text{g L}^{-1}$ NO_3^- -N defined as a threshold through different experimental methods for the Rocky Mountains (Nanus et al. 2012). The strength of the relative response continued to increase with nitrate above that level, with about a 25% increase in biomass at 20 $\mu\text{g L}^{-1}$ NO_3^- -N and an over 100% increase at 40 $\mu\text{g L}^{-1}$ NO_3^- -N.

A)



B)

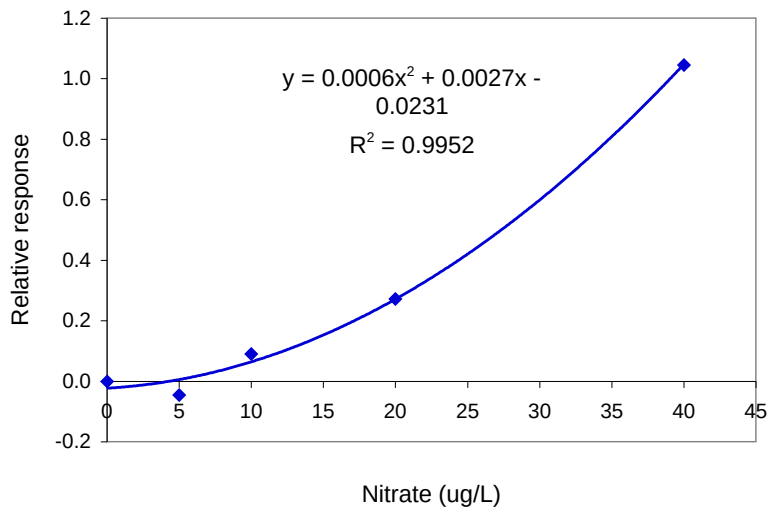


Figure 6. Results of nitrate gradient and DOC addition experiments for Sargent Lake, ISRO: A) changes in chlorophyll concentrations across the N gradient and with different DOC amendments; B) the percent change (i.e., change in chlorophyll relative to control) along the nitrate gradient.

Overall, the effects of *N additions are dependent on nutrient limitation status* (i.e., effect observed in N-limited lakes), whereas regardless of the nutrient limitation pattern, the effects of the *DOC treatments on algal biomass were similar across lakes*. ANOVA analyses revealed that in comparison to controls, the DOC addition nearly doubled algal biomass in both the N-limited ($p < 0.001$) and N&P co-limited lakes ($p = 0.004$), whereas the shading treatment had no effect on algal biomass in the N limited ($p = 0.679$) or N&P co-limited lakes ($p = 0.258$).

The relative abundances of phytoplankton phyla changed relatively little across the treatments in this experiment (Fig. 7). Looking at just the two ends of the nitrogen gradient (0 and 40 $\mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$) in conjunction with the DOC treatments, there was no change in Jordan Pond across the treatments (Fig. 7A). In Sargent Lake in ISRO, chlorophytes increased in relative abundance with the 40 $\mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$ addition, as well as in the DOC added treatments (Fig. 7B).

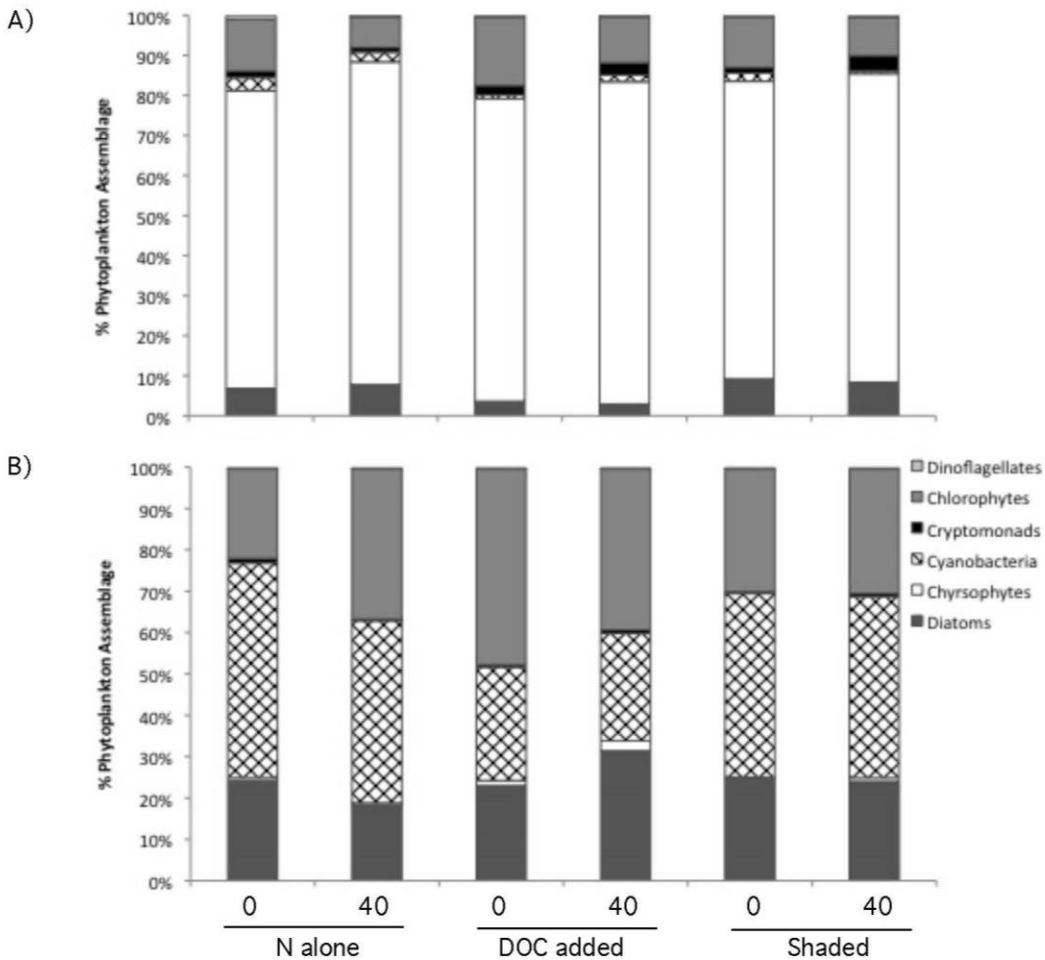


Figure 7. Relative abundance of algal phyla across the nitrate and DOC addition experiment: A) Jordan Pond in ACAD, B) Sargent Lake in ISRO. Nitrate concentration (either 0 or 40 $\mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$) indicated along x-axis, along with DOC treatment.

Implications for and Determination of N Critical Loads for Aquatic Resources

The lack of effects of N enrichment on algal growth in ACAD lakes suggests that N deposition alone is not affecting these lakes significantly, hence a critical load is not apparent for phytoplankton, which are some of the most sensitive organisms to N enrichment. In ISRO, the surface water concentrations of nitrate that stimulated algal growth ranged from 10 to 40 $\mu\text{g L}^{-1} \text{NO}_3^- \text{N}$, with approximately 10 to 100% stimulation across that gradient. For no observable effects to occur, a concentration just below 10 $\mu\text{g L}^{-1} \text{NO}_3^- \text{N}$ should be used as the critical level.

Data to relate N deposition to surface water nitrate concentrations are more widely available for ACAD than ISRO, but based on the results here, ISRO is the park in need of this link. Saros discussed this prospect with Dr. William Hobbs (Associate Scientist, St. Croix Watershed Research Station), who has another N project for parks in the Great Lakes region, and Dr. Leora Nanus (Research Scientist, San Francisco State University), who has previously conducted spatial modeling to relate N deposition to surface water nitrate concentrations (Nanus et al. 2003), to compile all available deposition and surface water nitrate concentrations for ISRO. At present, it does not seem that there are sufficient data available to use Dr. Nanus' modeling approach (Nanus et al. 2003) to relate N deposition to surface water nitrate concentrations in that park, but this is still being explored.

Lakebed Sediment Core

Jordan Pond, ACAD

The diatom profile (Fig. 8) reveals little change in species composition over the past century, with some fluctuation between the relative abundances of *Cyclotella stelligera* and *Cyclotella bodanica*. These two species have essentially the same pH optimum of 7.3 (Dixit & Smol 1994).

The pH reconstruction was calculated using the Maximum-likelihood (ML) model (Birks 1995). The ML model estimates pH optima and tolerances along a limnological gradient from a calibration set by fitting species-environment response curves. These curves are then used to calculate a most probable pH value for the species assemblage from each core assemblage. The calibration set and methodology used in this study was from Ginn et al. (2007), with pH inferences having a root mean squared error of prediction of 0.43.

The diatom-inferred pH for Jordan Pond indicates that lake water pH did not change during the 20th century (Fig. 9). This, however, does not imply that the lake is unaffected by sulfur and nitrogen deposition, just that lake water did not acidify. In the Northeast, it is not unusual to find systems in which lake water pH remained unchanged during the 20th century, but watershed acidification occurred and was evident via other changes in lake water chemistry, such as changes in DOC, aluminum and calcium (Davis et al. 2006). It is worth noting that, although diatoms do not reveal pH changes in this case, monitoring data from the park reveal that water transparency has declined in Jordan Pond (as well as other ACAD lakes) since 1996, and concentrations of dissolved organic carbon have been increasing.

The diatom profile does not suggest any effects of N enrichment on diatom community structure (Fig. 8). This is consistent with the results of our experiments, which indicated that Jordan Pond is co-limited by N and P, hence N alone should not induce enrichment effects on phytoplankton.

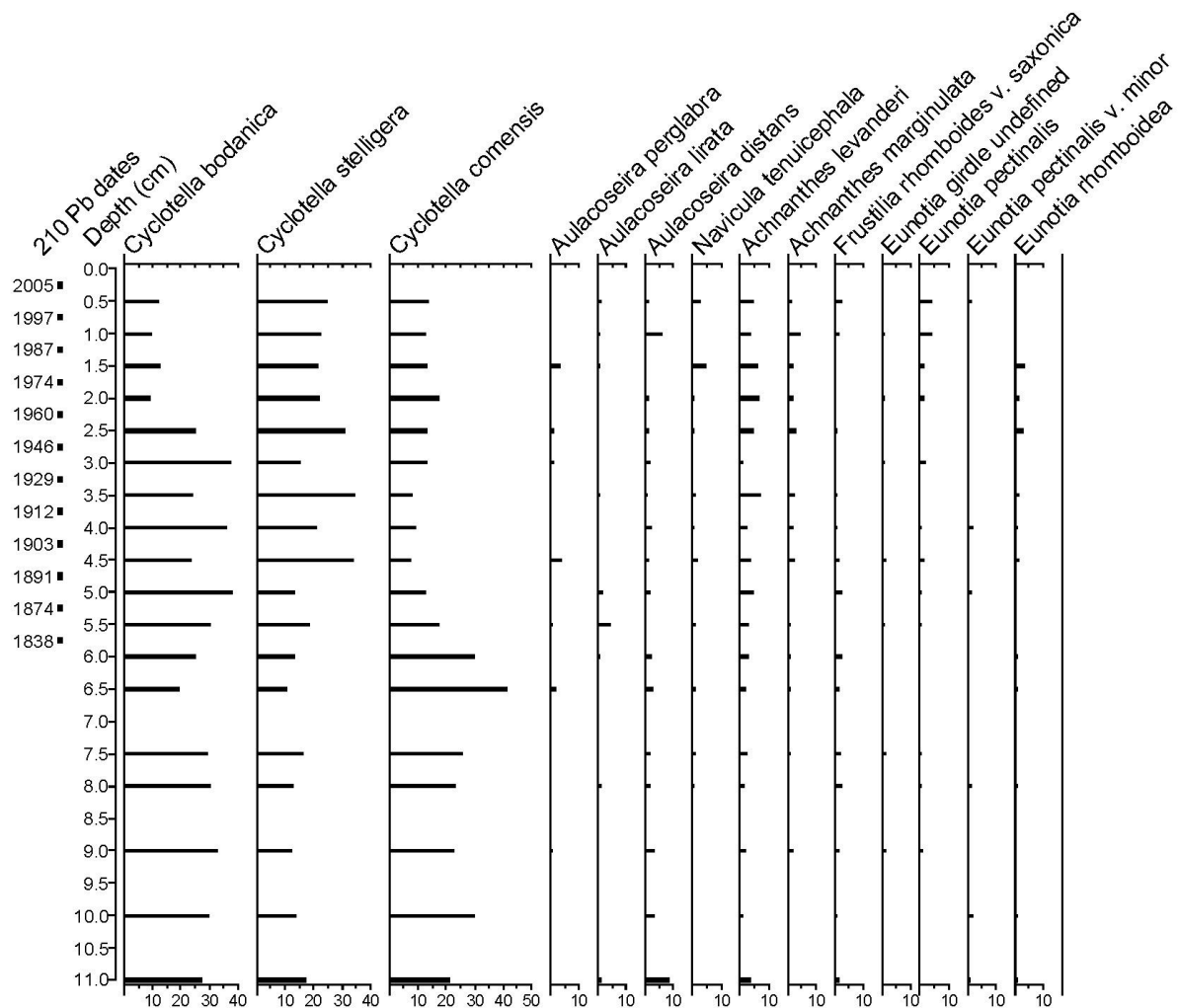


Figure 8. Sedimentary diatom profile from Jordan Pond, ACAD. Values for species are reported as percent relative abundance of each assemblage.

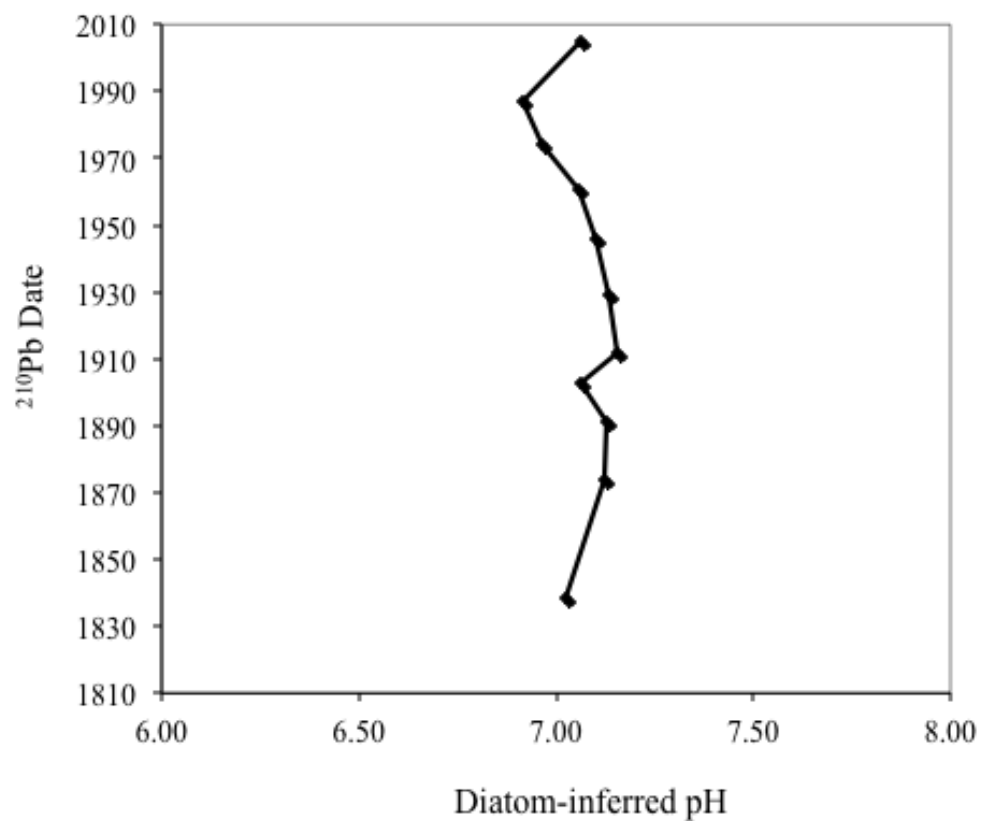


Figure 9. Diatom-inferred pH from Jordan Pond sediment core, based on recent diatom re-count of the 2008 Jordan Pond core.

The concentrations of various algal pigments in the Jordan Pond sediment core changed relatively little over the 20th century (Fig. 10), suggesting that algal production and species composition have changed little over this time. Some of the profiles (alloxanthin, canthaxanthin, and chlorophyll a) do indicate a possible increase in concentrations from 1950 to 1970 (indicated by blue box), and then a decline to present day. That increase may have been related to the 1948 fire in the Jordan Pond watershed.

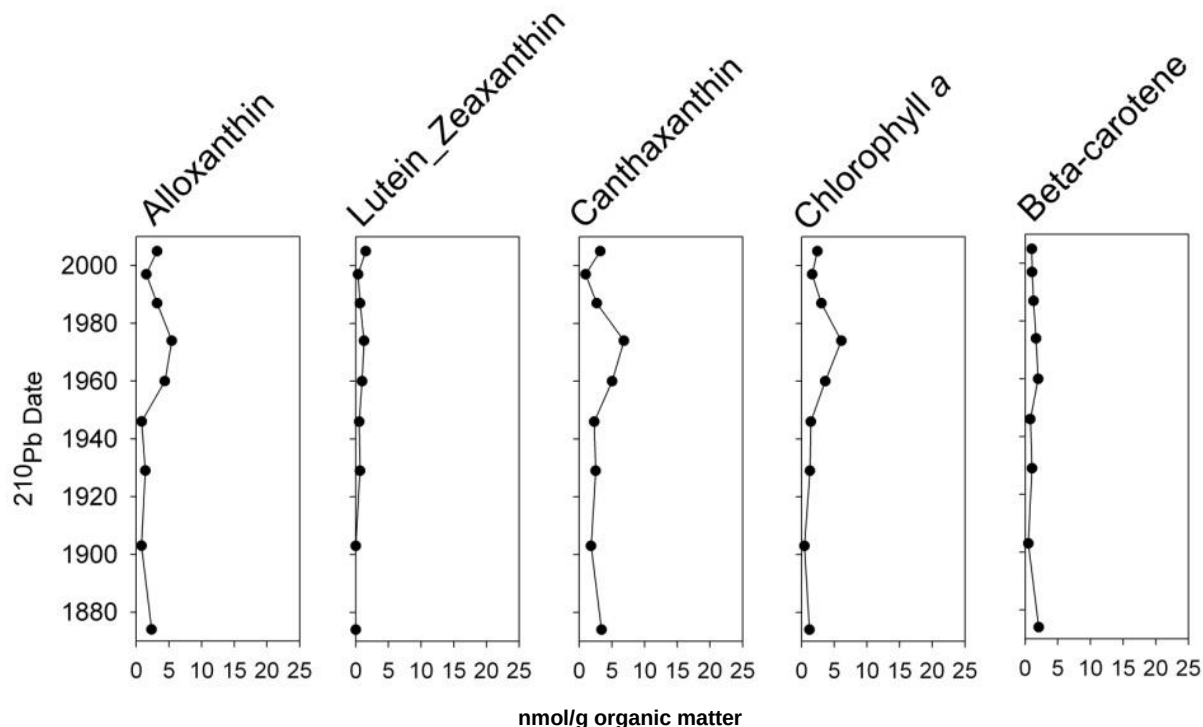


Figure 10. Algal pigments in the Jordan Pond core. From left to right, pigments are expressed as: 1) alloxanthin, from cryptophyte algae; 2) lutein-zeaxanthin, found in green algae, cyanobacteria, and euglenoids; 3) canthaxanthin, found in colonial cyanobacteria; 4) chlorophyll a, found in all algae; 5) beta carotene, found in all algae and plants. Blue box indicates period of slight increase in some pigments.

The percent organic matter in the Jordan Pond core varied little during the 20th century (Fig. 11), suggesting little change in total within lake productivity. The elemental data, however, reveal chemical changes in the lake's organic material as a result of N deposition, with $\delta^{15}\text{N}$ values becoming progressively lighter since 1900.

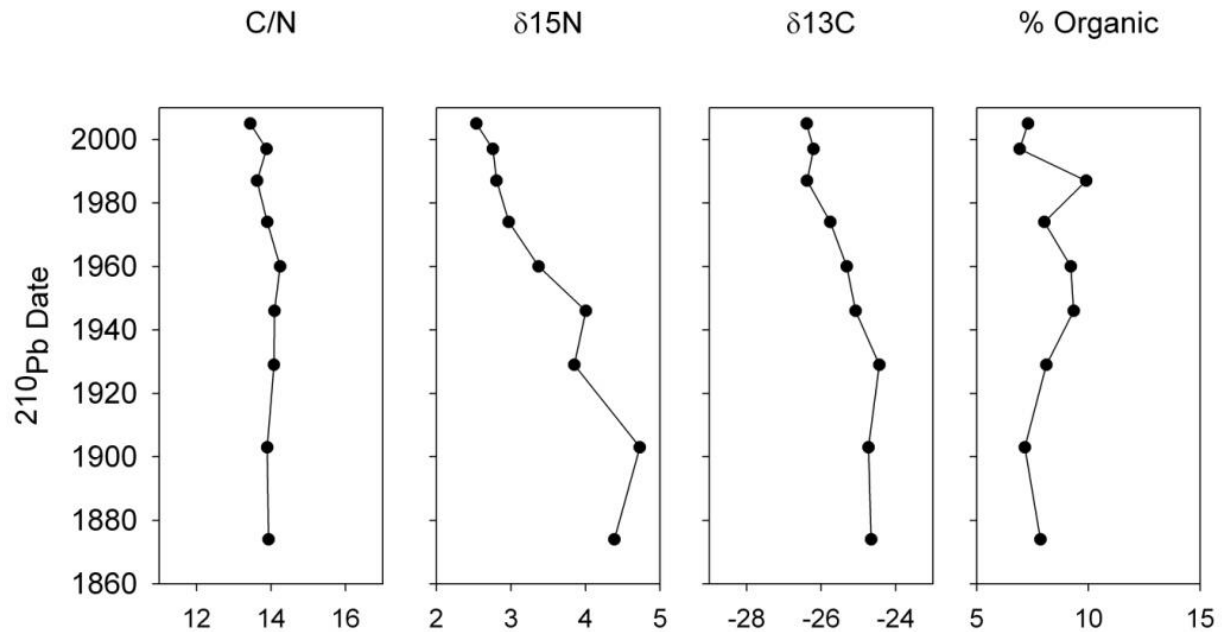


Figure 11. Elemental and organic composition of Jordan Pond core.

Bubble Pond, ACAD

The diatom profile from the Bubble Pond core (Fig. 12) was dominated by two species, *Cyclotella bodanica* and *Discostella stelligera* (also referred to as *Cyclotella stelligera*). These two species had relatively constant abundances up until about 1900, after which *C. bodanica* declined while *D. stelligera* increased up until the mid-20th century. At that point, *D. stelligera* declines while a variety of benthic species increase. The diatom-inferred pH suggests only slight acidification during the last half of the 20th century (Fig. 13). As in Jordan Pond, the diatom profile does not suggest N enrichment effects on diatom community structure in this lake.

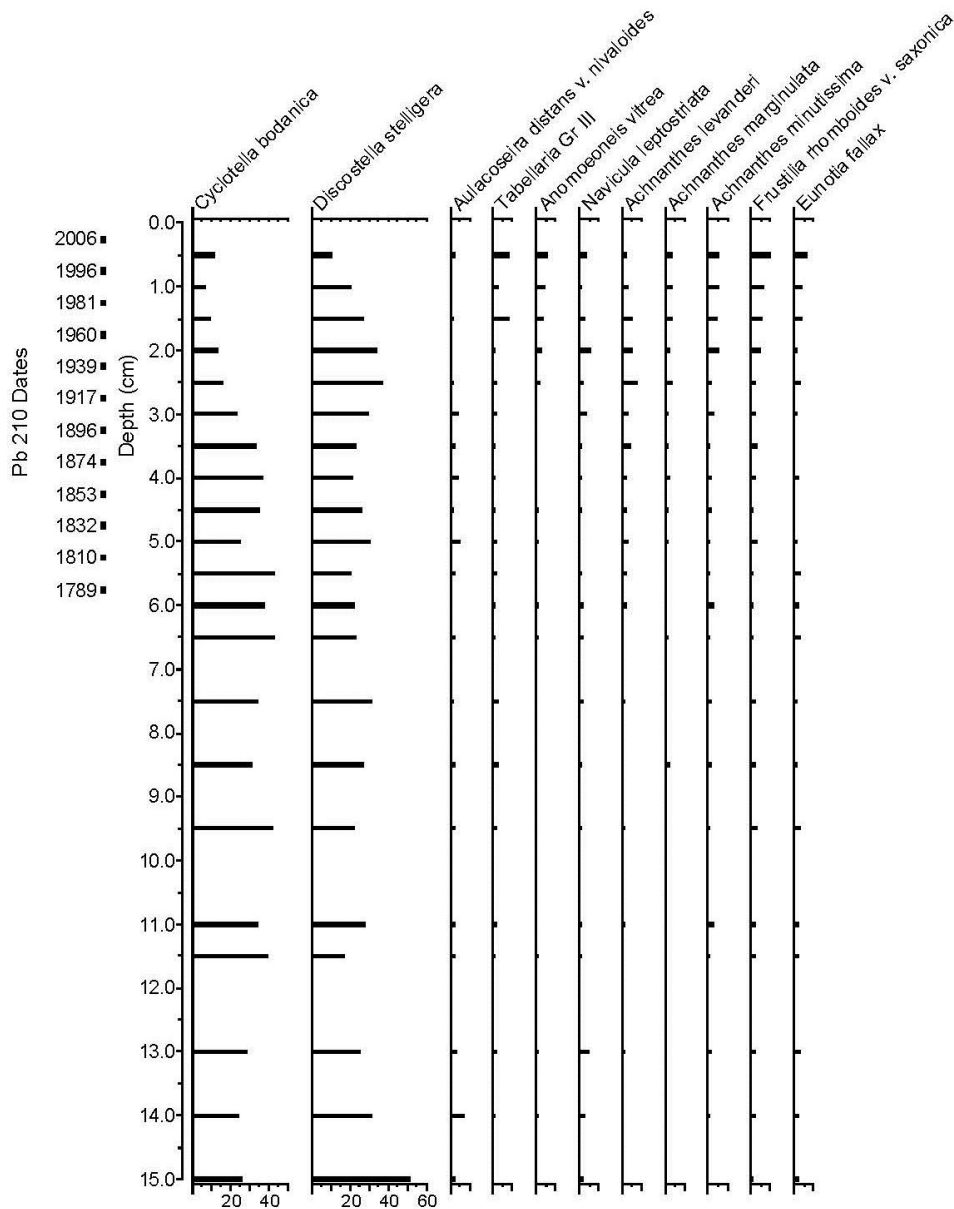


Figure 12. Diatom profile from the Bubble Pond core, with each increment on the x-axis representing 5% of relative abundance.

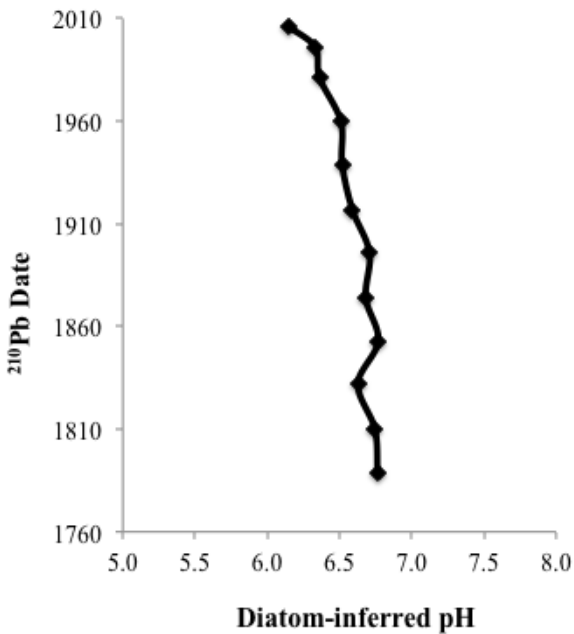


Figure 13. Diatom-inferred pH from Bubble Pond sediment core.

The pigment profile from Bubble Pond (Fig. 14) shows little change, except that alloxanthin (indicator of cryptophyte algae, a common group of algae in boreal lakes) increases toward the top of the core. The other pigments that increase towards the top of the core (e.g., fucoxanthin, chlorophyll a) often degrade over time, and hence often increase toward the top of the core, making it difficult to assess whether these changes reflect changes in algal species or simply degradation. Overall, the changes are small.

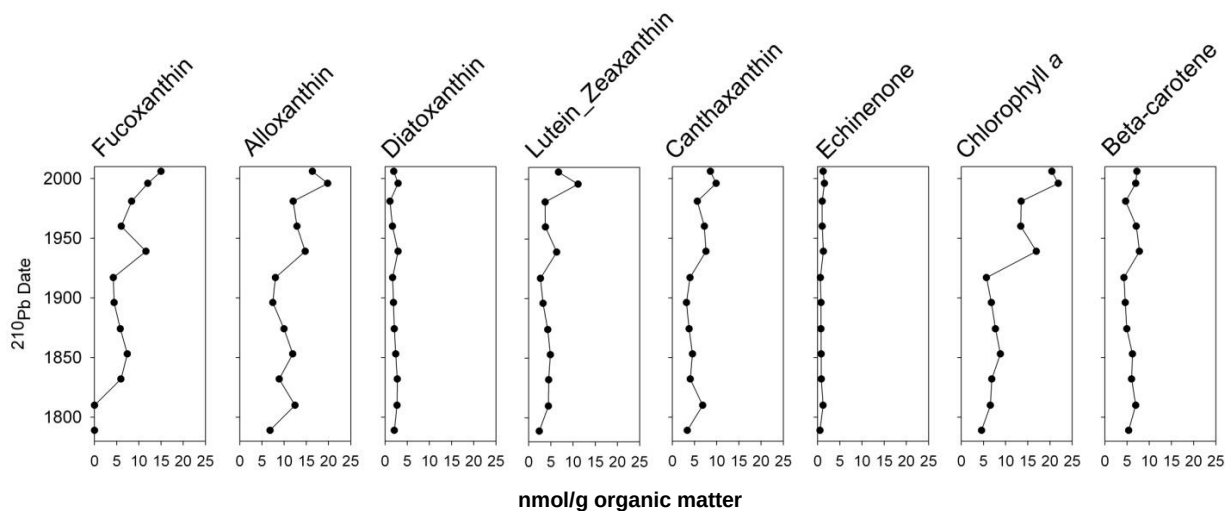


Figure 14. Algal pigments in the Bubble Pond core, with pigments as described in Fig. 10 along with: fucoxanthin, indicative of diatoms and other golden-brown algae; diatoxanthin, indicative of diatoms; echinenone, indicative of colonial cyanobacteria.

The percent organic matter in the Bubble Pond core fluctuated during the last 200 years (Fig. 15), without a clear direction of change in total within lake productivity over this time. Similar to Jordan Pond, the elemental data reveal chemical changes in the lake's organic material as a result of N deposition, with $\delta^{15}\text{N}$ values becoming progressively lighter since 1950 in this lake. In addition, the C:N ratio of organic material declines slightly in this lake, from 15 in 1800 to 13 at present.

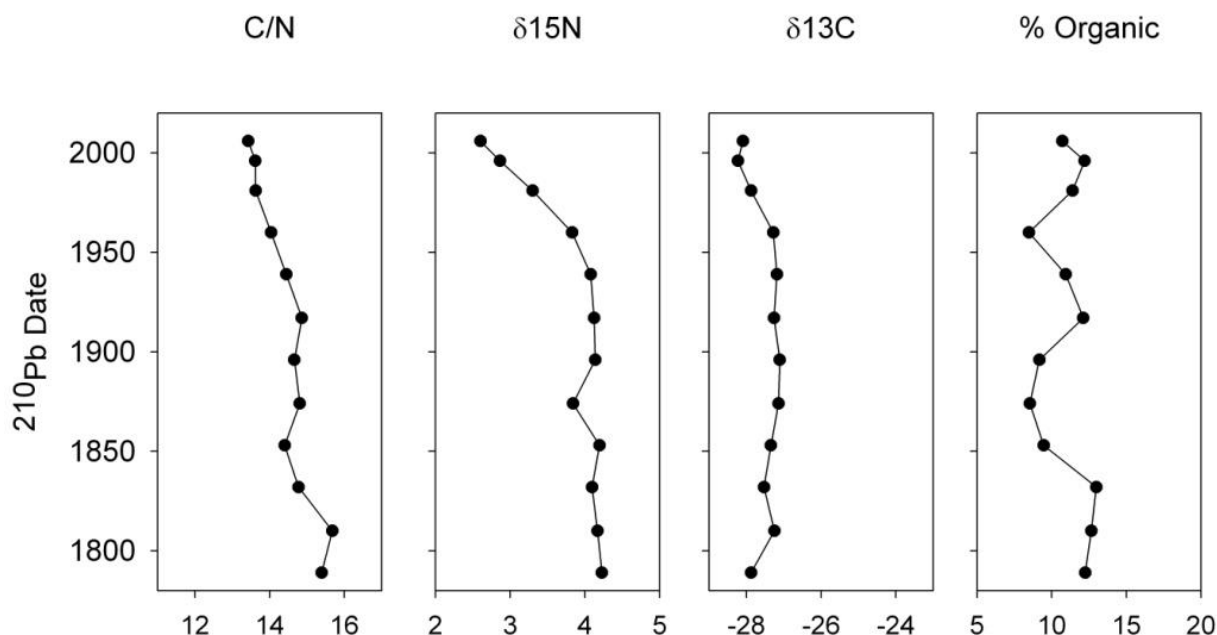


Figure 15. Elemental and organic composition of Bubble Pond core.

Siskiwit Lake, ISRO

The diatom profile from Siskiwit Lake (Fig. 16) shows remarkable change over the 20th century. The relative abundances of several *Cyclotella* species change directionally over this time, with larger taxa such as *Cyclotella bodanica* and *Cyclotella comensis* increasing and the smaller species *Discostella stelligera* declining. The results of an NSF-funded project to Saros in the Rocky Mountains to discern the ecology of these species revealed that these species respond strongly to climate-induced changes in lake mixing depths when nitrate concentrations are adequate (Saros et al. 2012). The species changes here suggest that mixing depths have become progressively deeper in Siskiwit Lake over the 20th century. Given the large surface area of Siskiwit Lake, wind strength would have to increase over the lake to produce deeper mixing depths. Desai et al. (2009) demonstrated that wind strength has been increasing over Lake Superior (and thus, likely over ISRO) in recent decades.

The diatom record also shows slight increases in *Asterionella formosa* and *Tabellaria* species, species indicative of nutrient enrichment, although their relative abundances remain very low.

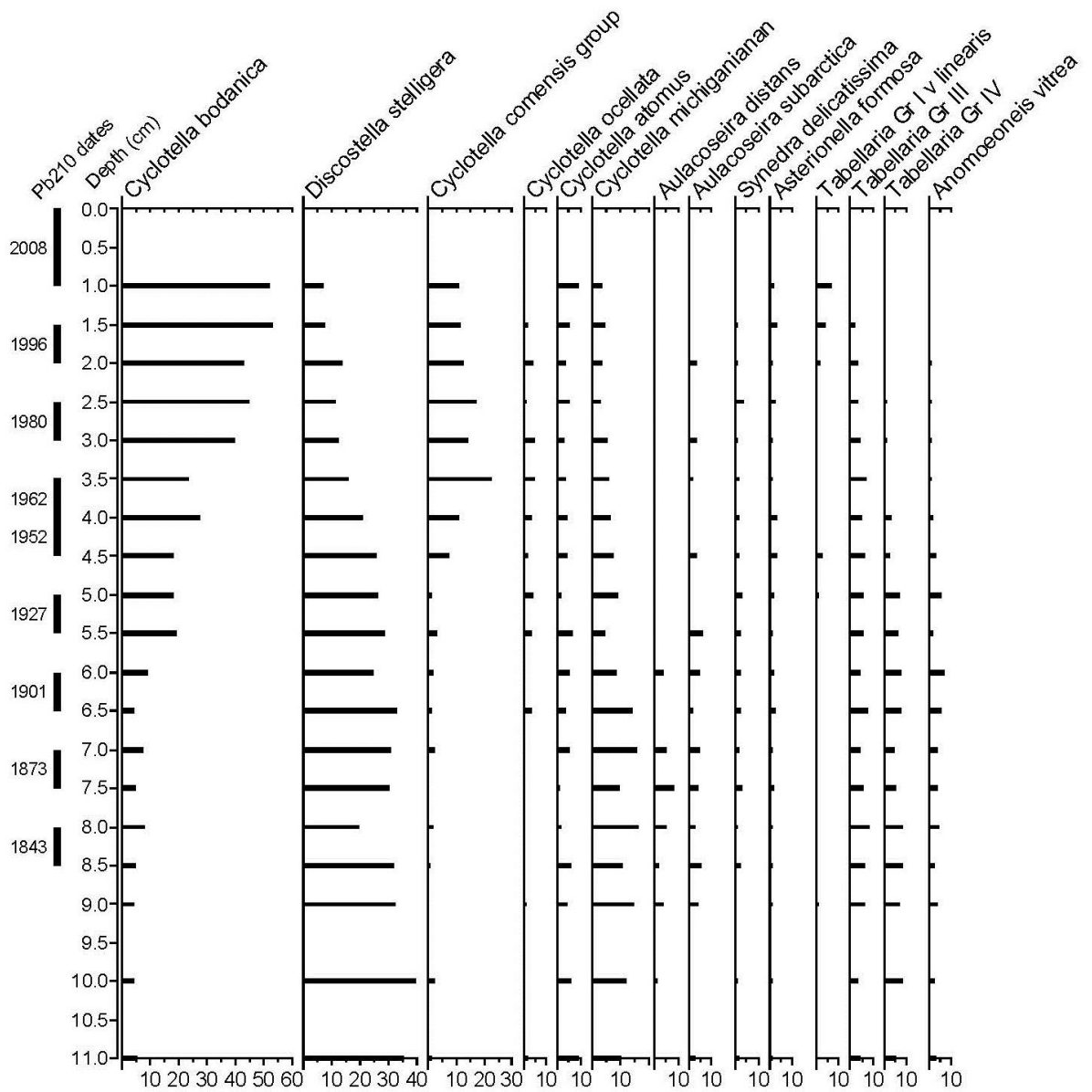


Figure 16. Diatom profile from the Siskiwit Lake core.

While the diatom record shows strong changes over the last century, the pigment record shows very little change (Fig. 17). Alloxanthin (indicative of cryptophyte algae) fluctuates moderately over the record, and lutein plus zeaxanthin (found in green algae, cyanobacteria, and euglenoids) increase slightly after 1960.

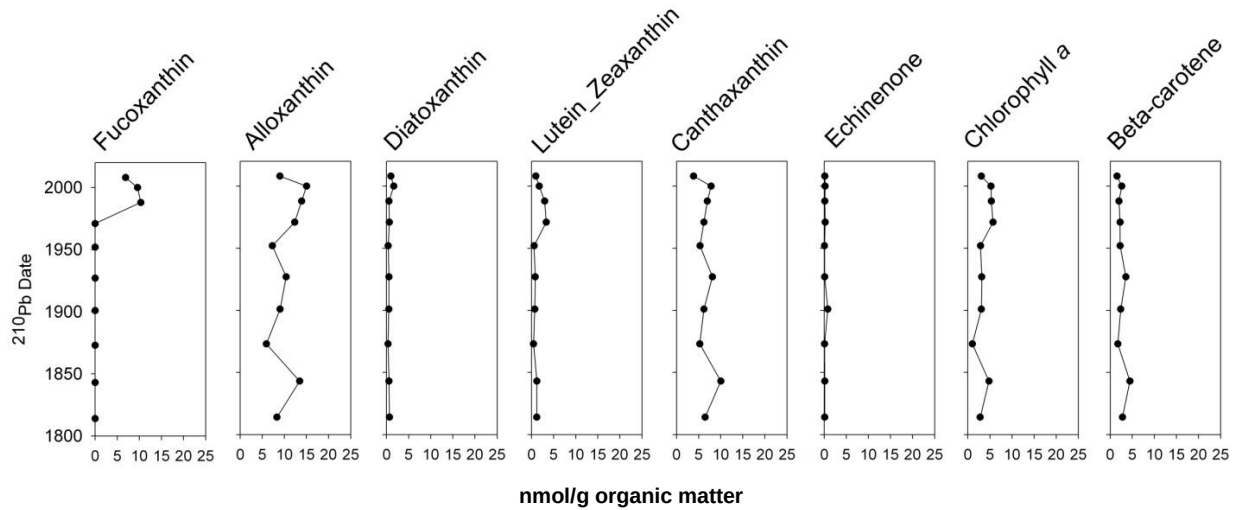


Figure 17. Algal pigments in the Siskiwit Lake core, with pigments as described in Figs. 10 & 14.

The percent organic material remains unchanged throughout the Siskiwit Lake core (Fig. 18). Similarly to the ACAD lakes, the elemental data reveal chemical changes in the lake's organic material as a result of N deposition, with $\delta^{15}\text{N}$ values becoming progressively lighter since 1960 in this lake. In addition, the C:N ratio of organic material declines very slightly in this lake, from 12.5 in 1950 to 11.7 at present.

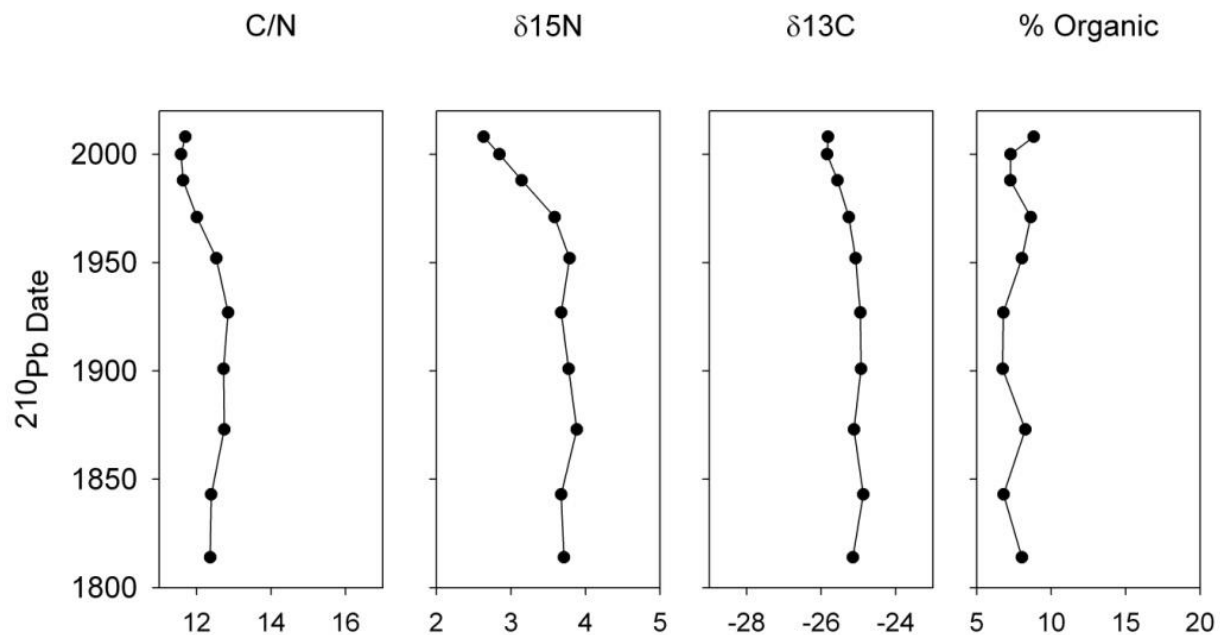


Figure 18. Elemental and organic composition of Siskiwit Lake core.

Clearly, the Siskiwit Lake record reveals that this lake is also being strongly influenced by the regional climate. It still remains unclear, however, to what extent changing nitrogen concentrations have played a role in biological changes.

Conclusions

In summary, this study demonstrated that algal growth in Jordan Pond and Echo Lake in ACAD was co-limited by N and P, whereas it was limited by N alone in Sargent Lake and Richie Lake in ISRO. There was thus no response to the N gradient experiment in ACAD, whereas algal biomass increased with N additions in Sargent Lake, a N-limited ISRO lake. The first positive response to N additions in Sargent Lake occurred at $10 \mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$, resulting in a 10% increase in biomass. The DOC shaded treatments had no effect in any case, whereas the DOC added treatments stimulated algal growth in both cases (in Jordan Pond and in Sargent Lake), indicating that DOC additions added nutrients. Overall, across both parks, the effects of N additions were dependent on nutrient limitation status (i.e., effect observed in N-limited lakes), whereas regardless of the nutrient limitation pattern, the DOC additions always stimulated algal growth. While the nitrogen isotopes in sediment cores reflect increasing N deposition since 1950 in all three lakes, there was generally little change in diatom fossils or algal pigments over the 20th century. The exception to this was the diatom fossil record of Siskiwit Lake in ISRO, in which changes in diatom community structure in this large, deep lake suggested climate-driven changes in this lake over the past century.

Overall, both atmospheric deposition and climate change have important implications for the base of lake food webs in both of these parks. Considering the environmental factors investigated here, the results of this study suggest that the greatest threat to lakes in ACAD is changes in DOC loading (which appear to be a consequence of both declining sulfur deposition and climate change), whereas the lakes of ISRO are vulnerable to increasing N deposition and climate change. Future research in ACAD should focus on the effects of increasing DOC concentrations on the biota of these lakes, and include considerations of episodic pulses of DOC given that storm frequency and severity has increased in this region. Future research in ISRO should focus on the effects of N deposition and climate change. Given the variation in lake morphometry and watershed influence across lakes on ISRO, research that identifies how vulnerability to N deposition and climate change varies across lake types may be useful for management purposes. In addition, ISRO is in need of better data capable of linking deposition rates to surface water concentrations. This might involve long-term monitoring across a set of sites on the island as N deposition varies. The Wallace Lake long-term study should be one key component of such monitoring.

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NPS 123/124334, 139/124334, April 2014

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