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DISSERTATION

MOUNTAIN LAKE RESPONSES TO ELEVATED NITROGEN DEPOSITION IN THE WEST: ALGAL PRODUCTIVITY AND NITROGEN RETENTION

Submitted by

Koren Risa Nydick

Graduate Degree Program in Ecology

In partial fulfillment of the requirements for the

Degree of Doctorate of Philosophy

Colorado State University

Fort Collins, Colorado

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY KOREN RISA NYDICK ENTITLED: MOUNTAIN LAKE RESPONSES TO ELEVATED NITROGEN DEPOSITION IN THE WEST: ALGAL PRODUCTIVITY AND NITROGEN RETENTION, BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTORATE OF PHILOSOPHY

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ABSTRACT OF DISSERTATION

WESTERN MOUNTAIN LAKE RESPONSES TO ELEVATED NITROGEN DEPOSITION: ALGAL PRODUCTIVITY AND NITROGEN RETENTION

Atmospheric nitrogen (N) deposition is increasing in many regions of the western United States. The objective of this work was to understand how increased N deposition inputs alter algal productivity and N uptake in shallow mountain lake ecosystems. Effects of elevated N, in combination with added phosphorus (P) and increased acidity were investigated with enclosure experiments. A large dataset, the Western Lake Survey, was then used to estimate the percentage of western mountain lakes likely to experience the observed experimental changes.

Study lakes east of the Continental Divide in the Colorado Front Range had high nitrate (NO_3) concentrations. Addition of NO_3 to bottle bioassays never caused detectable increases in phytoplankton biomass, but combined N and P amendments almost always increased phytoplankton biomass over controls. Larger enclosure experiments, which included benthic sediments and artificial tile substrates, yielded similar results. In contrast, study lakes in southern Wyoming had low NO_3 concentrations and were N limited. Phytoplankton biomass and photosynthetic rate increased up to four-fold in response to NO_3 amendments. Changes in phytoplankton community composition followed nutrient enrichment, but were more dramatic in response to increased acidity. Benthic algae rarely showed detectable changes in response to nutrients, alone or in combination with acid, but a ^{15}N isotope tracer addition

revealed that sediment dominated NO_3 uptake. Sediment organic matter and carbon explained over two-thirds of the variability in sediment NO_3 uptake, suggesting the importance of benthic microbial activity. These experiments showed that although phytoplankton were most sensitive to nutrient and acid additions, the benthos, and in particular, sediment microbial processes were likely the dominant regulator of ecosystem N uptake.

Using the Western Lake Survey, I estimated that 24% of mountain lakes without obvious land-based disturbances were N limited and likely would experience some degree of eutrophication from increased N input. Forty-eight percent of these lakes would not biologically buffer more than half of acid inputs from N deposition. Twelve percent also had very low measured alkalinities and were very acid sensitive. A substantial number of western lakes are likely to show some degree of observed experimental responses should N and acid inputs increase.

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1. Introduction

Although some mountain regions in the western United States are sheltered from many impacts of human use by designation as national parks, monuments, wilderness areas, recreations areas, or by shear difficulty of access, these lands are unprotected from air pollution. For example, Rocky Mountain National Park in Colorado is situated within 30-60 km of the Denver-to-Fort Collins urban/agricultural corridor and receives some of the highest levels of atmospheric nitrogen (N) deposition in the Rocky Mountain region. Deposition of 3-5 kg N ha^{-1} y^{-1} on the eastern slope of the Colorado Front Range has led to N saturation of old growth subalpine forests and altered plant communities in alpine tundra (Bowman and Steltzer 1998, Reuth and Baron 2002). Nitrogen deposition has elevated lake nitrate (NO_3) concentrations, increased diatom biovolumes, and altered diatom community composition (Baron et al. 2000, Wolfe et al. 2001). There are also reports of episodic acidification in headwater streams (Williams and Tonnesson 2000). The time scale of diatom shifts suggests that these alterations began affecting high elevation lake ecology approximately 50 years ago, coincident with intensive human population growth, increased animal production, and fertilizer usage in the South Platte Basin (Baron et al. 2000, Wolfe et al. 2001). Less extensive changes have been observed on the western slope, which receives only half as much N deposition.

Similar alterations have been documented in the eastern United States, but these generally have occurred at higher N deposition levels (Aber et al. 1998, Fenn et al.1998).

High elevation ecosystems in the western United States differ fundamentally from mountainous areas in the East because they include large expanses of bare rock, talus, and alpine tundra. The thin soils, sparse vegetation, snowmelt dominated hydrology, and short growing season of these alpine regions limit their ability to buffer against changes in atmospheric deposition (Baron et al. 1994, Bowman and Steltzer1992). Under these conditions terrestrial environments may leak excess NO_3 into lakes and streams, potentially causing eutrophication and acidification (Aber et al. 1998, Williams et al. 1996). Much attention has been given to the deleterious effects of acidification and aluminum toxicity on freshwater biota (Ingersoll et al. 1990, Schindler et al. 1989), but less is known about the consequences of N fertilization or the interactions between excess N and acid inputs on mountain lake ecology.

Nitrogen is an important regulating nutrient of freshwater algal growth (Axler et al. 1994, Elser et al. 1990, Morris and Lewis 1988), which suggests that excess N from atmospheric deposition could enhance algal primary productivity and biomass in certain lakes, and this has already been documented for Lake Tahoe in the Sierra Nevada (Goldman 1988, Jassby et al. 1994). Although phosphorus (P) often is not measured at low enough detection limits, it can be an important component of atmospheric deposition and also causes increased algal growth. A significant amount of P deposition was documented in montane regions in the Colorado Front Range and in the Lake Tahoe watershed (Lewis et al. 1985, Jassby et al. 1994). Furthermore, TP levels have increased since the mid-1980's at an intensively monitored alpine lake in the Sierra Nevada and at synoptic sampling sites in Rocky Mountain, Lassen Volcanic, Yosemite, and Sequoia/Kings Canyon National Parks (Clow et al. In Review, Sickman 2001).

The main objectives of my research were to determine how Rocky Mountain lakes respond biologically and biogeochemically to inputs of N, P, and acidity. I did this by comparing algal biomass, primary productivity, NO_3 uptake, and biological alkalinity generation between shallow mountain lakes with different ambient NO_3 levels, and manipulated conditions with controlled enclosure experiments. In particular, I compared the response of different algal habits (i.e. phytoplankton, algae on hard substrates, and algae on sediment) and determined the lake components most important in NO_3 uptake. Lastly, I used previously collected data to project the geographical extent of potential future alterations to western high elevation lakes from increases in both NO_3 and acid inputs.

This work resulted in several important findings. Perhaps most significantly, it showed that Rocky Mountain lakes can be N limited. Nitrogen amendments caused increased phytoplankton biomass and productivity in lakes with low ambient NO_3 concentrations. Benthic algae were relatively insensitive to nutrient inputs, however; we rarely detected changes in biomass. Although algal responses were far greater in the water column, the benthos dominated NO_3 uptake. Non-surface sediment was responsible for the greatest proportion of NO_3 uptake, and uptake was related to organic matter and carbon content, suggesting the importance of microbial activity. Thus, the second significant conclusion was that although phytoplankton were most sensitive to nutrient enrichment, non-photsynthetic benthic processes were most important in terms of ecosystem functions such as NO_3 uptake and associated biological alkalinity generation. Lastly, I estimated that a quarter of western mountain lakes without land-based disturbances were N limited and prone to some degree of N-induced eutrophication

observed in experimental enclosures. A substantial number of lakes were also acid sensitive and would be unable to buffer most of the acidity from potential future increases in N deposition.

This research was a collaborative effort between myself and another graduate student, Brenda Moraska Lafrançois. We used the same experimental designs, but investigated different aspects of mountain lake ecology. I focused on changes in algal biomass, productivity, N cycling, and water chemistry, while she was responsible for alterations in algal and zooplankton community composition (Lafrançois 2002). Together, we present a more complete picture of responses than would have been possible individually. Community composition data presented here is her work. Likewise, data that I collected is found in her dissertation. Ecological research is becoming more and more collaborative in nature, yet dissertations must represent the work of one graduate student. Our dissertations serve as an example of how graduate students can combine efforts, share data, still meet the individual requirements set forth by the graduate school, and produce more extensive and higher quality work than would have been possible if each had worked separately.

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2. Nitrogen enrichment in three subalpine lakes, Colorado Rocky Mountains, USA

2.1 Abstract

We explored variability and within-season coherence among subalpine lakes sharing very similar climate and atmospheric conditions, but differing in lake-specific factors. Special attention was given to nitrogen (N) dynamics because the study area receives some of the highest levels of atmospheric N deposition in the Rocky Mountains. We asked if the effect of regional N deposition would be manifested uniformly among neighboring lakes in terms of ambient conditions as well as potential response to greater nutrient inputs. Temperature was highly synchronous among all lakes, demonstrating that climate was indeed manifested similarly, but chemical and biological measurements, including nitrate (NO_3) levels, showed variable seasonal means and limited coherence. In general, NO_3 concentrations decreased and DOC increased along a continuum from a drainage lake with a non-vegetated watershed to seepage lake with a forested catchment and fringing wetlands. *In situ* bioassays conducted several times over the ice-free season showed that phosphorus (P) limitation decreased and N deficiencies increased along the same continuum. Enrichment with urea (CH_4N_2O) also caused increased phytoplankton biomass in the seepage lake, which points to the importance of the microbial loop as a source of algal nutrients. This study suggests that watershed characteristics and hydrology explain the main differences in subalpine lake NO_3 trends and algal

nutrient limitation. These factors, therefore, influence how individual lakes will respond to altered nutrient inputs from atmospheric deposition or climate change.

2.2 Introduction

An important theme in limnology concerns the variability of individual lakes within a region and the extent to which physical, chemical, and biological variables behave similarly. Lakes differ in predictable patterns in some regions depending on such factors as exposure to climactic conditions and hydrologic or landscape position (Magnuson et al., 1990; Kratz et al., 1997; Riera et al., 2000). In the Colorado Front Range, however, individual lake characteristics overwhelmed regional responses, and even lakes at similar altitudes or with similar exposure to the atmosphere did not show many strong correlations (Baron & Caine, 2000). Variability in lake response to elevated levels of atmospheric nitrogen (N) deposition is of particular concern because the eastern flank of the Colorado Front Range receives the highest N deposition in the Rocky Mountains (Baron et al., 2000). In this study, we focused on N dynamics and asked if the effect of regional N deposition would be manifested uniformly among neighboring lakes, and if lakes would respond similarly to further potential increases in nutrient inputs.

Mountain lakes are thought to be especially sensitive to atmospheric inputs; alpine watersheds have limited ability to immobilize nutrients due to steep slopes, sparse vegetation, shallow soils, a short growing season, and snowmelt dominated hydrology (Campbell et al. 2000; Williams et al., 1996). Even slight increases in N deposition are associated with measurable changes in mountain lake community and ecosystem properties (Jassby et al., 1994; Interlandi & Kilham, 1998; Baron et al., 2000), and N

deposition is already implicated in causing alterations in high elevation ecosystems of the Colorado Front Range east of the Continental Divide. Forests show signs of N saturation, many lakes have elevated NO_3 concentrations, and episodic acidification is reported to occur in some headwaters (Williams et al., 1996; Baron et al., 2000; Williams & Tonnessen, 2000). Analysis of sediment cores from two alpine lakes in this region show that a change in algal community composition and an increase in diatom biovolume occurred by about 1950-1970, coincident with intensified population growth, fertilizer usage, and cattle production in the Colorado Front Range (Wolfe et al., 2001).

Knowing what nutrient or nutrients limit algal growth in mountain lakes is crucial to predicting the consequences of increased atmospheric deposition and other possible perturbations such as altered nutrient supply resulting from climate change (Vinebrooke & Leavitt, 1998). Nitrogen limited lakes will respond to N enrichment by increasing primary productivity (i.e. eutrophication). Nitrogen fertilization will also increase the N:P ratio of inputs which could alter algal taxonomic composition (Tilman et al., 1986; Suttle & Harrison, 1988), with or without increasing productivity. A survey of nutrient enrichment experiments in North America showed that co-limitation by N and P was most common, but that N limitation occurred about as frequently as P limitation (Elser et al., 1990). Studies demonstrate that temporal changes in nutrient limitation status often take place seasonally within a single lake and that variability in nutrient limitation can occur among lakes within a region (Morris & Lewis, 1988; Axler et al., 1994). The effect of N enrichment may vary from lake to lake and over the course of a season even within a relatively small geographic area. In a review and critique of experimental enrichments,

Elser et al. (1990) urged investigators to characterize this seasonal and spatial variation in nutrient limitation.

We explored variability in ambient lake conditions and response to nutrient enrichment during the ice-free season for a small region on the east side of Rocky Mountain National Park, Colorado. Physical, chemical, and biological trends were monitored in three oligotrophic subalpine lakes located at about 3,100 m in the vicinity of Loch Vale Watershed, a long-term ecological research and monitoring site. We used *in situ* bioassays to assess phytoplankton biomass and community responses to further nutrient enrichment and measured particulate C, N, and P in epilithon to evaluate nutrient deficiencies of attached algae. We then project future changes given the likelihood of a continued rise in N deposition.

2.2 Study Sites

Loch Vale is a high elevation watershed (3,000-4,000 m) located on the east side of the Continental Divide in Rocky Mountain National Park, Colorado (Fig. 2.1). Geology is predominantly Precambrian granite, schist, and gneiss. Snowmelt occurs from April to July. Subalpine vegetation is Englemann spruce-subalpine fir; tundra occurs at elevations above approximately 3,300 m. Loch Vale is located within 60 km of the Denver to Fort Collins corridor, a region with many urban and agricultural sources of N emissions. Mean annual wet deposition to Loch Vale is 3.5 kg inorganic N $ha^{-1}y^{-1}$. More remote locations in Colorado report half as much wet N deposition (NADP, 1999).

The Loch, Mystery Pond, and Embryo Pond are shallow, oligotrophic subalpine lakes within 2 km of each other (Fig. 2.1). They do not stratify during summer, and ice cover

lasts from November to June. All are accessible only by hiking trails. Although the lakes are similar in certain respects, they vary in lake and catchment area and food web structure (Table 1). The Loch is a drainage lake (Pennak, 1969) with a permanently flowing inlet and outlet. It is the largest lake (5 ha) in this study and also has the largest watershed (660 ha). The Loch's drainage basin is comprised of 82% bare rock and talus, 12% tundra and meadow, and 6% forest (Baron & Cambell, 1997). Although originally fishless, The Loch was stocked with greenback cutthroat trout (*Oncorhynchus clarki stomias*), Colorado River cutthroat (*O. c. pleuriticus*), and rainbow trout (*O. mykiss*) in the early to mid 1900's (Rosenlund & Stevens, 1990). Currently, The Loch is dominated by rainbow x cutthroat hybrids. Embryo Pond is a small, fishless semi-drainage pond. It is very shallow and has a small watershed (21 ha) consisting of 89% bare rock and talus, 10% tundra and meadow, and <1% forest. Its inlet and outlet areas are marshy although a steep talus slope also drains into the lake. Mystery Pond is 1 ha in area and has a small watershed (18 ha) dominated by 66% meadow and 32% forest. The inlet and outlet areas of this fishless seepage pond are marshy without distinct channels. Sedges and macrophytes fringe a portion of the perimeter.

2.3 Methods

2.4.1 Ambient lake conditions

We monitored each lake weekly for temperature, pH, specific conductance, NO_3, NH_4, PO_4, dissolved organic carbon (DOC), and chlorophyll *a* during the summer of 1999. Silicate (SiO_2) was measured weekly in The Loch only. Samples were collected and processed using standard Loch Vale Watershed project methods (Allstott et al.,

1999). Nitrate, NH_4, and PO_4 were determined colorimetrically with a Perstorp Analytical Alpkem Spectrophotometer (Model 3590) using the cadmium reduction, salicylate, and ascorbic acid methods, respectively. Silicate was analyzed colorimetrically with the molybdate method modified by Hach, Inc. Dissolved organic carbon was analyzed on an Oceanographics International Model 700 Carbon Analyzer (U.S. Geological Survey, Boulder, CO). Temperature, specific conductance, and pH were measured with meters (Orion Model 128 and VWR Model 8000). Lake water samples for chl *a* analyses were immediately filtered through Whatman GF/C filters. Filters were frozen in dark containers and chl *a* was extracted with methanol (Riemann, 1980). Chl *a*, corrected for phaeophytin, was determined with a Sequoia-Turner Model 450 Digital Fluorometer.

Phytoplankton samples for taxonomic analysis were collected twice monthly from 1 m depth at the deepest area of each lake and preserved with Lugol's solution. Phytoplankton were identified to species when possible. Dufford Consulting, Fort Collins, CO performed identification and counting.

Zooplankton were sampled approximately weekly. Depth-integrated samples were collected by bilge pump at three representative sites within each lake until mid-summer. Representative locations in Mystery Pond included areas with macrophytes. Samples for each lake were pooled, collected on 80 μm mesh, and preserved with sugared buffered 4% formalin. Beginning on July 29, we collected zooplankton with an 80 μm mesh Wisconsin net in three to five locations for each lake (including macrophytes for Mystery Pond). The Wisconsin net allowed us to sample a greater lake volume. Both methods were used on July 29. Samples were rinsed of preservative and observed under a

dissecting scope to remove as much detritus as possible. Samples were then filtered onto Whatman GF/C filters and dried at 60 °C for 24 h to obtain dry mass. Chaoborids were present in late summer Mystery Pond samples; we removed them and measured chaoborid dry mass separately. Fairy shrimp, *Branchinecta coloradensis* (Anostraca) were observed in Embryo Pond, but were not captured in zooplankton samples. Although qualitative observations were made on all samples, zooplankton were enumerated for the June 29 sample date only. Rotifers were identified to genus, copepods to order, and cladocera as *Daphnia* sp. or Chydorinae. Immature copepods were identified as nauplii.

2.4.2 Phytoplankton Nutrient Bioassays

Phytoplankton nutrient bioassays were performed three times during the 1999 ice-free season in Embryo and Mystery Ponds and twice for The Loch. The early bioassay occurred from June 29 to July 5 during the end of the snowmelt period. The mid-summer bioassay ran from August 4 to 10, and we conducted the late-summer bioassay from September 11 to 18. For each experiment, a depth-integrated water sample was collected from the deepest area of each lake. Zooplankton grazers were removed by filtering through 80 μm mesh prior to distributing lake water into clear, plastic 2 liter bottles containing nutrient treatments. The bottles were previously acid-washed and rinsed three times with the filtered water sample. Treatments, in triplicate, were control, +2.26 mg l^{-1} NO_3-N, +0.19 mg l^{-1} PO_4-P, +NO_3 and PO_4 combined, +5 mg l^{-1} SiO_2, and +0.8 mg l^{-1} urea-N (CH_4N_2O). Nitrate was added as KNO_3 and PO_4 as K_2HPO_4. Bottles were shaken gently and incubated *in situ* at 0.5 to 1 m depth for 6 days. We measured chl *a*

and collected phytoplankton samples at the end of each experiment. Samples for phytoplankton taxonomic analysis were pooled among the three replicates and preserved with Lugol's solution. Cell counts and enumeration were conducted using Utermöhl settling chambers and a Leitz-Wetzlar Diavert inverted microscope with phase contrast. Phytoplankton were identified to species when possible, and diversity was calculated with the Shannon-Wiener Diversity Index (Pielov, 1977).

2.4.3 Epilithic Algal Nutrient Deficiencies

Algae were allowed to colonize on unglazed ceramic tiles and analyzed for particulate C, N, and P during mid-summer in all three lakes. Tiles (2" x 2") were glued to trays at three littoral sites (0.5 to 1.5 m depth) per lake. Tiles were installed in mid-June and retrieved after four weeks. Each tile was scraped with a razor blade to remove algae and rinsed with deionized water. Material from several tiles was pooled, dried, and ground. Particulate C and N were determined with a LECO CHN analyzer. Particulate P was oxidized to inorganic P and measured on a spectrophotometer (Murphy & Riley, 1962). Degree of nutrient deficiency was assigned based on indicator nutrient ratios for benthic freshwater algae developed by Kahlert (1998).

2.4.4 Statistical Analyses

Coherence of ambient lake conditions was assessed by calculating the Pearson product-moment correlation (rho) and corresponding p-value for each lake pair and each variable (Table 4; SAS Institute, Inc., Version 8.2). Correlations with p-values less than

or equal to 0.017 were considered significant at the alpha equals 0.05 level corrected for multiple comparisons.

Due to the uneven experimental design (no early bioassay in The Loch and silica treatment only during early and mid bioassays), we initially compared chl *a* responses to nutrient enrichment in three ways: 1) across all lakes for the mid- and late- summer bioassays (excluding the silica treatment), 2) across all bioassays for Embryo and Mystery Ponds (excluding the silica treatment), and 3) across all lakes and bioassays for the silica treatment only. We used lake, time (early, mid, or late summer), and treatment as factors in a full ANOVA with all interactions (SAS Institute, Inc., Version 8.2). The interaction of lake*time*treatment was important in the two analyses without silica ($p<0.0001$ and $p=0.048$, respectively). Because of this significant interaction, we combined all bioassays and compared responses for each experiment separately using lake*time and treatment as the two ANOVA factors. If the treatment main effect was significant at the alpha equals 0.05 level, we used least squared means to compare treatments against the control (and among treatments if more than one was different from the control) for each individual bioassay. One outlier was removed because its residual was more than four standard deviations from the predicted value. Chl *a* responses were log-transformed to meet the assumptions of normality and homogeneity of variance for all analyses. For each bioassay experiment, the mean percent of the control was used to indicate the magnitude of each treatment's chl *a* response.

2.5 Results

2.5.1 Ambient lake conditions

Mean daytime water temperatures over the summer were 8.5, 9.7, and 11.5 °C for The Loch, Embryo Pond, and Mystery Pond, respectively (Table 2.2, Figure 2.2). Temperatures were similar at the beginning and end of sampling, but Mystery and Embryo Ponds attained higher maximum mid-summer temperatures than The Loch. Seasonal mean pH's were 6.2, 6.1, and 5.9 for The Loch, Embryo and Mystery Ponds, respectively. All three lakes were dilute; average conductivity measurements were 11.2, 13.9, and 9.0 $\mu S\ cm^{-1}$. Mean DOC concentrations were 1.2, 4.0, and 9.2 $mg\ l^{-1}$ for The Loch, Embryo, and Mystery Ponds, respectively.

Mean NO_3-N concentration was highest for The Loch (224 $\mu g\ l^{-1}$), followed by Embryo Pond (111 $\mu g\ l^{-1}$), and lowest in Mystery Pond ($\leq 5\ \mu g\ l^{-1}$). Peaks of 350 and 300 $\mu g\ l^{-1}$ N occurred in mid to late June in The Loch and Embryo Pond, respectively (Fig. 2.2, Table 2.2). In The Loch, NO_3 remained elevated (>150 $\mu g\ l^{-1}$ N) until the end of sampling, but Embryo Pond experienced low NO_3 concentrations during mid-summer. Mystery Pond did not experience an early-summer NO_3 pulse. Mean NH_4 concentrations in The Loch, Embryo and Mystery Ponds were 12, 25, and 17 $\mu g\ l^{-1}$ N, respectively. Highest NH_4 concentrations occurred during or after chl *a* peaks. Mean summer PO_4 concentrations were 3, 6, and 8 $\mu g\ l^{-1}$ P for The Loch, Embryo, and Mystery Pond, respectively. Mean SiO_2 was 1.88 $mg\ l^{-1}$ for The Loch, and the lowest concentration (1.38 $mg\ l^{-1}$) was recorded during mid-summer.

Phytoplankton cell counts were correlated with chl *a* concentrations over time for The Loch (rho=0.92, p=0.0103) and Mystery Pond (rho=0.97, p=0.0011), but not for Embryo

Pond. Cell number per unit chl *a* varied greatly among lakes indicating species-specific differences in cell size or chl *a* allocation (Table 2.2). Mean summer chl *a* concentrations were similar (1.32-1.63 $\mu g\ l^{-1}$), but temporal dynamics and community composition varied among lakes (Fig. 2.4). The Loch had a mixed assemblage of mostly chrysophytes and non-heterocystous blue-green algae during the early to mid-summer. The diatom *Asterionella formosa* Hassall was also present in early summer and formed a small bloom on June 22 (1.35 $\mu g\ l^{-1}$ chl *a*; ~2,000 cells ml^{-1}). A late summer bloom of chrysophytes, mainly *Chrysochromulina parva* Lackey, occurred from mid-August until the end of sampling (max. 4.43 $\mu g\ l^{-1}$ chl *a*; ~7,000 cells ml^{-1}). The green alga *Chlorella* sp. also increased at the end of the summer in The Loch.

Embryo Pond was dominated by non-heterocystous blue-green algae, especially *Aphanothece smithii* Komarkova-Legnerova et Cronberg, in early June. Chrysophytes, mainly *Kephyrion boreale* Skuja and *Chromulina mikroplankton* Pascher, increased in June, and a bloom (2.74 $\mu g\ l^{-1}$ chl *a*; ~40,000 cells ml^{-1}) was detected on July 14, one week after the minimum summer NO_3 concentration. Cell numbers decreased rapidly, but chl *a* remained approximately 1.5 $\mu g\ l^{-1}$ for the remainder of sampling. Late season phytoplankton were composed mainly of chrysophytes, *Chromulina* sp., and a green alga, *Chlorella* sp.

Mystery Pond had a mixed phytoplankton assemblage during June and July, comprised mainly of chrysophytes, *Chromulina* sp., and non-heterocystous blue-greens, *Synechococcus sigmoideus* (Moore et Carter) Komarek and *A. smithii*, with fewer greens and cryptophytes. A large bloom of the non-heterocystous blue-green *Merismopedia*

glauca Ehrenberg began in August and continued into September (max. 3.44 μg l^{-1} chl *a*; ~110,000 cells ml^{-1}).

Both qualitative and quantitative observations indicated differences among lakes in zooplankton community composition and density (Table 2.3; Fig. 2.2). Mean dry mass was similar and highly coherent for Embryo and Mystery Ponds (0.228 and 0.231 g m^{-3}, respectively), but less and asynchronous for The Loch (0.051 g m^{-3}). Zooplankton generally increased over the summer in all lakes, although abundant early summer filamentous algae obscured this trend in terms of dry mass for The Loch since the algae could not be separated completely from the zooplankton on the filters (Fig. 2.2, Tables 2.2 & 2.4). The Loch had a sparse zooplankton community of rotifers and cyclopoid copepods, with *Daphnia* sp. appearing later in the summer. Zooplankton in Embryo Pond were mostly cyclopoid copepods in early summer with *Daphnia* sp. and fairy shrimp, *B. coloradensis*, gaining dominance later in the season. Mystery Pond had a community of mostly rotifers (*Keratella* sp.) with fewer cyclopoid copepods and nauplii, and *Daphnia* sp. *Chaoborus* sp., known to be a voracious predator of smaller zooplankton, were first observed on July 21 in Mystery Pond and appeared to grow larger over the course of the season. *Daphnia* sp. were less common during late summer, while copepods remained abundant. During this time we observed many copepod individuals associated with plant material, suggesting that many were captured from the macrophyte portion of Mystery Pond. Zoolankton density on July 29 (excluding *Chaoborus* and fairy shrimp) was 1, 10, and 189 l^{-1} for The Loch, Embryo, and Mystery Ponds, respectively (Table 2.3).

2.5.2 Temporal Coherence

Mean rho for all lakes and variables over the ice-free season was 0.37 (Table 2.4). Averaged over all parameters, individual lake pairs demonstrated low coherency (mean rho=0.32, 0.41, 0.37 for Embryo-Mystery, Embryo-Loch, and Mystery-Loch lake pairs, respectively). Temperature was by far the most coherent variable (mean rho=0.92), and the only measurement with significant correlations for all lake pairs. Ammonium and pH were also fairly synchronous (mean rho=0.47 and 0.61, respectively). No other variable had even moderate correlations for all lake pairs. Specific conductance was coherent between Mystery and The Loch (rho=0.60), less so for Embryo and The Loch (rho=0.44), and not at all for Embryo and Mystery Ponds. Nitrate was synchronous between Embryo Pond and The Loch (rho=0.84), but not for Mystery Pond pairings. In contrast, Mystery Pond's chl *a* trend correlated well with The Loch (rho=0.75), but Embryo Pond was not coherent with either lake. Zooplankton dry mass was coherent between Embryo and Mystery Ponds (rho=0.73), but not The Loch. Dissolved organic carbon and PO_4 were not synchronous among any of the lake pairs.

2.5.3 Phytoplankton Nutrient Bioassays

During the mid-summer bioassay in The Loch, NO_3 concentrations had decreased after the snowmelt pulse, but were still above 200 $\mu g\ N\ l^{-1}$. Phytoplankton chl *a* increased in response to PO_4 (203% of control, $p<0.0001$, Table 2.5), while NO_3 enrichment caused a negative response (29% of control, $p<0.0001$), and combined N+P caused a slight, insignificant increase (120% of control, $p=0.2426$). In the late summer bioassay, which occurred with similar NO_3 but greater chl *a* levels than the mid-summer

trial, both P and N+P treatments increased an average of 245% over the control ($p<0.0001$ for both).

Phytoplankton in Embryo Pond increased 136% over the control ($p=0.0429$) in response to P, but enrichment with both N+P caused a greater response (194% of control, NP-Control $p<0.0001$, NP-P $p=0.0336$) during the early summer experiment. Nitrate levels had decreased to 76 $\mu g\ N\ l^{-1}$ and chl *a* was increasing at the start of this bioassay. Similar responses were observed in Embryo Pond's mid-summer experiment; P elicited a 146% gain over control ($p=0.0209$), while N+P caused a 206% increase (NP-Control $p<0.0001$, NP-P $p<0.0001$). Urea addition led to a slight negative chl *a* response (79% of control, $p=0.0193$). During the late summer trial, the P and NP treatments caused similar phytoplankton gains (172%, $p=0.0012$; 160%, $p=0.0031$, respectively).

In the early-summer bioassay in Mystery Pond, fertilization with N+P caused a large algal response (369%, $p<0.0001$), while urea addition led to a smaller increase (156%, $p=0.0041$, NP-UR <0.0001). Nitrate caused a 125% gain over control, but this was not significant ($p=0.1397$). Mid-summer responses were similar although N+P response was lower in magnitude (227%, $p<0.0001$; 168%, $p=0.0011$; and 124%, $p=0.2451$ for NP, UR, and N treatments, respectively). In the late summer experiment during the large bloom of *M. glauca*, the N+P treatment was 126% of the control, but this response was only marginally significant ($p=0.0922$). Enrichment with either N or P alone gave a negative response, although only the P treatment was significant (57%, $p=0.0012$). No significant SiO_2 treatment effects were detected in any lake, although responses differed among lakes and over time.

Responses (mean percent of control) for phytoplankton chl *a* and cell density were positively correlated (rho = 0.65; $p<0.0001$) over all bioassays. Large changes in cell number (< 55% or >175% of control) corresponded to significant chl *a* responses roughly half the time. In general, when chl *a* and cell number responses were inconsistent, a major taxonomic shift occurred. Taxonomic shifts were common, but not consistent within particular nutrient treatments.

Shannon-Wiener Diversity Index differed from the control by 30% or more in only seven of 37 nutrient enrichments among all bioassay experiments. Diversity decreased in six of these seven instances. Nutrient enrichment reduced diversity most often in Embryo Pond (five of six cases). Fertilization with N+P resulted in two instances of lower diversity and a single instance of higher diversity. Diversity decreased twice with N amendment and once each with P and Ur enrichment. Substantial diversity changes were not always concurrent with substantial changes in cell number or significant chl *a* response.

2.5.4 Epilithic Algae Nutrient Deficiencies

Ratios of C:N in epilithic algae growing on unglazed ceramic tiles were 13.3, 11.1, and 18.7 for The Loch, Embryo, and Mystery Ponds, respectively (Table 2.6). These values were within the range for benthic algae reviewed by Kalhert (1998) and suggested slight N deficiency in Mystery Pond, consistent with bioassay results.

Ratios of C:P and N:P were greater than those previously published (Kalhert 1998, Hillebrand 1999). This discrepancy might indicate contamination by detritus, measurement errors, or severe P deficiencies. Detritus has more C in relation to N and P

than algae (Makarevich et al. 1993). Detritus contamination would therefore inflate both C:N and C:P ratios, although excess dissolved N might reduce C:N ratios to within range of published measurements . Studies reporting C:N:P for benthic freshwater algae are few, however, and may not include the true range of values (Kalhert 1998). Certain high-elevation lakes in areas of elevated N deposition could represent extreme situations of P limitation. Furthermore, attached algae may experience lower nutrient supply than other growth forms since phytoplankton suppress water column nutrient levels while solid substrates limit uptake from sediment sources. We used commonly employed methods to determine C, N, and P content of our attached algae samples and results generally agree with outcomes of our phytoplankton bioassays. Severe P limitation of attached algae in the study lakes is the most likely explanation.

2.4 Discussion

2.5.1 Variability in ambient conditions and response to nutrient enrichments

We asked how ambient conditions vary among neighboring subalpine lakes, if the effects of elevated N deposition are manifested uniformly among lakes, and if these lakes respond similarly to further addition of nutrients. Despite comparable climate, which was demonstrated by high coherency of temperature, the study lakes showed a large degree of individuality. Some of the few similarities were explainable, such as the comparable zooplankton means and trends in Embryo and Mystery Ponds, which were both fishless. Other patterns were not as decipherable and might be due to entirely different mechanisms acting in individual lakes. For example, The Loch and Mystery Pond, which were most different in terms of NO_3, DOC, and zooplankton, were the most coherent for

phytoplankton chl *a*, NH_4, and conductivity. Our study of seasonal coherency of lakes in and adjacent to the Loch Vale Watershed led to a similar conclusion as a previous investigation of year-to-year coherency conducted in the same basin (Baron and Caine, 2000). Both studies found that internal catchment and lake processes attenuated external influence of climate and atmospheric conditions.

In particular, differences in how elevated N deposition affected individual lakes were apparent in ambient NO_3 levels and trends, and a continuum was observed from the drainage lake with a rocky watershed (The Loch), to the semi-drainage lake with both a rocky catchment and adjacent wetlands (Embryo Pond), and to the seepage lake with both fringing wetlands and a forested catchment (Mystery Pond). High NO_3 levels, especially during snowmelt, were associated with the rocky watersheds, but presence of fringing marshes had the opposite effect. Non-vegetated terrain in the watershed has been linked to high autumn NO_3 levels in Tatra Mountain lakes (Slovakia and Poland; Kopáček et al., 2000) and high mean annual NO_3 concentrations in Colorado Rocky Mountain streams (Clow & Sueker, 2000). Lack of vegetation is often associated with steep terrain and short hydraulic residence times, which limit rock-water interactions. In addition, talus environments are sites of N mineralization and nitrification, processes that contribute to high NO_3 concentrations in drainage waters (Clow & Sueker, 2000). Low NO_3 levels, on the other hand, were measured in seepage lakes fringed by marshes in the Adirondack Mountains (Saunders et al., 2000). Adjacent marshes retain NO_3 (Baron et al., 1983) and export particulate P (Meili 1992). Furthermore, marshes may remove N at higher rates than P because of N limitation in wetland vegetation (Verhoeven et al., 1988) and/or enhanced denitrification (Saunders et al., 2000).

Embryo Pond had physical characteristics intermediate in nature between The Loch and Mystery Pond, and these features explain its corresponding NO_3 trend. In the early summer, Embryo Pond received snowmelt directly from adjacent talus slopes and had an associated NO_3 pulse. During this time, some NO_3 was probably retained in the inlet wetlands while P was flushed from marsh soils into the lake. As flow diminished, the wetlands were likely the main source of inflow, leading to low NO_3 inputs after snowmelt. Elevated DOC levels in Embryo Pond compared to The Loch support the idea of wetland P being exported into the pond. Both dissolved and particulate P were correlated with DOC concentrations in Adirondack lakes (Saunders 1992) and a similar relationship was found between PO_4 and DOC in the study lakes ($rho=0.69$, $p<0.0001$).

These watershed and hydrologic differences had implications concerning the nutrient limitation status of each study lake. We observed the greatest P deficiencies in The Loch (drainage), P limitation with secondary N limitation in Embryo Pond (semi-drainage), and slight N limitation, N+P "co-limitation", and nutrient sufficiency in Mystery Pond (seepage; Table 5). Specifically, high NO_3 concentrations caused P limitation in The Loch during both mid- and late-summer. Phosphorus also primarily limited Embryo Pond, but enrichment with both N+P caused greater biomass, signifying secondary limitation by N during the early and mid-summer experiments. Embryo Pond may have had relatively lower P deficiency than The Loch due to P input from the adjacent wetlands, as mentioned above. The positive (although statistically insignificant) responses to NO_3 in Mystery Pond, coupled with low ambient DIN: PO_4 ratios, suggest that N was the primarily limiting nutrient in early and mid-summer. Nitrogen limitation quickly was replaced by closely coupled N and P deficiencies, however, and a condition

of "co-limitation" followed. The positive responses to urea during this time also imply that the microbial community was limited by sources of labile organic N and/or C, and that the phytoplankton utilized bacteria as a supply of both N and P, either directly via mixotrophy or indirectly after mineralization. The lack of a significant response to N+P enrichment during Mystery Pond's late summer blue-green algal bloom suggests that N and P supply was sufficient at this time. Micronutrient deficiencies might have developed, but ambient DIN and PO_4 concentrations hint that macrophyte and epiphytic algae senescence released a pulse of nutrients in the late summer. In addition, the dominant late-summer alga, *Merismopedia* sp., is less capable of using NO_3 as its N source and may also control the system via allelopathy (Blomqvist, 2001).

In-lake nutrient assimilation and recycling also influence which nutrient or nutrients limit algal biomass, and zooplankton community composition and the presence of fish are integral to these processes. Trout stocking increases P availability by regenerating terrestrial and benthic nutrients (Schindler et al., 2001), while larger and more abundant zooplankton reduce P availability because of the low N:P ratio of their nutritional requirements (Elser et al., 1988). These relationships favor N limitation in The Loch, where trout were present but zooplankton were small and sparse, and P limitation in Mystery and Embryo Ponds, which did not have fish but had larger and more abundant zooplankton. We found just the opposite, however, and conclude that watershed vegetation and hydrology are more important regulators of nutrient limitation than internal processes in the study lakes.

2.5.2 Future considerations

Drainage lakes with rocky watersheds already have high NO_3 concentrations east of the Continental Divide (Baron et al., 2000), and this study shows that further increases in N deposition are unlikely to enhance algal biomass in these lakes, unless P inputs increase as well. Phytoplankton species may change with altered nutrient supply, but the nature of this response was not predictable from short-term studies. It is unlikely that benthic algae will respond to the additional N because nutrient ratios of attached algae indicated severe P limitation. In addition, epipelic algae usually are not often stimulated by water column nutrient enrichment because they obtain ample nutrients from the sediment (O'Brien et al., 1992; Blumenshine et al., 1997; Vinebrooke & Leavitt, 1998).

We hypothesize that lakes with vegetated catchments and fringing marshes are more protected from atmospheric N inputs, but because of wetland P sources, are also most prone to future eutrophication from rising N deposition if the terrestrial buffering processes are overwhelmed. Lakes with these characteristics are often found at lower elevations in the montane zone, and N limitation has indeed been reported for several montane lakes in the Colorado Front Range (Morris & Lewis, 1988). Like Mystery Pond, these lower elevation lakes have watersheds dominated by soil cover that dampen NO_3 input. The single P limited lake studied by Morris and Lewis (1988) was a drainage lake located at 3,160 m elevation in the subalpine zone, which gives further support for the idea that high elevation lakes having rocky catchments are not likely to respond to future increases in N alone.

Finally, it is apparent from this study that increases in P input combined with rising N deposition could cause substantial eutrophication in Colorado Front Range lakes.

Atmospheric P deposition to high elevations has been given little attention and is not routinely measured with low enough detection limits to be useful. Hence, it is not known if human activities have altered atmospheric P inputs to high elevation systems. Isolated studies have shown atmospheric deposition to be a substantial source of P to high elevations, however, and provide evidence for terrestrial P sources such as volatile organic compounds from soil or plant biota or leachate from windblown dust (Lewis et al., 1985; Jassby et al., 1994). Sickman (2001) recently observed decreases in the DIN:TP ratio for high elevation Sierra Nevada lakes and suggests that increased P loading is coming from windblown agricultural dust from fertilized fields. The Colorado Front Range is also located adjacent to an intensively agricultural region. Given already elevated N deposition in the region and the high likelihood of a strong algal response to increases in combined N and P supply, greater attention to P deposition to these high elevation systems is warranted.

2.6 Acknowledgments

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Table 2.1. Characteristics of the study lakes.

	The Loch	Embryo Pond	Mystery Pond
Lake Type	Drainage	Semi-drainage	Seepage
Inlet/Outlet	Permanent streams	Marsh/ Stream	Marsh/ Marsh
Lake Area (ha)	5.0	0.2	1.0
Lake Elevation (m)	3,102	3,164	3,090
Watershed Area (ha)	660	21	18
Mean / Max. Depth (m)	1.5 / 4.7	0.8 / 2	1.0 / 3
Lake Area : Mean Depth	3.33	0.25	1.00
Watershed Area: Lake Area	132	105	18
% watershed rock & talus	82	89	<1
Fish	Rainbow & greenback cutthroat trout	none	none
Other		Talus drains directly into lake; wetlands	Wetlands and macrophytes

Table 2.2. Mean summer 1999 measurements for The Loch, Embryo Pond, and Mystery Pond. *Pearson product-moment correlation.

	The Loch	Embryo Pond	Mystery Pond
Temperature (°C)	8.5	9.7	11.5
pH	6.2	6.1	5.9
Conductivity ($\mu S\ cm^{-1}$)	11.2	13.9	9.0
NO_3-N ($\mu g\ l^{-1}$)	224	111	5
NH_4-N ($\mu g\ l^{-1}$)	12	25	17
PO_4-P ($\mu g\ l^{-1}$)	3	6	8
SiO_2 ($mg\ l^{-1}$)	1.88	--	--
DOC ($mg\ l^{-1}$)	1.2	4.0	9.2
Chlorophyll *a* ($\mu g\ l^{-1}$)	1.35	1.32	1.66
Cells per ml (x 1000)	3.33	11.0	44.2
Cell #:Chlorophyll *a* ratio	2.5	8.3	26.6
Zooplankton dry mass (g/m^3)	0.051	0.228	0.231
*Cell density and chlorophyll *a*	0.92 (p=0.0103)	0.17 (p=0.7465)	0.97 (p=0.0011)

Table 2.3. Composition of zooplankton assemblages sampled from the three study lakes on June 29, 1999. Numbers are density (organisms/L) except for major taxonomic groups, which are percent composition.

Taxon	The Loch	Embryo Pond	Mystery Pond
Rotifers (%)	69	1	80
Asplanchna	0.18	0	0
Keratella	0.08	0.07	151.5
Nothalca	0.32	0	0
Copepods (%)	31	92	18
Cyclopoids	0	8.6	11.2
nauplii	0.28	0.82	22.9
Cladocera (%)	0	7	2
Daphnia	0	0.6	3.5
Chydorinae	0	0.1	0
Total Crustacean zooplankton (#/L)	0.28	10.13	37.6
Total for all taxa (#/L)	0.9	10.2	189.1

Table 2.4. Pearson product-moment correlations (rho), p-values, and number of values used in correlation for lake pairs. Significant positive correlations ($p \leq 0.017$ at the alpha=0.05 level corrected for multiple comparisons) are shown in bold.

	Embryo & Mystery	Embryo & The Loch	Mystery & The Loch	MEANS
Temperature	**0.90**	**0.92**	**0.92**	0.92
	<0.0001	**<0.0001**	**<0.0001**	
	16	**15**	**15**	
PH	**0.76**	0.46	**0.61**	0.61
	0.0006	0.0868	**0.0158**	
	16	15	**15**	
Conductivity	-0.02	0.44	**0.62**	0.35
	0.9282	0.0966	**0.0145**	
	16	15	**15**	
NO_3-N	0.13	**0.84**	0.07	0.35
	0.6230	**0.0001**	0.8000	
	16	**15**	15	
NH_4-N	0.41	0.34	**0.66**	0.47
	0.1108	0.2210	**0.0081**	
	16	15	**15**	
PO_4-P	0.15	0.00	0.03	0.06
	0.5919	1.000	0.9235	
	16	15	15	
DOC	0.13	0.41	-0.74	-0.07
	0.6644	0.1488	0.0028	
	14	14	14	
Phytoplankton	0.09	-0.11	**0.75**	0.24
Chlorophyll *a*	0.7396	0.6777	**0.0008**	
	16	15	**15**	
Zooplankton	**0.73**	0.23	-0.34	0.21
dry mass	**0.0171**	0.09464	0.3033	
	10	11	11	
MEANS	0.36	0.39	0.29	0.35

Table 2.5. Nutrient limitation status of each lake determined from bioassay experiments conducted during early, mid, and late-summer 1999. "P lim" is P limitation, "2nd N lim" refers to secondary N limitation, and "N+P co-lim" means closely coupled N and P limitation. Also shown are the magnitude and statistical significance of chl *a* responses for each bioassay. Percentage (magnitude) is the mean percent of control at the end of the experiment. P-values (n =3) are for the treatment compared to control, except where indicated as a comparison between treatments (i.e. "P-NP" or "NP-UR"). Positive responses with p-values ≤0.05 are shown in bold; negative responses with p-values ≤0.05 are italicized. Treatments are: N = NO_3, P = PO_4, NP = NO_3 + PO_4, UR = urea, and Si = SiO_2.

	Early			Mid			Late		
	Trts	%	p-value	Trts	%	p-value	Trts	%	p-value
The Loch					P lim			P lim	
				N	*29*	*<0.0001*	N	87	0.3850
				P	**203**	**<0.0001**	**P**	**245**	**<0.0001**
				NP	120	0.2426	**NP**	**245**	**<0.0001**
				UR	117	0.3255	UR	110	0.5564
				Si	82	0.1860			
Embryo	P lim, 2nd N lim			P lim, 2nd N lim			P lim		
	N	92	0.6758	N	100	0.9900	N	89	0.5017
	P	**136**	**0.0429**	**P**	**146**	**0.0209**	**P**	**172**	**0.0012**
	NP	**194**	**<0.0001**	**NP**	**206**	**<0.0001**	**NP**	**160**	**0.0031**
	UR	78	0.1258	*UR*	79	*0.0193*	UR	131	0.0883
	Si	87	0.4034	Si	100	0.9691			
	P-NP		**0.0336**	**P-NP**		**<0.0001**	P-NP		0.7703
Mystery	N lim, N+P "co-lim"			N lim, N+P "co-lim"			N and P sufficiency		
	N	125	0.1397	N	124	0.2451	N	71	0.0593
	P	96	0.8003	P	110	0.5195	*P*	*57*	*0.0012*
	NP	**369**	**<0.0001**	**NP**	**227**	**<0.0001**	NP	12	0.0922
	UR	**156**	**0.0041**	**UR**	**168**	**0.0011**	UR	6	0.5115
	Si	90	0.4752	Si	98	0.5864		10	
	NP-UR		**<0.0001**	NP-UR		0.0551		5	

Table 2.6. Molar ratios of C, N, and P for epilithic algae grown on unglazed ceramic tiles. Number in parentheses is standard error. Nutrient deficiencies were assigned based on Kahlert (1998).

	C:N	C:P	N:P	Nutrient Deficiency
The Loch	13.3 (0.4) n=6	880 (106) n=3	66 (12) n=3	P (severe)
Embryo Pond	11.1 (0.16) n=3	1637 (450) n=3	146 (38) n=3	P (severe)
Mystery Pond	18.7 (2.03) n=2	1916 (573) n=2	107 (42) n=2	P (severe) N (slight)

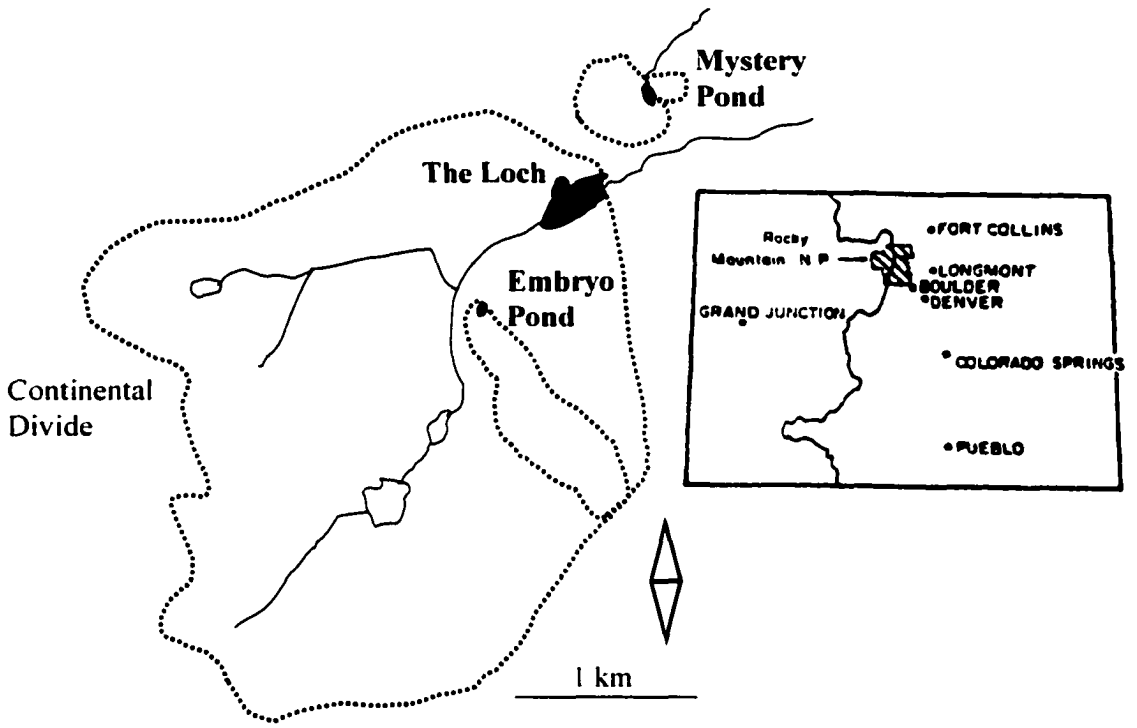

Figure 2.1 Map showing location of study lakes and Rocky Mountain National Park. Approximate extent of drainage basin is illustrated for each lake.

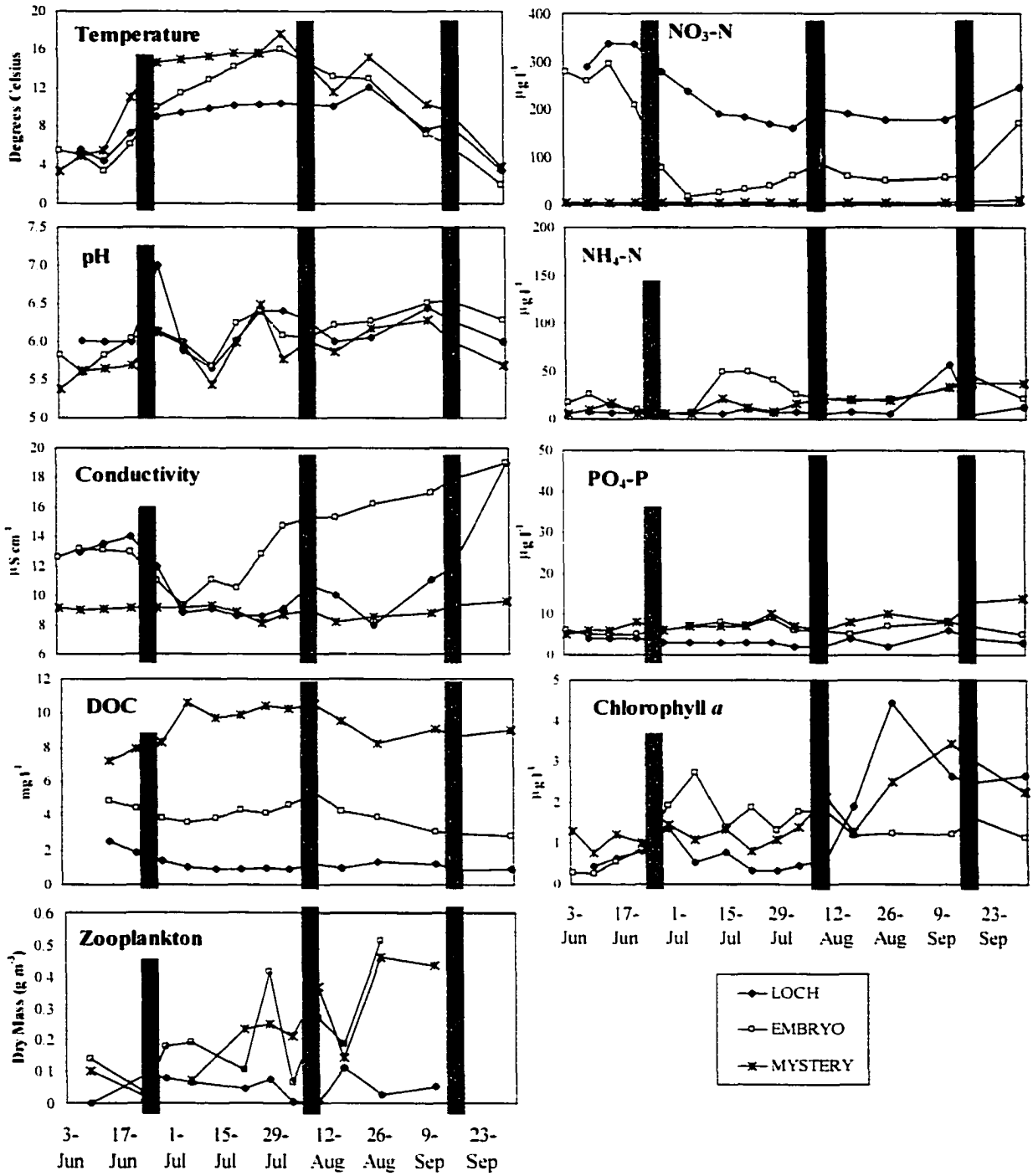

Figure 2.2 Summer trends in ambient lake conditions for The Loch, Embryo Pond, and Mystery Pond. The timing of the early, mid, and late bioassays is indicated with shaded bars.

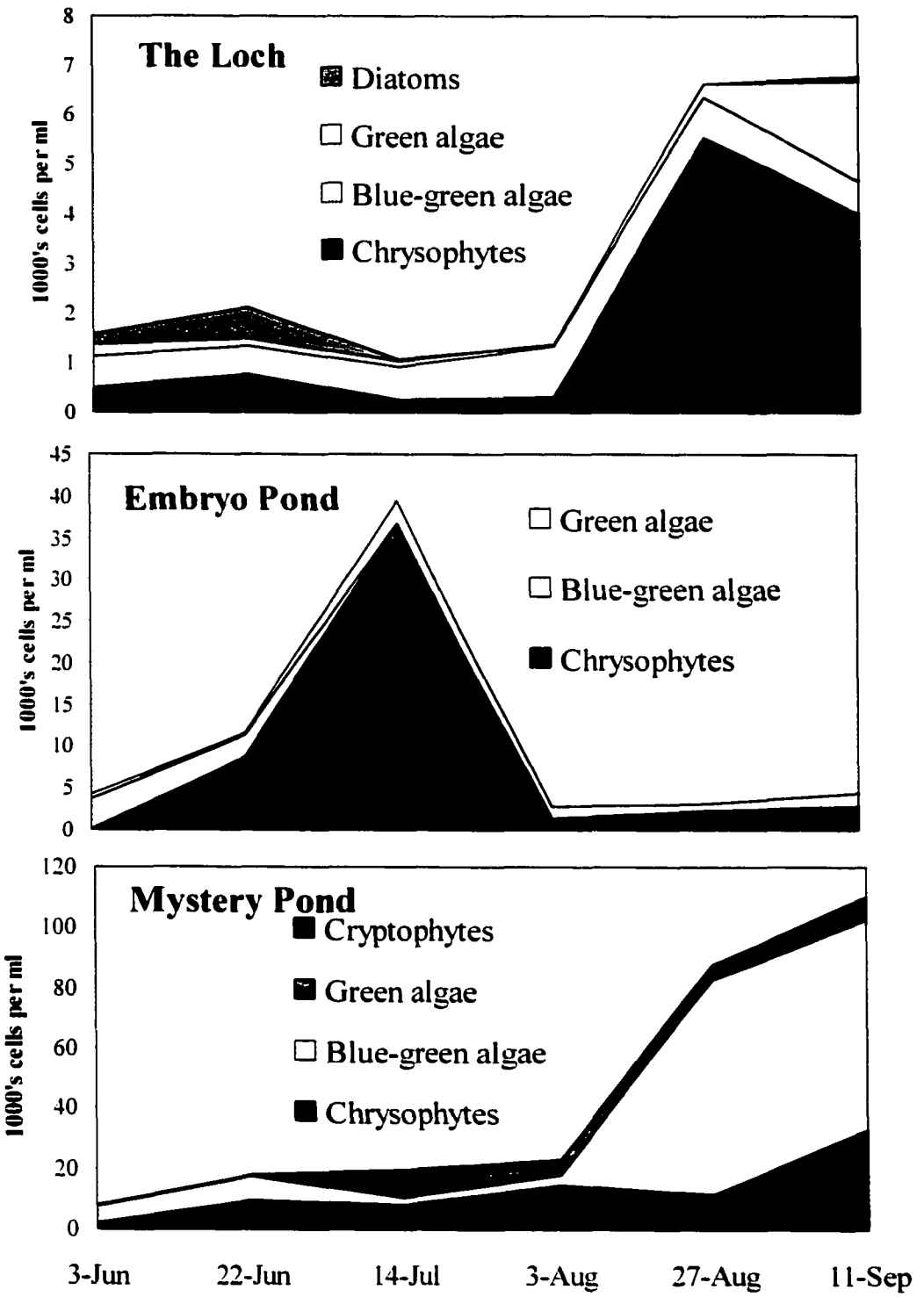

Fig. 3.3 Abundance of major phytoplankton divisions for (a) The Loch , (b) Embryo Pond, and (c) Mystery Pond.

3. Nitrogen regulation of algal biomass, productivity, and composition in small mountain lakes, Snowy Range, Wyoming, USA

3.1 Abstract

Nitrogen (N) may be an important regulator of mountain lake ecology, especially where ambient nitrate levels are low and future increases in atmospheric N deposition are likely. We investigated the effects of increased N inputs, alone and in combination with phosphorus (P), using enclosure experiments in small mountain lakes of the Snowy Range, Wyoming. Each set of experiments lasted two weeks in the early and late summer, during which we measured a suite of ecosystem and community variables with special attention to algal responses across habitat types (phytoplankton, epilithon, and epipelon). Phytoplankton responded most strongly to N and N + P enrichment, showing increased photosynthetic rate, chlorophyll *a*, particulate carbon (PC), and cell density. Nitrogen and N+P enrichment also led to more alkaline and light-limited environments, and phytoplankton composition shifted from chrysophytes to cyanophytes, chlorophytes, and diatoms. Epilithon responded to a lesser degree and only in the late summer experiment. Epipelon dominated chl *a* stock, but replicates were highly variable and responses inconsistent. Nutrient enrichment did not alter zooplankton biomass, abundance or composition. The relative increases in photosynthetic rate, chl *a*, and PC suggest that a large degree of photosynthetic carbon gain was lost via respiration or excretion in the dark, and that a

portion of phytoplankton chl *a* gain and probably all of the epilithic chl *a* gain was physiological and not a result of increased algal biomass. These results suggest that increased N deposition can alter multiple components of mountain lake ecology.

3.2 Introduction

Alpine watersheds are characterized by steep slopes, sparse vegetation, shallow soils and a short growing season that limit their capacity to immobilize nutrients and heighten their sensitivity to atmospheric pollutants (Campbell et al. 2000, Williams et al. 1996). This sensitivity has important implications for mountain lakes, many of which lie close to urban and agricultural sources of nitrogen (N) emissions in the western United States. For example, high-elevation sites on the eastern flank of the Colorado Front Range, closer to the Denver-Fort Collins urban corridor, receive mean annual wet deposition of about 3.5 kg inorganic N ha^{-1} y^{-1}, compared with less than half that in more remote locations in Colorado (NADP 1999). Furthermore, N deposition has been increasing at several high-elevation sites in the Colorado Front Range since monitoring began in the early to mid-1980's (Baron et al. 2000).

Even slight increases in N deposition are associated with measurable changes in mountain lake community and ecosystem properties (Baron et al. 2000, Interlandi and Kilham 1998, Interlandi et al. 1999, Jassby et al. 1994). In the Colorado Front Range east of the Continental Divide, forests show signs of N saturation, many lakes have elevated nitrate (NO_3) concentrations, and episodic acidification is reported to occur in some headwaters (Baron et al. 2000, Williams et al. 1996, Williams and Tonnessen 2000). Analysis of sediment cores from two alpine lakes in this region show that changes

in algal community composition occurred by about 1950-1970, coincident with intensified population growth, fertilizer usage, and cattle production in the Colorado Front Range (Baron et al. 2000, Wolfe et al. 2001). Diatoms shifted from a typical oligotrophic assemblage to dominance by disturbance species *Asterionella formosa* and *Fragilaria crotonensis*. Diatom valve concentrations and biovolumes also increased dramatically, suggesting increased productivity.

Despite historical biological responses, however, current high NO_3 conditions likely inhibit further fertilization effects due to N alone. For example, nutrient enrichment experiments in Rocky Mountain National Park (RMNP) lakes with up to 1 mg l^{-1} ambient NO_3 suggest that additional N inputs will alter phytoplankton community composition, but will not increase phytoplankton biomass without commensurate phosphorus (P) inputs (Nydick et al. 2002). Similarly, nutrient ratios of dissolved inorganic N to total P (DIN:TP) in RMNP lakes indicate that limitation by N alone is rare regionally (Sickman 2001).

How continued increases in N inputs will affect present-day mountain lakes depends on a variety of additional factors, including abundance of grazers, lake hydrology, and interactions between benthic and pelagic responses. Trophic structure, particularly the presence/absence of large zooplankton and benthic grazers, may influence the fate of excess N by limiting algal standing stock and altering species composition (Carpenter et al. 1987, Cottingham and Schindler 2000, Vanni 1987) or regulating nutrient supply via recycling (Elser et al. 1987, Sterner 1986). Hydrological differences among lakes also have implications for nutrient cycling. For example, denitrification rates are generally higher in shallow lakes with short hydraulic residence times (Kelly at al. 1987).

Additionally, morphometric differences among mountain lakes result in variation in the relative importance of benthic versus pelagic habitat. Differences in species' nutrient requirements and life histories between these habitats (Vinebrooke and Leavitt 1998), as well as competition for nutrients between habitats (Blumenshine et al. 1997, Hansson 1990), may affect the fate of excess N and its impact on lake ecology.

We used littoral mesocosm enclosures to examine the effects of nutrient additions to shallow mountain lakes with low NO_3 concentrations in order to: 1) explore potential effects of N deposition on low NO_3 lakes, 2) determine the relative impacts of N and P, alone and in combination, on low NO_3 lakes, and 3) investigate competition for nutrients between the benthos and water column. We hypothesized that addition of NO_3 would increase algal biomass and productivity and alter community composition in a manner similar to changes documented in the fossil record since the 1950's (Wolfe et al. 2001), and that N+P additions would amplify those patterns. We measured both phytoplankton and benthic responses to investigate possible shifts in habitat dominance, and predicted that nutrient additions would favor epilithic algae growing on hard substrates (Axler and Reuter 1996, Blumenshine et al. 1997, Vinebrooke and Leavitt 1998).

2.3 Methods

2.3.1 Study area

We conducted enclosure (mesocosm) experiments in Shelf Lakes 4 and 5, located adjacent to one another at 3,313 meters in the Snowy Range, Medicine Bow National Forest, Wyoming, about 55 km west of Laramie. We selected these lakes because they had very low NO_3 concentrations (unpublished data) and were relatively accessible via hiking trail. The lakes share essentially the same watershed, which drains the NW slope

of a quartzite ridge composed chiefly of talus and exposed bedrock, as well as some meadow vegetation and krummholz (Engleman spruce and subalpine fir). Spring snowmelt dominates lake hydrology, with surface water inflow/outflow diminishing throughout the summer. The ice-free season lasts from early July to mid-October, and cool temperatures, high winds and summer frosts are typical during these months (Musselman 1992). Hikers or anglers seldom visit either lake. No record of fish stocking exists for either lake, but brook trout (*Salvelinus fontinalis*) are present and naturally reproducing in both lakes. Surface areas are 1.2 and 0.4 ha and maximum depths are 3.4 and 1.8 m, for Shelf Lakes 4 and 5, respectively. Littoral zones are extensive and are characterized by large rocks and substantial fine sediment. A fisheries survey documented summer alkalinities at or less than 100 $\mu eq\ l^{-1}$, circumneutral pH's, and Secchi depths reaching the lake bottoms (Snigg 1989).

2.3.2 Experimental Design

We conducted two mesocosm experiments during the summer of 2000. The early summer experiment in Shelf Lake 4 lasted four weeks (11 July – 8 Aug). Because NO_3 and phosphate (PO_4) concentrations fell to near-ambient levels in enriched mesocosms after two weeks during this experiment, we conducted the late summer experiment in Shelf Lake 5 for two weeks only (22 Aug – 5 Sep). We performed the experiments in 500-l cylindrical enclosures, approximately 1.5 m deep. Frames were constructed with vertical PVC tubing and horizontal plastic hoops. PVC was attached on the bottom to a semi-rigid plastic cylinder that extended at least 10 cm into the sediments. Clear, cylindrical polyethylene plastic was fitted tightly around the cylindrical frame and

attached to the top hoop. The enclosure extended about 10-20 cm above water level. Plastic tarps formed a skirt around the bottom of the enclosure, and rocks were piled on the tarps to secure the enclosures in place. We documented enclosure effects by including a lake (La) treatment, consisting of the frame only (i.e. no polyethylene plastic).

Mesocosms were installed in three blocks of five, and treatments were randomly assigned within each block. Care was taken to minimize sediment disturbance during installation and enclosures were allowed to sit for one day (early experiment) and three days (late experiment) prior to taking initial measurements to permit settling of fine sediments. Nitrogen (N) and phosphorus (P) enclosures received a single pulse of approximately 1 mg l^{-1} NO_3-N and 0.1 mg l^{-1} PO_4-P, respectively, at the beginning of the experiment. Combined N and P (NP) mesocosms received both additions in a N:P molar ratio of about 22. Treatments were added to the water surface and mixed carefully with a clean boat paddle. Specific conductance was measured concurrently and mixing was continued until conductivity at 1 m depth reached a steady value.

2.3.3 Sample collection, processing, and analysis

Field measurements, incubations, and sampling were conducted just prior to adding treatments and at regular intervals thereafter (every two weeks and every week for the early and late experiments, respectively). Sampling included epilithic algae, which were colonized on unglazed ceramic tiles (2" x 2") glued to trays. The tile trays had been placed in the lakes one month prior to each experiment. Tile trays were randomly assigned to mesocosms and initial samples were collected prior to lowering trays to the

bottom of each enclosure. Each sampling event lasted two days; pelagic samples (water and plankton) were taken on the first day, followed by benthic samples on the second. Nitrate, PO_4, ammonium (NH_4), and conductivity were also measured immediately after adding treatments. Zooplankton were sampled at the beginning and end of each experiment. Samples of epipelic algae (on sediment) and epi-plastic algae (on mesocosm walls) were collected at the end of each experiment.

Samples were collected and processed using standard Loch Vale Watershed project methods (Allstott et al. 1999). Nitrate, PO_4, and NH_4 were determined colorimetrically with a Perstorp Analytical Alpkem Spectrophotometer (Model 3590) using the cadmium reduction, salicylate, and ascorbic acid methods, respectively. Silica was analyzed colorimetrically with the molybdate method modified by Hach, Inc. Dissolved organic carbon was analyzed on an Oceanographics International Model 700 Carbon Analyzer (U.S. Geological Survey, Boulder, CO). Alkalinity was measured by titration (APHA 1995). Temperature, specific conductance, dissolved oxygen (DO) and pH were measured with meters (Orion Model 128, Hach Sension6, VWR Model 8000).

Depth-integrated water samples for phytoplankton analyses were collected by hand-pump. Epilithic algae was scraped from tiles with a razor blade and rinsed with deionized water. One tile was collected per response variable on each sampling date. Samples for both epilithic and phytoplankton chlorophyll *a* analyses were immediately filtered through Whatman GF/C filters. Filters were frozen in dark containers and chl *a* was extracted with methanol (Riemann 1980). Chl *a*, corrected for phaeophytin, was determined with a Sequoia-Turner Model 450 Digital Fluorometer. Particulate C on filters was oxidized and measured with a LI-COR Model LI-6252 CO_2 Analyzer (Lapman

et al. 2001). Photosynthetic rate was measured using the ^{14}C light/dark technique (Pregnall 1991). Clear borosilicate bottles suspended at 1m depths were used for phytoplankton incubations. Clear Whirlpaks™ containing lake water and an algae tile were employed to measure epilithic photosynthesis. Incubations lasted four to six hours after which containers were kept cold and dark in coolers while they were transferred to the laboratory.

Samples for both phytoplankton and epilithic taxonomic analyses were preserved with Lugol's Iodine in a 1:100 ratio. For phytoplankton taxonomic analyses, samples were allowed to settle in sedimentation chambers (Utermöhl 1958). Several transects were counted under low magnification (150x or 480x) and high magnification (1500x) using a Leitz Diavert inverted microscope with phase contrast and oil immersion. A minimum of 400 cells were enumerated per sample and identified to genus and species where possible. Species richness and diversity (Simpson's *D*; Washington 1984) were calculated for all samples.

For epilithic taxonomic analyses, samples scraped from tiles were sonicated for 1-2 minutes to homogenize contents. Three replicate aliquots were removed from each sample, diluted as necessary and settled in Utermöhl chambers. They were then enumerated as described above for phytoplankton, and identified to Division.

Zooplankton were collected twice from each mesocosm (initial and final sampling dates) by pumping mesocosm water with a bilge pump into a four liter container. Contents of the container were filtered onto 80 μm mesh, rinsed into Whirlpaks™ and preserved in 4% sugared buffered formalin. In the laboratory, samples were poured through a 40 μm sieve, rinsed into 50 ml plastic centrifuge tubes, diluted to 15 ml with

deionized water and shaken to suspend. From this suspended sample, five 1-ml aliquots were removed with a Hensen Stempel pipette and placed in Sedgewick-Rafter cells for identification and enumeration. Zooplankton biomass was estimated using length-weight regression (Copepoda and Cladocera) or biovolume equations (Rotifera), following (Bottrell et al. 1976, Dumont et al. 1975, McCauley 1984).

At the end of each experiment we collected epipelic and epi-plastic algal samples. We sampled epipelic algae from the sediment of each mesocosm by cutting a hole in the plastic enclosure and slowly embedding a plastic petri dish into the sediment. A thin plastic plate was slid under the petri dish. The sample was retrieved and sealed in the petri dish (Vinebrooke and Leavitt 1998). The sample included the top 1.0 cm of epipelon. In the lab, a sub-sample was thoroughly mixed with 100 ml deionized water and 10 ml of this homogeneous mixture was filtered onto a Whatman GF/C filter. Epi-plastic algae growing on the enclosure walls were also sampled. Both filters and plastic strips were analyzed for chl *a* as described above for epilithic algae and phytoplankton.

2.3.4 Statistical Analyses

To facilitate comparisons between the two experiments of different durations, we analyzed results from only the first two weeks of the early experiment. Each experiment was analyzed separately because they occurred in different lakes and at different times. Repeated-measures analyses of variance (RM-ANOVA) were used to test the effects of treatments (excluding lake mesocosms) on response variables that were measured at regular time intervals. If the treatment main effect or the treatment*week interaction was significant at the $\alpha = 0.1$ level, we used a priori comparisons of each treatment with the

control (ls means) and set the significance level at $\alpha = 0.05$. If more than one treatment was different than the control, we compared these treatments to each other to determine if a certain treatment had a significantly larger response than the other treatment. An analysis of variance (ANOVA) was used to test for treatment effects on final epipelic and epi-plastic chl *a*. A Tukey correction for multiple comparisons was used to compare individual treatments. We also used RM-ANOVA's and ANOVA's to identify enclosure effects by comparing response variables between control and lake mesocosms. Data were transformed when necessary to meet the assumptions of normality and homogeneity of variance. Analyses were performed with SAS Version 8e (SAS Institute, 1999-2001).

Statistical analyses of phytoplankton community data were performed using CANOCO version 4 (ter Braak and Smilauer 1998). Principal response curves (PRC) were used to examine algal community response to experimental manipulations over time (Van den Brink and ter Braak 1998, Van den Brink and ter Braak 1999). PRC is a derivation of partial redundancy analysis (RDA) designed to overcome visual limitations of RDA and display major treatment effects on species composition over time. The resulting diagram plots time (week of experiment) on the horizontal axis and treatment effect (expressed as deviation from control) on the vertical axis. Controls (in this case, lake mesocosms) were set to zero and used as the baseline in order to simultaneously compare treatment response curves and examine potential container effects. Individual species scores identify which taxa are most involved in the observed community response patterns. Species abundance data were log-transformed prior to analysis (Leps and Smilauer 1999). Significance of the first axis of the PRC diagram was tested by performing 199 Monte Carlo permutations of the mesocosms using an *F*-type,

eigenvalue-based test statistic (Van den Brink and ter Braak 1999). The null hypothesis predicts no deviation of treatment communities relative to lake controls. Diagrams of second and higher order axes were also constructed and tested to examine residual variance (Van den Brink and ter Braak 1999).

3.4 Results

Ambient lake conditions were similar for the early experiment in Shelf Lake 4 and the late experiment in Shelf Lake 5 (Table 3.1). Temperature averaged 15.7 °C in the early experiment and 11.4 °C in the late experiment. Both lakes experienced low ambient ion concentrations, and low conductivity and alkalinity. Both had NO_3 concentrations below detection limit and circumneutral pH. Phytoplankton chl *a* concentrations were characteristic of oligotrophic lakes, while TP levels were indicative of slightly mesotrophic systems. Phytoplankton photosynthetic rate during the late experiment was nearly double that of the early experiment, but epilithic photosynthetic rates were comparable.

3.4.1 Enclosure Effects

Conditions in the control mesocosms varied slightly from the lakes, although differences were orders of magnitude less than treatment responses. For the early summer experiment in Shelf Lake 4, NH_4, PO_4, conductivity, DOC, alkalinity, pelagic PC, and phytoplankton chl *a* and photosynthetic rate were all slightly, yet significantly, greater in the controls than in the lake for at least one of the two sampling dates (Table 3.2). Silica concentration was slightly, yet significantly, lower after two weeks in the

control. Temperature was marginally cooler (about 0.1-0.3 °C) in the control during initial sampling. Nitrate, pH, DO, and epilithic tile chl *a* and photosynthetic rate did not differ between controls and the lake. Significantly more chlorophytes and fewer cryptophytes were present in control mesocosms after two weeks, but no significant enclosure effects were noted for zooplankton abundance or biomass. For the late experiment in Shelf Lake 5, temperature was slightly cooler in the controls at all three sampling dates (Table 3.2). Dissolved oxygen was significantly greater in the controls for initial conditions only. Control phytoplankton photosynthetic rate and pelagic PC were greater, but the difference with the lake diminished over the course of the experiment. Conductivity was greater in the controls after both weeks one and two. Significantly higher densities of planktonic diatoms and benthic cyanophytes were found in control mesocosms; no enclosure effects were detected for other response variables including zooplankton. Differences were attributed to release of nutrients due to sediment disturbance during installation, lack of mixing, and slight shading by the mesocosm plastic.

3.4.2 Treatments

Initial mean NO_3-N and PO_4-P concentrations were < 5 and 8 $\mu g\ l^{-1}$ respectively, prior to adding treatments. Mean NO_3-N concentrations after adding treatments were 1,041 and 1,090 $\mu g\ l^{-1}$ for N and NP treatments in the early experiment and 999 and 927 $\mu g\ l^{-1}$ for the late experiment. Nitrate-N levels were < 5 $\mu g\ l^{-1}$ after two weeks for the NP treatments, but were 17 and 125 $\mu g\ l^{-1}$ in the N treatments for the early and late experiments, respectively (Table 3.3). Phosphate-P concentrations after adding

treatments were 127 and 123 μg l^{-1} for the P and NP treatments in the early experiments and 96 and 115 μg l^{-1} in the late experiment. Phosphate was reduced to 17 and 12 μg l^{-1} P for the NP treatments and 38 and 13 μg l^{-1} P for the P treatments after two weeks for the early and late experiments, respectively.

3.4.3 Water Quality Responses

Prior to adding nutrient amendments, water quality conditions among the enclosures (excluding lake mesocosms, but see *Enclosure Effects* above) did not differ significantly among treatments for either experiment with the exception of alkalinity at the onset of the late summer experiment. Temperature measurements did not differ significantly among the enclosures during either experiment. Temperature across treatments increased during the early summer experiment, however, and decreased during the late summer experiment (Tables 3.4 and 3.5). Ammonium, silica, and DOC concentrations did not differ among treatments for either experiment. Ammonium increased over time during the early summer experiment, but decreased during the late summer experiment. Silica decreased over time in both experiments while DOC increased in just the early experiment.

Alkalinity and pH in the N and NP treatments were greater than control and P treatments at the end of both experiments, reaching concentrations of about 7 and 9 mg l^{-1} $CaCO_3$ (i.e. pH's above 9; Fig. 3.1). As mentioned above, initial alkalinity concentrations differed among treatments for the late experiment, but by the end of the late experiment, the N and NP treatments clearly had gained more alkalinity than both the control and P treatments. DO (percent saturation) did not vary among treatments nor

over time during the early experiment. Levels remained at about 90% saturation. DO saturation increased dramatically from about 100 to 115% for the N and NP treatments during the first week of the late experiment. By the end of two weeks, DO saturation had further increased to over 140% in response to the NP amendment, but remained at week one levels in the N mesocosms.

3.4.4 Phytoplankton

Phytoplankton did not differ among enclosed mesocosm treatments prior to either experiment, with the exception of fewer phytoplankton cells in P treatments at the start of the early experiment and larger chl *a*:C ratios in the N and NP treatments prior to the late experiment. Nitrogen and NP amendments increased total phytoplankton abundance, chl *a*, photosynthetic rate, and PC over controls (Tables 3.4 and 3.5, Fig. 3.2) by the end of both experiments. The patterns of change in chl *a*, photosynthetic rate, and pelagic C were very similar, although photosynthetic rate increased more than chl *a*, yielding higher photosynthetic efficiency (C uptake per unit chl *a*) by the end of both experiments. Similarly, chl *a* increased more than pelagic PC, so that chl *a*: C ratios increased (far beyond the differences present at the start of the late experiment). Addition of PO_4 alone did not cause any significant responses.

In the early summer experiment, the N and NP treatments showed similar results, although N responses were greater for some variables. Responses were also similar between N and NP treatments for the first week of the late summer experiment, but chl *a*, photosynthetic rate, and PC continued to increase in the NP treatment and were significantly different than the N treatment after two weeks (similar to the DO response

during this experiment). Means, after two weeks during early summer, reached about 3.2 x 10^5 and 1.6 x 10^5 cells ml^{-1} (respectively for N and NP), 11 μg l^{-1} chl *a*, 400 mg C m^{-3} h^{-1}, and 0.55 and 0.96 mg C l^{-1} (respectively for N and NP). Responses to N and NP treatments reached approximately 2.7 and 3.9 x 10^5 cells ml^{-1}, 7 and 17 μg l^{-1} chl *a*, 230 and 760 mg C m^{-3} h^{-1}, and 0.73 and 1.06 mg C l^{-1}, respectively, after two weeks during late summer.

Dramatic changes in phytoplankton composition also accompanied N and N+P additions. Phytoplankton in the lake, control and P treatments were relatively sparse and dominated by small chrysophytes throughout both experiments. Nitrogen and NP enclosures, on the other hand, caused declines in chrysophyte abundance and significant increases in chlorophytes, cyanophytes, diatoms, and total cell abundance in both experiments. Species shifts in N and NP treatments were accompanied by significant declines in species richness in the early experiment and declines in both richness and diversity in the late experiment (Tables 3.4 and 3.5, Fig. 3.2).

PRC analysis of the early experiment explained significant variation in phytoplankton composition ($F=6.596$, $p=0.005$) and displayed the strong divergence of phytoplankton in N and NP enclosures from phytoplankton in the lake, control, and P treatments (Fig. 3.3). Time (differences between weeks) explained 25% of the total variance in phytoplankton composition (Table 3.6). Treatment regime (time*treatment interaction) explained another 40% of the variance. Species with high positive weights showed a strong affinity for the patterns displayed in the PRC diagram, indicating that they increased in abundance in N and NP treatments relative to the lake. Similarly, negative weights denoted species that decreased in relative abundance in N and NP treatments. Only taxa

with weights greater than | 1 | were included in diagram. Taxa that increased with N and NP enrichment tended to be small colonial Chroococcales (Cyanophyta) and chlorophytes, whereas taxa that diminished in relative importance were mainly chrysophytes and two species of *Monoraphidium* (Fig. 3.3a). The second PRC explained a significant amount of the residual variance (p=0.005), but explained less of the treatment variance than the first axis (Table 3.6).

PRC analysis for the late experiment explained significant variation in phytoplankton composition (F=7.900, p=0.005) and, as in the early experiment, displayed a strong divergence of N and NP treatments from lake, control and P mesocosms (Fig. 3.4). Time (differences between weeks) explained 21% of the total variance in phytoplankton composition (Table 3.6). Treatment regime (the time*treatment interaction) explained another 36% of the variance. Taxa negatively associated with the PRC diagram (i.e., those that decreased in abundance in N and NP treatments) were again chrysophytes, notably the initially dominant *Ochromonas* sp. With the exception of *Nitzschia acicularis* and a colonial *Cryptomonas* sp., all taxa that increased with N and NP enrichment were chlorophytes, whereas taxa that diminished most in relative importance were chrysophytes (Fig. 3.4). The second PRC for the late experiment explained a significant amount of the residual variance (p=0.01), but explained less of the treatment variance than the first axis (Table 3.6).

3.4.5 Zooplankton

Zooplankton abundance and biomass did not differ among mesocosms at the start of either experiment (Tables 3.4 and 3.5). Additionally, no significant differences in

abundance, biomass, richness, or diversity were detected across treatments at the end of either experiment. Instead, time accounted for the majority of changes in zooplankton variables, particularly biomass (Tables 3.4 and 3.5).

Pre-treatment zooplankton assemblages for the early experiment were dominated by copepods, mainly nauplii and *Paracyclops* sp. By week four, zooplankton assemblages in all mesocosms were dominated by *Keratella* sp. Abundance and biomass (particularly of rotifers) increased across all treatments from beginning to end (Table 3.4), and diversity, but not richness, increased ($p < 0.0001$ for all treatments). In the late experiment, pre-treatment zooplankton consisted of a mixed assemblage of copepod nauplii and rotifers (*Polyarthra* sp. and *Keratella* sp.). By week two, overall zooplankton abundance and biomass had decreased across all treatments (Table 3.5), and diversity had increased slightly over pre-treatment samples.

2.5.6 Epilithic Algae

In the early summer experiment, epilithic chl *a* did not vary among treatments nor over time (Table 3.4, Fig. 3.5). A slight, yet significant, decrease in photosynthetic rate occurred in the P treatment. This caused a decrease in photosynthetic efficiency for the P amendments. This ratio increased in the N and NP treatments, however. The chl *a*:pheophytin ratio was similar among treatments although it increased significantly over time. Particulate C and community composition were not measured in the early experiment.

After two weeks in the late summer experiment, both N and NP treatments had significantly greater epilithic chl *a* and total cell abundance than the control (Table 3.5,

Fig. 3.5). Magnitude of the mean NP response was twice as large as the N response, but variability among replicate mesocosms precluded detection of significant difference between N and NP treatments. Photosynthetic rate increased for the NP treatment only during the first week and was significantly different from the control, but this effect was temporary. The chl *a*: phaeophytin ratio was significantly greater in the NP treatment, suggesting more vigorous growth or less senescence. Epilithic PC did not respond to nutrient enrichments, however, yielding greater chl *a*:C ratios in both the N and NP treatments after two weeks.

Epilithic algal taxonomic responses were comparable to phytoplankton at the Division level. Significant increases in cyanophyte and chlorophyte abundance occurred in N and NP treatments, contributing to the increase in overall epilithic algal abundance in N and NP treatments by week two.

3.4.7 Epipelic Algae

Mean epipelic chl *a* concentrations in the top cm of sediment were 150 ± 7.6 and 156 ± 9.1 mg m^{-2} across all mesocosms at the end of the early summer (four weeks) and late summer (two weeks) experiments, respectively (mean ± 1 SE). Variability was substantial, even among replicates, and individual concentrations ranged from 16 to 478 mg m^{-2}. At the end of the early experiment, means were not significantly different among treatments (p=0.7726). At the end of the late experiment, chl *a* was marginally different among treatments (p=0.0569). Means for the N, NP, and lake treatments (246, 213, and 189 mg m^{-2}) were much larger than the control and P treatments (86 and 48 mg

m^{-2}), but substantial variability among replicates prevented detection of significant differences among individual treatments.

3.4.8 Epi-plastic Algae

Mean epi-plastic chl *a* concentrations across all mesocosms were 0.58 ± 0.03 and 0.26 ± 0.01 mg m^{-2} at the end of the early and late experiments, respectively (mean ± 1 SE). Individual concentrations ranged from 0.128 to 1.207 mg m^{-2}. We detected significant treatment differences for epi-plastic chl *a* at the end of both the early and late summer experiments (p=0.0026 and 0.0126, respectively). The direction of these differences was opposite, however. In the early trial, both the N and NP treatments were significantly lower than the control (p=0.0101 and 0.0427, respectively), probably because the plastic strips were sampled at four weeks, after excess nutrients had been consumed and attached algae could have sloughed off. In the late experiment, NP was significantly greater than the control (p=0.0162). The N treatment was intermediate; N was not significantly different than the control, but also only marginally different from NP (p=0.0691).

3.4.9 Algal Responses Across Habitat

When scaled to mesocosm size (assuming half of the benthos is epipelon and half is epilithon), epipelic chl *a* clearly dominated over phytoplankton, epitlithic algae, and epi-plastic algae in all treatments for both experiments (Fig.3.6a). When we consider algal responses as percent of control at the end of the experiments, however, epipelic algae did not experience much *relative* chl *a* gain from N or N+P enrichment compared to other algal types. After two weeks in the early summer experiment, phytoplankton chl *a* was

about 4.5 times greater than the control in both the N and NP treatments, but only a *maximum* of about two times greater than the control for epilithon, epipelon (after four weeks), and "epi-plaston" (after four weeks; p=0.0147; Fig 3.6a). In the late summer experiment, there were negligible differences in relative chl *a* gain among algal types for the N treatment, but both phytoplankton and epilithic algae gained significantly more relative chl *a* than epipelic or epi-plastic algae in the NP treatment (p=0.0031; Fig. 3.6a). If we consider the photosynthetic rate of treatments compared to control, however, phytoplankton clearly dominated relative gain in C uptake compared to epilithic algae for both experiments (p=0.0161 and p<0.0001 for early and late experiments, respectively; Fig. 3.6b). Furthermore, phytoplankton particulate C at the end of the late summer experiment was two-thirds and two times greater than the control in the N and NP treatments, respectively, while epilithic PC was similar among all treatments (Fig. 3.6c).

3.5 Discussion

Nitrogen limitation of freshwater productivity is now considered more common than previously thought (Elser et al. 1990, Guildford and Hecky 2000). In our study of shallow mountain lakes, we found N to be the nutrient most limiting to algal biomass and productivity in both the early and late summer. Phosphorus was secondarily limiting during late summer as ambient productivity increased and inflow diminished. Additionally, a survey of 15 lakes in the Snowy Range found that N regulation was common regionally (Lafrançois et al., in prep). Nitrogen limitation has also been documented by experimental enrichments in lakes in Yellowstone National Park, WY (Interlandi and Kilham 1999); the Sawtooth Mountains, ID (Wurtsbaugh et al. 1997); the

Sierra Nevada Mountains, CA (Axler et al. 1981; Goldman et al.1993); and in montane lakes on the western slope of the Front Range, CO (Morris and Lewis 1988).

We found that although epipelon dominated algal biomass in our mesocosms, phytoplankton responded most consistently to N enrichment. Several other studies have also found that the linkage between water column nutrients and algal biomass was stronger for phytoplankton rather than periphyton (Cattaneo 1987, Hansson 1992, O'Brien et al. 1992). Light limitation, resulting from pronounced increases in phytoplankton biomass, may restrict periphyton response to nutrients (Hansson 1992, O'Brien et al. 1992). This scenario may have occurred in our mesocosms. N and NP mesocosms were noticeably cloudier than the lake, control, and P mesocosms. Tiles on the N and NP substrates were not visible, and the Secchi disk depth would have been less than 1.5 m. Greatly increased productivity also led to very high pH levels and, therefore, low CO_2 concentrations. The dramatic decline in epilithic productivity in the NP treatment after the second week in our late summer experiment could be explained by shading and dissolved inorganic carbon (DIC) limitation (Turner et al. 1994), since phytoplankton biomass reached its peak at this time.

Other studies contrast with our findings and document increased periphytic biomass with nutrient enrichment. Specifically, N + P amendments fertilized epilithon and, to a lesser extent, epipelon, rather than phytoplankton in shallow, alpine Pipit Lake (Vinebrooke and Leavitt 1998). Similarly, accumulation of chl *a* in epi-plastic algae greatly exceeded that of phytoplankton and epipelon for shallow mesocosms in an oligo-mesotrophic lake (Blumenshine et al. 1997). There are several factors that may contribute to contrasting results among studies. First, adsorptive properties of the

sediment may differ among lakes, altering transport of nutrients to the benthos. The form of N amendment may also affect its affinity toward the benthos since NH_4, but not NO_3, binds to clay sediments (Horne and Goldman 1994). Our N amendments were as a nitrate salt (KNO_3), so as to mimic N deposition transformed by the terrestrial environment, while NH_4NO_3 or NH_4Cl have typically been used in other studies. Differences in grazer density and composition might also influence pelagic versus benthic responses to nutrient enrichment. Enclosures in our study lakes had low populations of planktonic copepods and rotifers, which are inefficient phytoplankton grazers compared to *Daphnia*. This was also the case in Pipit Lake, however, where phytoplankton biomass did not respond to nutrients (Vinebrooke and Leavitt 1998). Benthic grazers, not sampled in our study, may similarly regulate the magnitude of benthic algal response to nutrient enrichment. Nevertheless, Blumenshine et al. (1997) found that epi-plastic chl *a* increased following nutrient enrichment despite increases in epi-plastic invertebrate density.

Although the factors determining pelagic versus benthic responses to nutrient enrichment are not fully understood, our results as well as others (Blumenshine et al. 1997, Vinebrooke and Leavitt 1998) do indicate differences within benthic algal types. Epilithic and epi-plastic algae responded more frequently or to a greater degree than epipelic algae. Epipelon benefit from access to nutrients diffusing upward from the sediment, while algae attached to inert surfaces such as rocks, tiles, and plastic rely on nutrients from the water column or from regeneration within the mat itself (Burkholder 1996, Wetzel 1996). Thus, nutrient availability most likely rarely limits epipelon growth.

Most other mesocosm nutrient experiments did not include multiple indices of algal biomass and productivity. Chl *a* is most often used as an index for algal biomass, but it is well known that factors such as light and physiological cell condition can alter chl *a* concentrations without impacting biomass (Laws and Bannister 1980, Chapra 1997). Particulate C is not a perfect indicator of algal biomass either because it can include non-algal C, although we suspect that in our experiments increased PC following nutrient enrichment was due to accumulation of algal C. By combining indices, our measurements of chl *a*, PC, and photosynthetic rate highlight certain factors not often considered. First, increased chl *a*:C ratios suggest that a portion of the phytoplankton chl *a* gain was due to a physiological response to increased nutrients followed by reduced light availability. Epilithic PC did not increase, and it is likely that all of the epilithic chl *a* gain was physiological, and that nutrient enrichment did not cause any actual biomass gain. The increase in epilithic cell density likely reflects the proliferation of small cyanophytes. Second, photosynthetic efficiency (i.e. C uptake per unit chl *a*) was higher for phytoplankton than epilithon. Means ± 1 SE across all mesocosms and all sampling dates were 18.9 ± 0.2 and 11.0 ± 0.2 mg C mg Chl a^{-1} h^{-1}, respectively for phytoplankton and epilithon. This may reflect gradients within the epilithic mat that reduce DIC, nutrient, and light availability at depth (Lowe 1996). We expect that algal cells lower in the mat would, therefore, be less photosynthetically efficient despite containing living chl a. Chl *a* measurements might be misleading in terms of both biomass gain and ecological function. Thirdly, gains in photosynthetic rate greatly exceeded gains in PC. This suggests that much of the additional C uptake was lost to dark respiration, excretion, or grazing. Because zooplankton biomass did not increase, we suspect that, at least for

phytoplankton, most of this loss occurred via dark respiration or excretion. Since DOC levels were not altered, it is possible that the microbial component, not measured in this study, was greatly augmented by nutrient enrichment.

Changes in phytoplankton community composition and diversity with nutrient enrichment have been described from nutrient gradient studies across many lakes (Ilmavirta 1990, Moss 1973, Reynolds 1998, Watson et al. 1997, Willén et al. 1990) as well as experimental nutrient additions. We found that addition of N and N+P to mesocosm enclosures caused a shift from chrysophytes toward assemblages dominated by cyanophytes and/or chlorophytes and diatoms. These shifts were accompanied by declines in species richness and diversity. Similar responses have followed experimental additions of N and P to whole oligotrophic lakes (Cottingham et al. 1998b, Findlay and Kasian 1987, Johannessen et al. 1984, Schindler 1975) and to mesocosm enclosures in other regions (Paul et al. 1995, Vanni 1987). Increases in chlorophytes and small Chroococcales (Cyanophyta) also followed experimental additions of N to water from several Michigan lakes (Hough and Thompson 1996). In the absence of a strong grazing effect, these division-level shifts may be explained largely by competitive differences among algal divisions. Chrysophytes, the dominant pre-treatment phytoplankton in the above studies and ours, are good competitors in low nutrient, low pH conditions due to their small size and large surface area:volume ratios. Increased N and N+P availability following treatment addition led to replacement of chrysophyte taxa by larger cyanophyte and chlorophyte taxa with generally higher N optima (Reynolds 1984). Increased NO_3 uptake resulted in higher pH levels and, consequently, lower availability of free CO_2. These conditions favored cyanophytes and chlorophytes, which unlike chrysophytes can

utilize bicarbonate in photosynthesis (Shapiro 1973, Shapiro 1990) and thrive in high pH environments (Levine and Schindler 1999, Moss 1973). Increased abundance of diatoms following N enrichment in our experiments has been documented in the fossil record of other mountain lakes (Wolfe et al. 2001) and may relate to the high competitive ability of diatoms at elevated N:P ratios (Tilman et al. 1986). Similarly, paleolimnological studies in various Connecticut lakes showed that *Nitzschia acicularis*, which responded positively to N and N+P in our experiments, was most often found lakes with high total N content (Siver 1999). Overall, phytoplankton community responses to N and N+P in our experiments closely matched eutrophication responses observed at different spatial and temporal scales and in lakes of different geographic regions. Individual species responses, while somewhat similar in our two experiments, appear to be lake- and region-specific, and remain difficult to predict (Cottingham et al. 1998a).

Previous studies have also noted changes in benthic algal composition in response to nutrient enrichment, although these have generally been slight (Peterson et al. 1993, Vinebrooke and Leavitt 1998). Unlike other investigations, which have demonstrated increased density of benthic diatoms (Vinebrooke and Leavitt 1998) with enrichment, we found no evidence of increased diatom density among our treatments. Instead, benthic chlorophytes and cyanophytes responded most to N and NP additions. Although shading by phytoplankton likely limited epilithic biomass in N and NP treatments, it may have also protected chlorophytes and cyanophytes from UV light damage and accounted for their relative increase in those treatments (Vinebrooke and Leavitt 1999).

Zooplankton and phytoplankton biomass are often closely coupled (McCauley and Kalff 1981), and bottom-up effects on zooplankton biomass and density following algal

nutrient enrichment have been reported (Cottingham et al. 1997, Paul et al. 1995, Vanni 1987). Nonetheless, zooplankton have also been shown to *not* respond significantly to nutrient enrichment (Brett and Goldman 1997, Vinebrooke and Leavitt 1998), to respond unevenly among taxa (Baxter 1959, Hansson and Carpenter 1993) or even to respond negatively (Malley et al. 1988), indicating some degree of frailty in the phytoplankton-zooplankton relationship. Factors limiting the ability of zooplankton to respond to increased nutrients and algal biomass include composition and size structure of the zooplankton community (Bergquist et al. 1985, Carpenter et al. 2001, Cottingham and Schindler 2000, Vanni 1987), shifts toward poorer quality food items such as cyanobacteria and chlorophytes (Brett et al. 2000), chemical stress related to greatly elevated primary productivity (Malley et al. 1988), and phytoplankton responses that are too rapid or large for zooplankton populations to effectively graze (Scheffer et al. 1993). Additionally, slow growing copepods may not have responded due to the limited duration of the experiments. A combination of the above factors likely accounted for the insignificant effect of nutrient enrichment on zooplankton biomass and composition in our lake enclosures. Sparse populations of copepods and rotifers dominated enclosures in both study lakes, rather than Cladocera, whose parthenogenetic reproduction and large body size enable them to respond efficiently to increased algal biomass (Cottingham and Schindler 2000, Elser and Goldman 1991). Likewise, shifts in phytoplankton composition from highly edible chrysophytes toward cyanobacteria and chlorophytes, accompanied by pH levels exceeding nine in fertilized mesocosms, may have dampened effects of enrichment on zooplankton biomass. Further, the magnitude of the phytoplankton response (which involved four to eleven-fold increases in algal chl *a*) may

have simply exceeded the grazing capacity of sparse zooplankton assemblages in our enclosures.

Enclosure experiments occupy small spatial and temporal scales that complicate generalization to whole lake ecosystems (Carpenter 1996, Carpenter 1998). For example, our experiments took place in only two lakes and lasted only two weeks, during the relatively warm and calm summer months. If N supply were instead to increase during periods of rapid hydrologic flushing, effects on algal biomass and productivity would likely be reduced due to loss of phytoplankton via lake outlets (Keefer and Pennak 1977, Reynolds 1984), and sloughing of benthic algal mats (Peterson 1996, Vinebrooke and Leavitt 1996). Further, if nutrient increases were sustained over longer time periods, changes in slower-growing benthic algal mats and responses of invertebrate grazers may become more apparent (Fairchild and Sherman 1992, Hershey 1992).

Similarly, applicability of our results to other mountain lakes depends on inter-lake differences in chemistry, morphometry, and food web structure. Many mountain lakes already have high NO_3 levels due to atmospheric inputs (Baron et al. 2000), and NO_3 addition to these lakes would not be expected to alter algal biomass or composition as it did in our enclosures (Nydick et al., submitted). Further, our study indicates that even primarily N limited lakes may quickly become P limited with continued N additions. Differences in lake morphometry may affect the magnitude of benthic versus pelagic responses, and nutrient losses to the hypolimnion of stratified lakes may alter apparent enrichment effects (Proulx et al. 1996). Finally, the presence of fish in our study lakes minimized zooplankton grazing pressure. Other mountain lakes are fishless and

dominated by large Daphnid grazers, which could place greater constraints on algal nutrient responses.

Despite the limitations of our mesocosm approach, several factors suggest that other low-NO_3 mountain lakes are likely to respond in a manner similar to our enclosures, and that the nature of the response may be generally predictable. First, enclosure effects were minimal compared to nutrient enrichment responses. Secondly, N limitation has been documented in other western mountain lakes and is quite common among Snowy Range lakes (Lafancois et al. in prep). Additionally, we found very similar responses to N addition in two mountain lakes and during two different time periods, demonstrating that strong effects of N addition may override lake-to-lake differences and seasonal variation in water chemistry and community composition. Furthermore, changes in algal biomass and composition following N and N+P additions in our experiments were consistent with P or N+P enrichment studies from geographically diverse regions and whole-lake experiments. We conclude that increased N influx will alter algal productivity and composition in many N limited lakes of the mountain West, and may trigger eutrophication responses both alone and in conjunction with additional P.

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Table 3.1. Ambient lake conditions during the two experiments. Variables are reported as mean ± 1 SE for all measurements taken in lake mesocosms. n = 9 for most variables (3 replicates x 3 sampling dates). Epipelic chl *a* is the mean of 3 replicates at the end of each experiment.

VARIABLE	UNIT	EARLY EXPERIMENT	LATE EXPERIMENT
Temperature	^{o} C	15.7 ± 0.2	11.4 ± 0.5
Conductivity	$\mu S\ cm^{-1}$	12.5 ± 0.3	14.3 ± 0.6
pH		7.1 ± 0.1	7.1 ± 0.1
Alkalinity	$mg\ l^{-1}$ as $CaCO_3$	4.35 ± 0.09	5.45 ± 0.16
Dissolved Oxygen	$mg\ l^{-1}$	6.88 ± 0.32	8.64 ± 0.09
NO_3-N	$\mu g\ l^{-1}$	≤ 5	≤ 5
NH_4-N	$\mu g\ l^{-1}$	43 ± 21	18 ± 8
PO_4-P	$\mu g\ l^{-1}$	4 ± 1	5 ± 1
TP	$\mu g\ l^{-1}$	20 ± 0.5	26 ± 0.9
Silica	$mg\ l^{-1}$	2.85 ± 0.23	2.56 ± 0.15
DOC	$mg\ l^{-1}$	2.18 ± 0.09	2.74 ± 0.20
Pelagic Particulate C	$mg\ l^{-1}$	0.44 ± 0.03	0.38 ± 0.03
Epilithic (tile) Particulate C	$mg\ m^{-2}$	- -	254 ± 39
Phytoplankton Chl a	$\mu g\ l^{-1}$	1.41 ± 0.12	1.87 ± 0.15
Epilithic (tile) Chl a	$mg\ m^{-2}$	0.84 ± 0.33	1.32 ± 0.29
Epipelic Chl a	$mg\ m^{-2}$	125 ± 76	189 ± 98
Phytoplankton Photosynthetic Rate	$mg\ C\ m^{-3}\ h^{-1}$	16.7 ± 2.7	35.6 ± 3.8
Epilithic (tile) Photosynthetic Rate	$mg\ C\ m^{-2}\ h^{-1}$	10.2 ± 1.6	9.03 ± 1.98

Table 3.2. Enclosure, time, and enclosure*time effects for chemical and biological variables during early and late summer mesocosm experiments. Differences between controls and un-enclosed lake mesocosms are reported as p-values; values ≤ 0.05 are in bold.

	EARLY EXPERIMENT			LATE EXPERIMENT		
VARIABLE	ENCL	TIME	ENCL* TIME	ENCL	TIME	ENCL* TIME
NH_4-N	0.0884	**0.0055**	0.0884	0.8996	**0.0149**	0.9625
NO_3-N	All below 5 μg l^{-1}			All below 5 μg l^{-1}		
PO_4-P	**0.0200**	0.2787	0.6200	0.3574	0.2063	0.4985
DOC	0.0680	**0.0046**	0.1562	0.4948	0.8872	0.7779
Silica	0.0611	0.0549	0.1309	0.4381	**<0.0001**	0.6375
Alkalinity	0.1078	**0.0106**	**0.0295**	0.7564	**0.0103**	0.1297
pH	0.9509	0.0594	0.9074	0.1431	0.2946	0.1600
DO	0.3532	0.0910	0.8598	0.0734	**<0.0001**	**0.0038**
Temperature	0.0810	**0.0395**	0.6030	**0.0176**	**<0.0001**	0.1626
Conductivity	**0.0292**	**0.0061**	**0.0381**	**0.0301**	**0.0029**	0.0752
Pelagic Particulate C	**<0.0001**	**0.0040**	0.0769	0.2586	**0.0003**	**0.0032**
Phytoplankton Chl *a*	**0.0155**	0.6230	0.1978	0.2285	**0.0125**	0.5220
Phytoplankton Photosynthesis	**0.0063**	**<0.0001**	**0.0179**	**0.0311**	**<0.0001**	0.5561
Pelagic Chl *a*:C	0.5936	0.0587	0.2344	0.1299	0.0908	0.1538
Phytoplankton Photosynthetic Efficiency	0.7177	**0.0009**	**0.0432**	0.1004	0.2399	0.6487
Phytoplankton Cell Abundance	0.5013	**0.0050**	0.5843	0.2088	0.1597	0.1421
Epilithic Particulate C	--	--	--	0.9919	**0.0119**	0.0571
Epilithic (tile) Chl *a*	0.7280	0.5057	0.3376	0.4583	**<0.0001**	0.1121
Epilithic (tile) Photosynthetic Rate	0.4926	**0.0439**	0.1307	0.1600	**<0.0001**	0.9739
Epilithic Chl *a* : C	--	--	--	0.5611	0.0862	0.1105
Epilithic Photosynthetic Efficiency	0.5934	0.3047	0.1045	0.9275	0.1912	0.0829
Epilithic Cell Abundance	--	--	--	**0.0087**	**0.0348**	0.1868
Zooplankton Abundance	0.1219	0.2381	0.2381	0.6433	0.1868	0.8683
Zooplankton Biomass	0.0969	**<0.0001**	0.0958	0.9202	0.2730	**0.0170**

Table 3.3. Nitrate, ammonium and phosphate concentrations at the start and end of the 14-d period for A) the early summer experiment in Shelf Lake 4, and B) the late summer experiment in Shelf Lake 5. Units are $\mu g\ l^{-1}$. Numbers in brackets show SE ($n = 3$).

		START of EXPERIMENT		END of EXPERIMENT	
	Treatment	NO_3-N	PO_4-P	NO_3-N	PO_4-P
A)	Lake	5 (0)	5 (0)	5 (0)	2 (0)
	Control	5 (0)	10 (2)	5 (0)	8 (3)
	N	1,041 (71)	11 (3)	17 (9)	8 (3)
	P	5 (0)	127 (2)	5 (0)	38 (10)
	NP	1,090 (17)	123 (3)	5 (0)	17 (7)
B)	Lake	5 (0)	5 (2)	5 (0)	5 (0)
	Control	5 (0)	9 (3)	5 (0)	5 (0)
	N	999 (23)	11 (2)	125 (171)	7 (2)
	P	93 (88)	96 (21)	5 (0)	13 (7)
	NP	927 (145)	115 (7)	5 (0)	12 (2)

Table 3.4. Treatment effects for the early summer experiment in Shelf Lake 4. Ø = no significant differences among treatments (see methods – statistical analyses). For individual week responses, letters indicate which treatment means were different than control (N=nitrate, NP = nitrate + phosphate, P= phosphate). Arrow indicates direction of difference (↑ = greater than control, ↓ = less than control). N↑ > NP↑ indicates that N response was greater than NP response, although both were significantly greater than the control. For time main effect, arrow indicates direction of change (↑ = means increase over time).

RESPONSE VARIABLE	WEEK 0 (lsmeans)	WEEK 2 (lsmeans)	TIME MAIN EFFECT
Temperature	Ø	Ø	↑
NH_4	Ø	Ø	
Silica	Ø	Ø	↓
DOC	Ø	Ø	↑
pH	Ø	N↑ NP↑	↑
Alkalinity	Ø	N↑ NP↑	↑
Dissolved Oxygen (% Saturation)	Ø	Ø	
Phytolankton Cell Abundance	P↓	N↑ > NP↑	↑
Pelagic Particulate C	Ø	N↑ > NP↑	
Phytoplankton Chl *a*	Ø	N↑ NP↑ P↓	↑
Phytoplankton Photosynthesis	Ø	N↑ NP↑	↑
Pelagic Chl *a* : C	Ø	N↑	↑
Phytolankton Photosynthetic Efficiency	Ø	N↑ NP↑	↑
Epilithic Chl *a*	Ø	Ø	
Epilithic Photosynthesis	Ø	P↓	↑
Epilithic Photosynthetic Efficiency	Ø	N↑ NP↑ P↓	
Epilithic Chl *a* : Pheophytin ratio	Ø	Ø	↑
Zooplankton Abundance	Ø	Ø	
Zooplankton Biomass	Ø	Ø	↑
Epipelic Chl *a* (after four weeks)	--	Ø	--
Epi-plastic Chl *a* (after four weeks)	--	N↓ NP↓	--

Table 3.5. As Table 4, but for the late summer experiment in Shelf Lake 5. Ø = no significant differences among treatments (see methods – statistical analyses). For individual week responses, letters indicate which treatment means were different than control (N=nitrate, NP = nitrate + phosphate, P= phosphate). Arrow indicates direction of difference (↑ = greater than control, ↓ = less than control). N↑ < NP↑ indicates that N response was less than NP response, although both were significantly greater than the control. For time main effect, arrow indicates direction of change (↑ = means increase over time).

RESPONSE VARIABLE	WEEK 0 (lsmeans)	WEEK 1 (lsmeans)	WEEK 2 (lsmeans)	TIME MAIN EFFECT
Temperature	Ø	Ø	Ø	↓
NH_4	Ø	Ø	Ø	↓
Silica	Ø	Ø	Ø	↓
DOC	Ø	Ø	Ø	
pH	Ø	N↑ NP↑	N↑ < NP↑	↑
Alkalinity	N↑ NP↑ P↑	N↑ NP↑	N↑ NP↑	↑
Dissolved Oxygen (% Saturation)	Ø	N↑ NP↑	N↑ < NP↑	↑
Phytoplankton Cell Abundance	Ø	Ø	N↑ < NP↑	↑
Pelagic Particulate C	Ø	N↑ NP↑ P↑	N↑ < NP↑	↑
Phytoplankton Chl *a*	Ø	N↑ NP↑	N↑ < NP↑	↑
Phytoplankton Photosynthesis	Ø	N↑ NP↑	N↑ < NP↑	↑
Pelagic Chl *a* : C	N↑ NP↑	N↑ NP↑	N↑ < NP↑	↑
Phytolankton Photosynthetic Efficiency	Ø	Ø	N↑ NP↑	↑
Epilithic Cell Abundance	Ø	P↓	N↑ < NP↑	↑
Epilithic Particulate C	Ø	Ø	Ø	↓
Epilithic Chl *a*	Ø	NP↑	N↑ NP↑	↑
Epilithic Photosynthesis	Ø	NP↑	Ø	↓
Epilithic Chl *a* : C	Ø	Ø	N↑ < NP↑	
Epilithic Photosynthetic Efficiency	Ø	Ø	Ø	↓
Epilithic Chl *a* : Pheophytin ratio	Ø	NP↑	NP↑	↑
Zooplankton Abundance	Ø	Ø	Ø	
Zooplankton Biomass	Ø	Ø	Ø	↓
Epipelic Chl *a*	--	--	Ø	
Epi-plastic Chl *a*	--	--	NP↑	

Table 3.6. Percentages of the total variance attributed to time and treatment regime in Principal Response Curves (PRC) for phytoplankton assemblages during the early and late experiments. The treatment component includes time x treatment interactions; variance remaining after time and treatment effects are summed is residual. The fraction of treatment variance captured by the first and second PRC's is also noted and tested, with respective p-values in parentheses.

	% Variance accounted for by:		% Variance explained by treatment regime in PRC:	
Experiment	Time	Treatment	First PRC	Second PRC
Early	25	40	47 (p=0.005)	20 (p=0.005)
Late	21	36	46 (p=0.005)	13 (p=0.01)

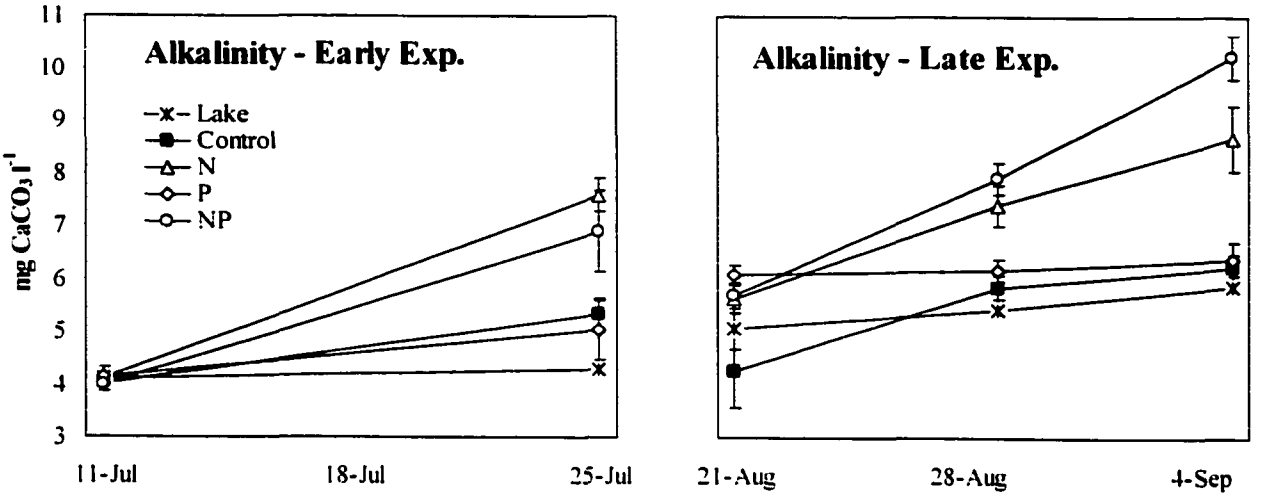

Figure 3.1. Effects of nitrate (N), phosphate (P), and combined nitrate + phosphate (NP) fertilization on alkalinity. Bars indicate ±1 SE (n = 3).

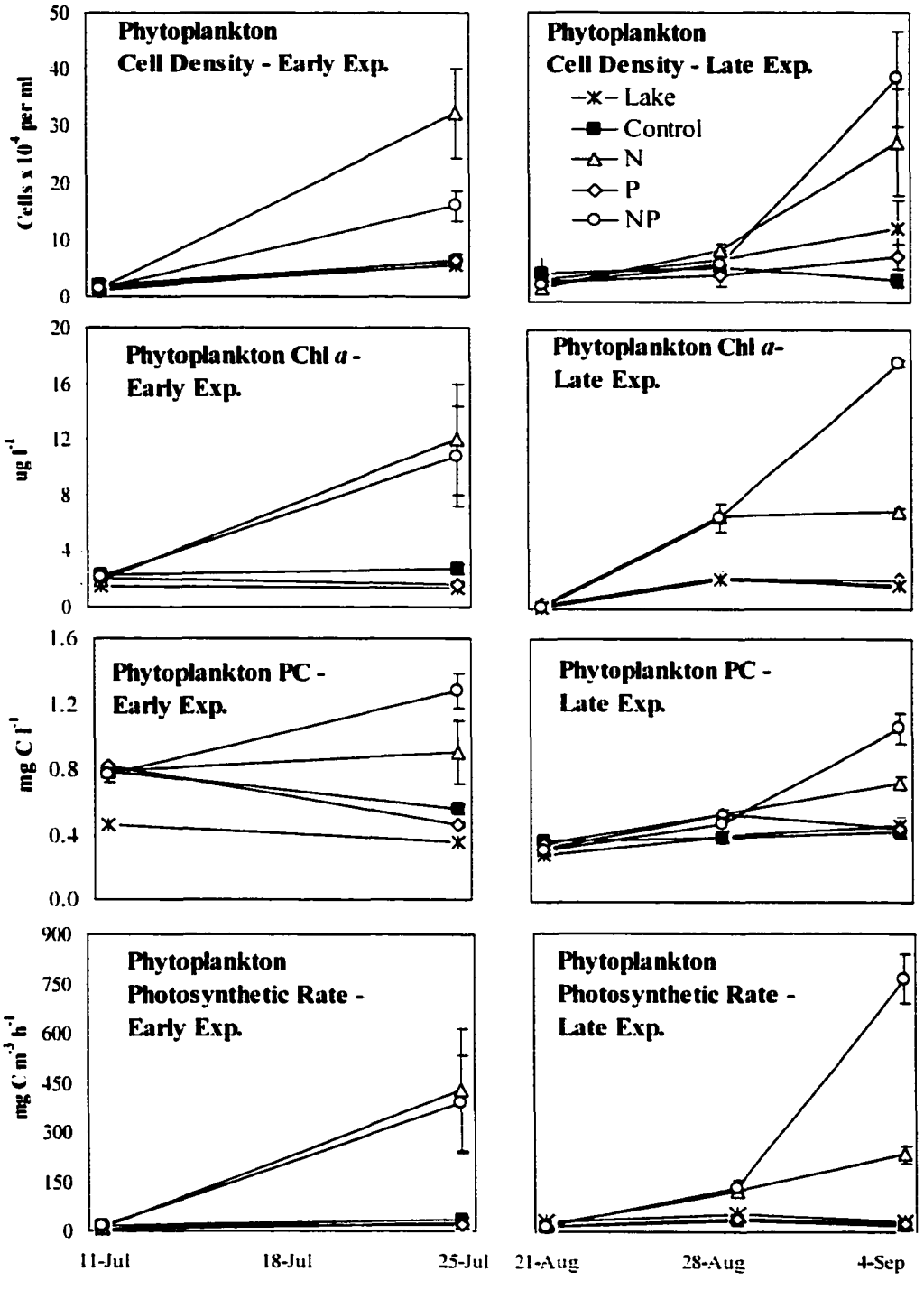

Figure 3.2. Effects of nitrate (N), phosphate (P), and combined nitrate + phosphate (NP) fertilization on phytoplankton cell density, chl *a*, pelagic particulate C, and photosynthetic rate. Bars indicate ±1 SE ($n = 3$).

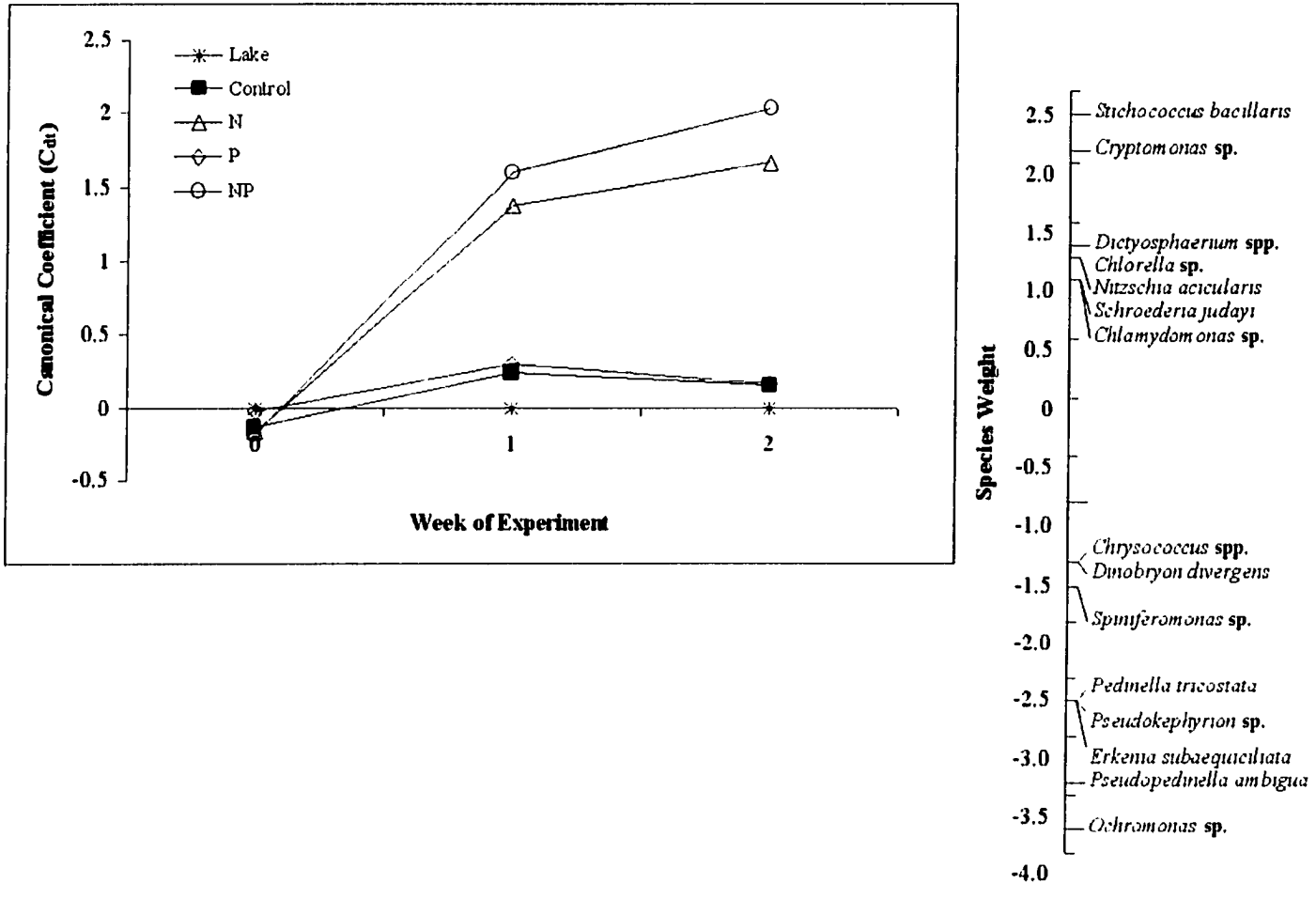

Figure 3.3. First Principal Response Curves (PRC) with species weights for the early experiment showing strong effects of N and N+P additions on phytoplankton composition. Lake controls are set to zero. Percentages of variance accounted for and significance levels are found in Table 5. See text for discussion of species weights.

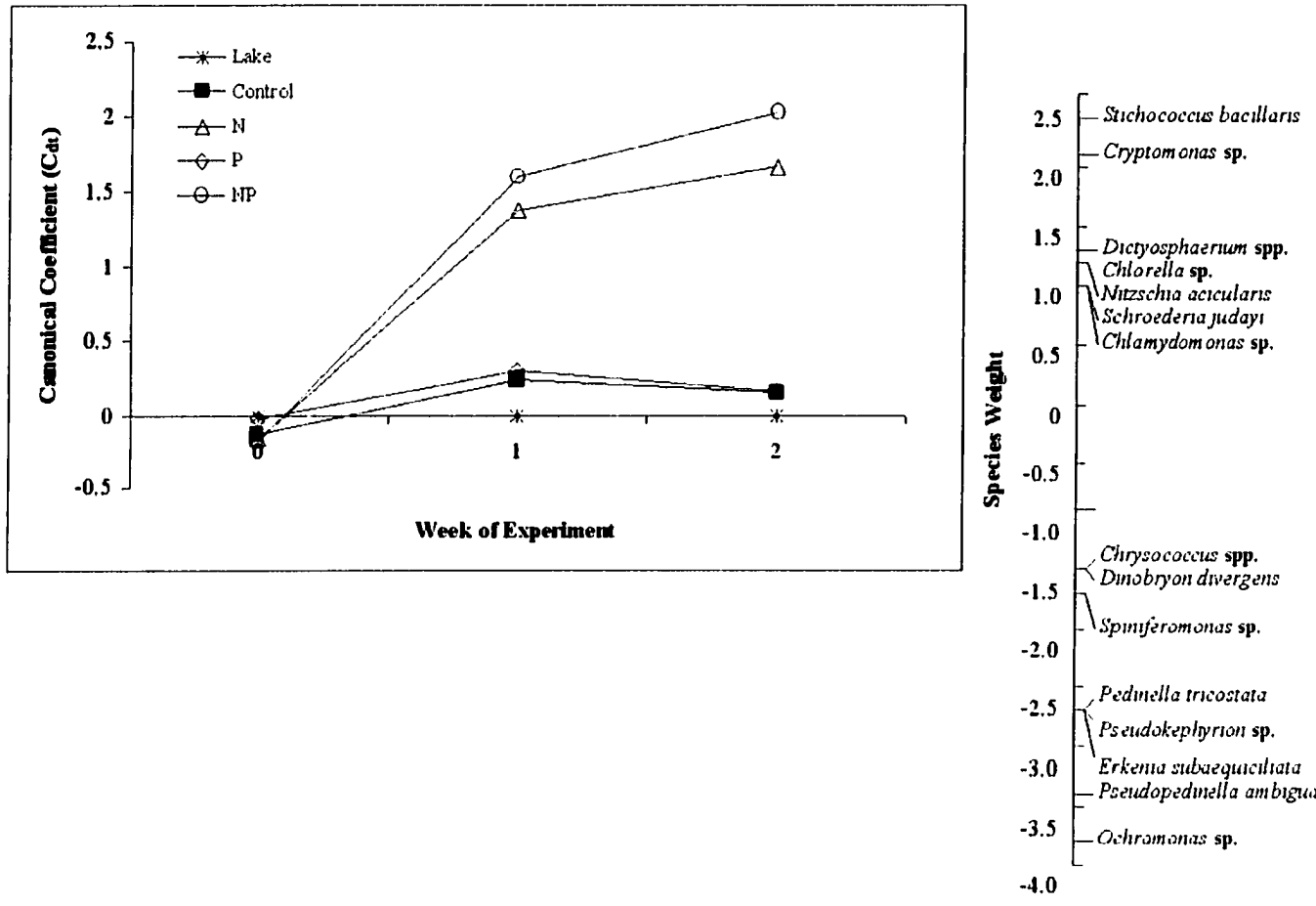

Figure 3.4. First Principal Response Curves (PRC) with species weights for the late experiment showing strong effects of N and N+P additions on phytoplankton composition. Lake controls are set to zero. Percentages of variance accounted for and significance levels are found in Table 5. See text for discussion of species weights.

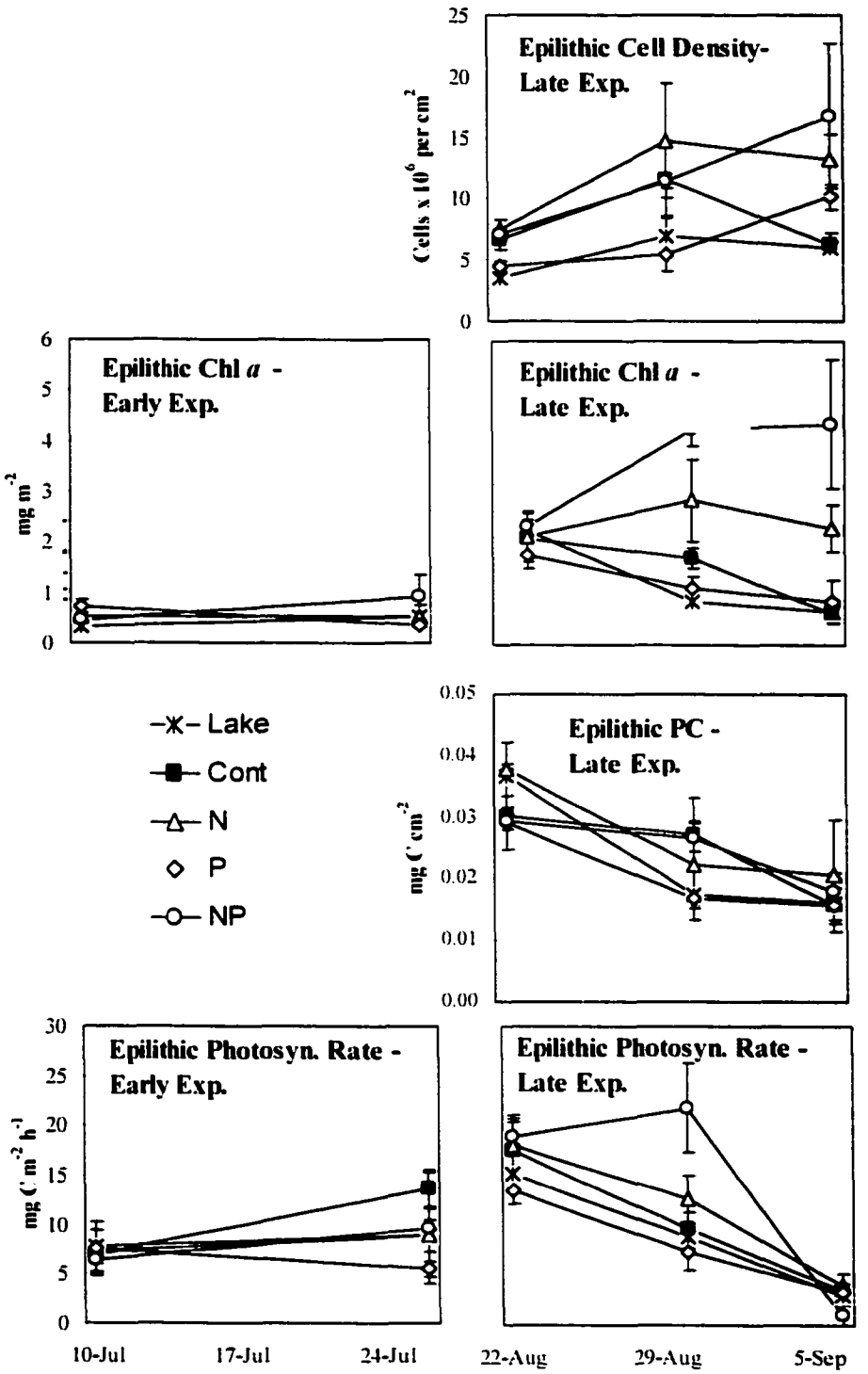

Figure 3.5. Effects of nitrate (N), phosphate (P), and combined nitrate + phosphate (NP) fertilization on epilithic algal cell density, chl *a*, particulate C, and photosynthetic rate. Bars indicate ±1 SE ($n = 3$).

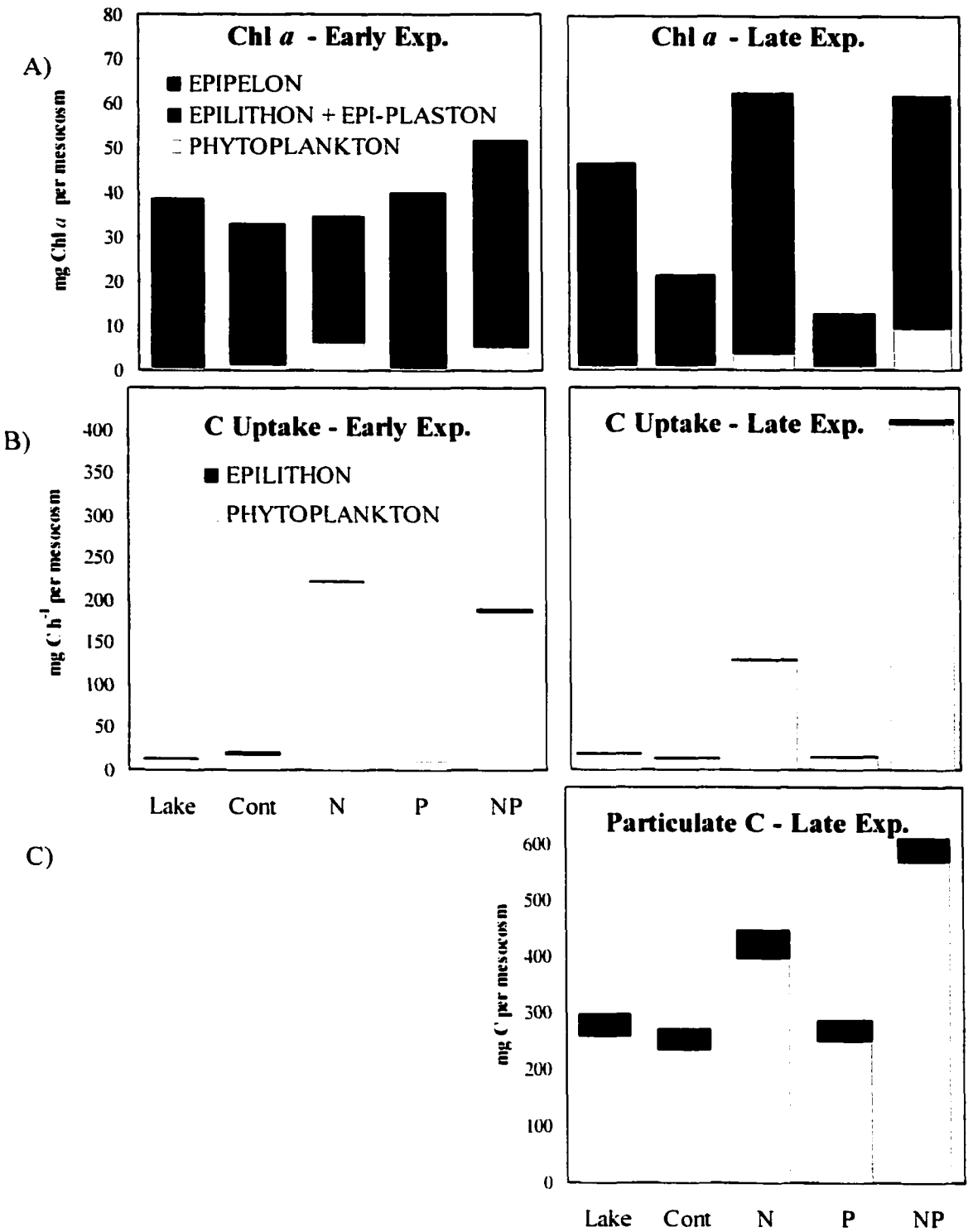

Figure 3.6. Mesocosm-scale budgets of different algal types at the end of the experiments for A) chl *a*, B) carbon uptake, and C) particulate carbon.

4. Phytoplankton versus benthos: Indicators and regulators of nutrient and acid stress in shallow mountain lakes

4.1 Abstract

We used enclosure experiments and ^{15}N isotope tracer to assess how various lake components (phytoplankton, epilithon, surface sediment, and deeper sediment) function as indicators and regulators of increased nutrient and acid inputs to shallow, oligotrophic mountain lakes (>3,000m ASL). Indicators have easily detectable responses to nutrient and acid additions, while regulators control nitrate (NO_3) uptake and, hence, biological alkalinity generation. Phytoplankton were the best indicators; increases in chlorophyll *a* and photosynthetic rate were easily detected in response to addition of nutrients, while dramatic taxonomic shifts occurred following addition of both acid and nutrients. Increased phytoplankton biomass and photosynthetic rate were also strongly correlated with enhanced NO_3 uptake by phytoplankton. Despite these strong responses, phytoplankton only accounted for 1-26% of total NO_3 uptake after two weeks, depending on the nutrient treatment. Surface sediment (0-0.5 cm) and epilithon on artificial tile substrates accounted for 7-34% and 1-2% of NO_3 uptake, respectively. Algal biomass was not well correlated to the amount of NO_3 uptake in either surface sediment or epilithon, however, and these algal types showed no detectable response to treatments. Much of the NO_3 (22-82%) was recovered from subsurface sediments (0.5-5 cm). Furthermore, in deeper sediment, both organic matter and total carbon explained about two-thirds of the variability in NO_3 uptake, suggesting that benthic microbial activity

exerted strong control over ecosystem NO_3 uptake capacity. Our experiments showed that although phytoplankton were most sensitive to nutrient and acid additions, the benthos, and in particular, sediment microbial processes, were the dominant regulator of ecosystem N uptake and acid neutralization.

4.2 Introduction

Mountain lakes can interrupt downstream transport of nitrate (NO_3) through uptake and denitrification. This is especially important in regions of high atmospheric nitrogen (N) deposition. For example, lakes and streams were the second largest reservoir (after tundra) for incoming N and retained the most N per surface area in the Loch Vale Watershed, Rocky Mountain National Park (Baron and Campbell 1997). As NO_3 loading to a lake increases, however, it is hypothesized that the efficiency of NO_3 retention decreases because algal N requirements are exceeded and because denitrification is not as efficient as algal removal (Kelly et al. 1990). In soils, however, microbial immobilization of N is probably the most significant N retention process (Holmes and Zak 1999, Nadelhoffer et al. 1995). Nitrate uptake by sediment might also be an important N retention process in aquatic ecosystems, although this is generally not considered (Jansson et al. 1994, Saunders and Kalff 2001).

Since reduction of NO_3 via mechanisms such as algal assimilation, microbial immobilization, and denitrification produces alkalinity, NO_3 retention is also a key process in terms of ecosystem resistance to acidification (Reuss and Johnson 1986). Acid neutralizing capacity in lakes is often assumed to originate from geochemical weathering and ion exchange in the watershed. In-lake biological processes can account for a

substantial proportion in low alkalinity lakes, however (Schindler et al. 1986), and are expected to be important in many western mountain lakes because total alkalinity is often less than 200 $\mu eq\ l^{-1}$ (Landers et al. 1987).

Benthic algae in shallow, oligotrophic lakes often dominate whole-system primary productivity (Lodge et al. 1998, Vadeboncoeur et al. 2002). Increased nutrient loading often enhances phytoplankton photosynthesis, however. Benthic algal response can be positive or negative depending on whether the added nutrient has any direct effect and whether there are indirect effects such as decreased light, dissolved inorganic carbon (DIC), and UV radiation (Vinebrooke and Leavitt 1998). As the lake eutrophies, phytoplankton become more dominant and productivity shifts to the water column. We've seen the beginning of this progression in a previous enclosure experiment (Nydick et al. 2002a). In a whole lake manipulation, this shift actually reduced whole-ecosystem productivity because of light limitation in the benthos (Vadeboncoeur et al. 2001).

Inorganic N and acid concentrations in atmospheric deposition have risen since the 1980's at many locations in the western United States, including the Colorado Front Range (Baron et al. 2000, Lynch et al. 1996, Nilles and Conley 2001). Atmospheric deposition has also been postulated as a significant source of phosphorus (P) to mountain lakes (Lewis et al. 1985, Sickman 2001). Given the possibility of increasing nutrient and acid inputs to western mountain lakes, we asked how different components of the lake system (phytoplankton, epilithon, surface sediment, and deeper sediment) function as both indicators and regulators of increased nutrient and acid stress. Indicators have easily detectable responses to nutrient and acid additions, while regulators are important in terms of ecosystem NO_3 retention and, hence, alkalinity generation. Enclosure

experiments were conducted in two mountain lakes with high and low NO_3 concentrations. The stable isotope ^{15}N was used as a tracer to measure NO_3 uptake. We hypothesized that high NO_3 concentrations would limit NO_3 uptake and associated biological alkalinity generation. We also expected that adding P would increase NO_3 uptake and alkalinity generation, especially under high ambient NO_3 conditions.

4.3 Methods

4.3.1 Study Sites

Shelf Lake 4 is located at 3,313 m in the Snowy Range, Medicine Bow National Forest, Wyoming, about 55 km west of Laramie. Its watershed drains the NW slope of a quartzite ridge of talus and exposed bedrock, with some meadow vegetation and krummholz. The lake surface area is 1.2 ha. The mean and maximum depths are 1.5 m and 3.4 m. Previous enclosure experiments documented N limitation of phytoplankton primary productivity and biomass (Nydick et al. 2000a). Nitrate levels were ≤ 0.36 μeq l^{-1} and mean chlorophyll *a* and alkalinity were 1.41 μg l^{-1} and 86.9 μeq l^{-1}, respectively. The ambient phytoplankton were mostly small chrysophytes. Zooplankton were dominated by copepods, mainly nauplii and *Paracyclops* sp., and rotifers, primarily *Keratella* sp. (Lafrançois et al. 2002). No record of fish stocking exists, but brook trout (*Salvelinus fontinalis*) are present and self-sustaining (Snigg 1989).

The Loch is located at 3,102 m on the east side of the Continental Divide in Rocky Mountain National Park, Colorado. The Loch's watershed is largely bare granitic rock and talus with some tundra, meadow, and subalpine forest. The lake surface area is 5 ha. The mean and maximum depths are 1.5 m and 4.7 m. Alkalinity averaged 42.6 μeq

l^{-1} from 1983-1988 (Baron 1992). Previous nutrient bioassay experiments documented P limitation of phytoplankton (Nydick et al. 2002b). Mean NO_3 and chl *a* levels were 15.99 μeq l^{-1} and 1.35 μg l^{-1}, respectively. Ambient phytoplankton were mostly chrysophytes and non-heterocystous blue-green algae. Zooplankton were very sparse and dominated by copepods, mainly nauplii, and rotifers, primarily *Nothalca* sp. and *Asplanchna* sp. (Lafrançois et al. 2002). Although originally fishless, The Loch was stocked with greenback cutthroat trout (*Oncorhynchus clarki stomias*), Colorado River cutthroat (*O. c. pleuriticus*), and rainbow trout (*O. mykiss*) in the early to mid 1900's (Rosenlund and Stevens 1990). Currently, The Loch is dominated by rainbow x cutthroat hybrids.

The littoral zones of both lakes are extensive and are characterized by large rocks and substantial fine, flocculent sediment. Neither lake stratifies during summer. Spring snowmelt, from April to July, dominates lake hydrology. The ice-free season lasts from early July to mid-October, and cool temperatures, high winds and intense solar radiation often occur during these months. Both lakes are only accessible by trail.

4.3.2 Experimental Design

We conducted enclosure experiments in both Shelf Lake and The Loch during the summer of 2001. The experiment in Shelf Lake lasted four weeks from July 19 to August 16. A large rainstorm truncated experiments in The Loch by flooding the enclosures, so data are presented for two weeks only, from July 17 to 31. Enclosures were constructed with vertical PVC tubing and horizontal plastic hoops (Nydick et al. 2002a). PVC was attached on the bottom to a semi-rigid plastic cylinder that extended at

least 10 cm into the sediments. Clear, cylindrical polyethylene plastic was fitted tightly around the cylindrical frame and attached to the rigid plastic piece. The enclosure extended about 10-20 cm above water level. Plastic tarps formed a skirt around the bottom of the enclosure, and rocks were piled on the tarps to secure the enclosures in place. Once installed in 1.5 m of water, they had a volume of approximately 450-l.

Enclosures were installed in two blocks of five, and treatments were randomly assigned within each block. The treatments were control (C), $+NO_3$ (N), $+PO_4$ (P), $+NO_3$ + acid (NA), and $+NO_3$ + acid + PO_4 (NAP). Care was taken to minimize sediment disturbance during installation, and enclosures were allowed to sit for two days prior to taking initial measurements to permit settling of fine sediments. Treatments were added to enclosures each week, starting immediately after initial measurements. Target NO_3-N and PO_4-P concentrations were 1 mg l^{-1} (71.4 μeq l^{-1}) and 0.032 mg l^{-1} (3.1 μeq l^{-1}) over ambient, respectively. The target in the acid treatments was pH 5. Nitrate was added as $K^{15}NO_3$ (10% atom), PO_4 as K_2HPO_4, and acid as HCl. We used $^{15}NO_3$ to allow calculation of NO_3 uptake. A small amount (<10% of ambient NO_3) of 99% atom $^{15}NO_3$ was added to the control and P enclosures in order to measure uptake. This trace amount of 99% atom $^{15}NO_3$ was also included in all the N treatments. Treatments were poured onto the enclosure water surface and mixed with a clean boat paddle; care was taken not to disturb sediments. Specific conductance was measured concurrently and mixing was continued until conductivity at 1 m depth reached a steady value. We also included a lake control, consisting of the frame only (i.e. no polyethylene plastic), in block one in order to measure ambient conditions.

4.3.3 Sample collection, processing, and analysis

Field measurements, incubations, and sampling were conducted prior to treatments and weekly thereafter for most variables. Benthic sampling was conducted every two weeks, and algae growing on the enclosure plastic were sampled after four weeks only. Benthic sampling included epilithic algae, which were colonized on unglazed ceramic tiles glued to trays. The tile trays were placed in the lakes one month prior to each experiment and were randomly assigned to enclosures. Algal and zooplankton community changes were investigated and are reported separately in Lafrançois et al. (2002).

4.3.4 Water chemistry

Samples were collected and processed using standard Loch Vale Watershed project methods (Allstott et al. 1999). Nitrate, PO_4, and NH_4 were determined colorimetrically with a Perstorp Analytical Alpkem Spectrophotometer (Model 3590) using the cadmium reduction, salicylate, and ascorbic acid methods, respectively. Dissolved organic carbon (DOC) was analyzed on an Oceanographics International Model 700 Carbon Analyzer (U.S. Geological Survey, Boulder, CO). Alkalinity was determined by Gran titration for low alkalinity samples (APHA 1995). We computed alkalinity generation as the difference between treatment applications. Temperature, specific conductance, dissolved oxygen (DO) and pH were measured with meters (Orion Model 128, Hach Sension6, VWR Model 8000). Calcium (Ca) and sulfur (S) were

determined by inductively coupled plasma-atomic emission spectroscopy (Thermo Jarrell Ash IRIS Advantage dual view high resolution ICP) for initial and end of experiment on filtered water samples only. Total dissolved nitrogen was determined by a Schimadzu TOC-V total organic carbon/nitrogen analyzer for initial and end of experiments filtered water samples as well.

4.3.5 Algal analyses

Depth-integrated water samples for phytoplankton analyses were collected by hand-pump. Tiles were scraped and rinsed with deionized water. At the end of each experiment, sections of plastic were cut from the wall of each enclosure and were analyzed for chl *a*. Phytoplankton and tile samples for chl *a* analyses were immediately filtered onto Whatman GF/C filters. Filters and plastic sections were frozen in dark containers and chl *a* was extracted with methanol (Riemann 1980). Chl *a*, corrected for phaeophytin, was determined with a Sequoia-Turner Model 450 Digital Fluorometer. Particulate C, N, and P on pre-combusted filters were oxidized with a potassium persulfate digest (Lampman et al. 2001). Oxidized C was then measured with a LI-COR Model LI-6252 CO_2 Analyzer. Nitrate and PO_4 in the digest were determined colorimetrically as described for water samples. Atom % ^{15}N of algae on replicate filters was measured with an isotope ratio mass spectrometer (VG-903).

Photosynthetic rate for phytoplankton and algae on tiles was measured weekly for block one enclosures only using the ^{14}C light/dark technique (Pregnall 1991). Incubations occurred at 1 m depth and lasted four to six hours after which containers were kept cold and dark in coolers while they were transferred to the laboratory.

Sediment cores were collected initially and every two weeks thereafter with a clear plastic tube, 2.85 cm in diameter. The surface sediment (top 0.5 cm) was separated and analyzed for chl *a*. The 0.5-5, 5-10, and 10-15 cm core sections were analyzed for percent organic matter (Steinmann and Lamberti 1996). Percent C and N, and atom % ^{15}N were determined by isotope ratio mass spectrometry as described above, coupled to a Carlo Erba elemental analyzer (NA-1500).

4.3.6 Denitrification

Denitrification rates were measured initially and every two weeks thereafter following methods adapted from Rudd et al. (1986). We collected a 3.2 cm diameter core containing 10 cm of sediment, 31.5 cm (250 ml) overlying water, and 50 cm of airspace from each enclosure prior to adding the week's enclosure treatment. Four mg l^{-1} of $^{15}NO_3$-N (99% atom) was added to all N treatments. An amount of $^{15}NO_3$-N (99% atom) equal to 5% of ambient NO_3 concentration was added to controls and P treatments. After treatment addition, the water column was mixed, taking care not to disturb the sediment. Each tube was sealed with a rubber stopper fitted with a septum and incubated vertically for 18-24 h in the lake. Three reference air samples were collected with an airtight syringe and stored in evacuated serum vials. Each sediment tube was shaken vigorously for three minutes at the end of the incubation to equilibrate N_2 and N_2O in the sediment, water, and headspace. A sample of the headspace was then taken with a syringe and stored in an evacuated serum vial. The tube was replaced in the lake, allowed to settle, and water temperature was measured. Atom% ^{15}N of N_2 and N_2O gases in serum vials was determined by isotope ratio mass spectrometry. Denitrification (N flux m^{-2} d^{-1}) was

calculated following methods of Mosier and Schimel (1993). Denitrification was estimated in the enclosures by assuming that N flux was linearly related to NO_3 concentration in the water column (Andersen 1977).

4.3.6 Statistical analyses

Repeated-measures analyses of variance (RM-ANOVA) were used to test the effects of treatments on response variables that were measured at regular time intervals for each lake. If the treatment main effect or the treatment*week interaction was significant at the $\alpha = 0.1$ level, we used *a priori* comparisons of each treatment with the control and set significance level at $\alpha = 0.05$. If more than one treatment was different than the control, then we compared these treatments to each other. An analysis of variance (ANOVA) was used to test for treatment effects on response variables measured at the end of the experiment, and comparisons among treatments were done in the same manner as for the RM-ANOVA. Nitrate uptake after two weeks duration was adjusted for loading and compared between lakes with a t-test. Data were transformed when necessary to meet the assumptions of normality and homogeneity of variance. Regression analysis was used to determine relationships between algal biomass and NO_3 uptake. All analyses were performed with SAS Version 8e (SAS Institute 1999-2001).

4.4 Results

4.4.1 Ambient conditions

Both lakes were cold and dilute throughout the experimental period (Table 4.1). Shelf Lake was warmer (14.7 vs. 11.5 °C) and had more than double the alkalinity (93 vs.

38 μeq l^{-1}) and DOC (2.0 vs. 0.8 mg l^{-1}) as The Loch (Table 4.1). Nitrate was elevated in The Loch compared to Shelf Lake (15.6 vs. 0.17 μeq l^{-1}) while total phosphorus (TP) was greater in Shelf Lake (22 vs. 9 μg l^{-1}), yielding a much higher dissolved inorganic N (DIN):TP ratio in The Loch (25.1 vs. 0.3). Phytoplankton chl *a* and photosynthetic rates were similar. Tile chl *a* was slightly higher in The Loch (5.7 vs. 4.4 mg m^{-2}), but tile photosynthetic rate was only about one-third of Shelf Lake (10.8 vs. 28.5 mg C m^{-2} h^{-1}). Chl *a* in Shelf Lake's surface sediment (205 mg m^{-2}) was almost double that of The Loch (122 mg m^{-2}), although percent organic matter in The Loch's deeper sediment was double Shelf Lake's (20.1 vs. 11.4 %). Zooplankton biomass was nearly 200 times higher in Shelf Lake than in The Loch (88.5 vs. 0.44 μg l^{-1}; LaFrançois et al. 2002). Shelf Lakes' zooplankton community was comprised mainly of cladocerans (*Daphnia* sp.) and copepods, while rotifers dominated The Loch (Lafrançois et al. 2002).

4.4.2 Enclosure effects

Enclosures enhanced water column productivity, while the benthos became less productive, although these changes were generally orders of magnitude less than treatment effects (Table 4.1). Conductivity, alkalinity, DOC, and phytoplankton chl *a*, photosynthetic rate, and particulate P were greater in the controls compared to the lakes. Tile chl *a* and photosynthetic rate were lower in the controls. Chl *a* in surface sediment was similar between control and the lake for The Loch, but lower in the control for Shelf Lake. Zooplankton biomass was also lower in the controls compared to the lakes, probably because some organisms were able to escape during enclosure installation (Lafrançois et al. 2002). Controls accumulated more chl *a* on plastic walls in The Loch

over the four weeks than in Shelf Lake. Dissolved nutrients were similar between the lake and controls in low-NO_3 Shelf Lake, but NO_3 in The Loch's control and P enclosures were well below lake levels after a week.

4.4.3 Treatments

Mean NO_3 concentrations in Shelf Lake were 99, 122, and 121 $\mu eq\ l^{-1}$ in the N, NA, and NAP treatments, respectively, immediately after amendments were added. Phosphate concentrations were 2.47 and 4.89 $\mu eq\ l^{-1}$ in the NAP and P treatments. The NA and NAP treatments had pH's of 4.76 and pH 4.81, respectively. Mean NO_3 levels in The Loch's N, NA, and NAP treatments were 92, 92, and 89 $\mu eq\ l^{-1}$, respectively, immediately after adding treatments. Phosphate concentrations were 2.16 and 2.35 $\mu eq\ l^{-1}$ in the P and NAP treatments. The NA and NAP treatments had pH's of 4.9 and pH 5.2. We added an average of 95 and 63 $\mu eq\ H^{+}\ l^{-1}\ wk^{-1}$ to acid treatments in Shelf Lake and The Loch, respectively.

4.4.4 Effects of treatments on algae

Shelf Lake

Phytoplankton chl *a* in the control treatments averaged 2.8 $\mu g\ l^{-1}$over four weeks. Enclosures did not differ in phytoplankton chl *a* prior to adding treatments or after one week of the experiment, but after two weeks, chl *a* increased significantly over the control in the NA and NAP treatments (Table 4.2A; Fig 4.1A). All N treatments were greater than the control after three weeks, but only NA and NAP remained significantly greater after four weeks. The N treatment was marginally greater than the control after

week one (p=0.07), week two (p=0.10), and week four (p=0.05), however. At the end of the experiment, mean phytoplankton chl *a* levels were three times greater than the control in the N (13 $\mu g\ l^{-1}$) and NA (15 $\mu g\ l^{-1}$) treatments and about nine times the control in the NAP (27 $\mu g\ l^{-1}$) treatment. Phytoplankton chl *a* did not respond to the P treatment.

Phytoplankton photosynthetic rate was similar among enclosures at the onset of the experiment and followed a pattern analogous to chl *a* (Fig. 4.1A). The control averaged 25 $\mu g\ C\ l^{-1}\ h^{-1}$. After week two, the N, NA, and NAP enclosures were 5, 16, and nearly 30 times greater than the control, respectively. Photosynthetic rate subsequently decreased in the NA and NAP enclosures, but continued to increase in the N treatment. After four weeks, N (202 $\mu g\ C\ l^{-1}\ h^{-1}$), NA (173 $\mu g\ C\ l^{-1}\ h^{-1}$), and NAP (320 $\mu g\ C\ l^{-1}\ h^{-1}$) were 7, 6, and 11 times the control, respectively.

Variability among replicate benthic measurements of tile, plastic, and surface sediment was substantial, such that even seemingly large distinctions among means were not significantly different (Table 4.2A, Figure 4.2A).

The Loch

Phytoplankton chl *a* concentrations in The Loch were similar among enclosures prior to adding treatments (Table 4.2B, Fig. 4.1B). The sediment below the block two control (C2) enclosure was rocky, and the frame moved slightly in response to wave action. There was a large increase in phytoplankton chl a (26 $\mu g\ l^{-1}$) in this replicate during the first week, which we attribute to suspension of benthic algae from disturbance. The mean chl *a* in the controls was 7.5 $\mu g\ l^{-1}$ during the entire experiment, but this average was enlarged because of the sediment disturbance C2. After two weeks, the

NAP1 enclosure had mean chl *a* of 35 $\mu g\ l^{-1}$, which was 12.5 times greater than the control. Phytoplankton chl *a* for the NAP2 enclosure was lower than the control, but chl *a* plus phaeophytin (a chlorophyll degradation product) was similar between NAP replicates and greater than the control. This suggests senescence of the NAP2 algal bloom that began forming the prior week. The N, NA, and P treatments had phytoplankton chl *a* levels similar to the control.

Phytoplankton photosynthetic rate was similar among treatments prior to adding nutrient and acid amendments (Fig 4.1B). The control averaged 19 $\mu g\ C\ l^{-1}\ h^{-1}$ during the experimental period. After the first week, photosynthetic rate in the P treatment (80 $\mu g\ C\ l^{-1}\ h^{-1}$) was about three to four times greater than the other enclosures. After two weeks, however, the P treatment decreased (44 $\mu g\ C\ l^{-1}\ h^{-1}$) and the NAP treatment increased to almost 27 times the control (665 $\mu g\ C\ l^{-1}\ h^{-1}$). Like Shelf Lake, no statistically significant treatment effects were detected for tile, plastic, and surface sediment response variables in The Loch (Table 4.2B, Fig. 4.2B).

4.4.5 Effects of treatments on N loss and Alkalinity Generation

Enclosures in Shelf Lake lost an average of 53 to 80% of the added NO_3 in the water column during the first two weeks of the experimental period. Percentages were similar over the four-week period during which all N treatments lost, on average, between 55 and 76%. One of the enclosures (N, block 2) deviated from the others and lost 98% of its added NO_3 during the first two weeks. In comparison, The Loch's N treatments lost 60-85% of the NO_3 added during the first two weeks. Differences in NO_3 loss among the N, NA, and NAP treatments were not significant for either lake.

Alkalinity generation occurred in all enclosures, but generally more so for the NA and NAP treatments, and to a lesser extent the N treatments. After correction for different experimental NO_3 loadings, alkalinity generation did not differ among lakes when NO_3 was added and averaged 42 $\mu eq\ l^{-1}$ per week.

In Shelf Lake, the NA and NAP treatments had weekly alkalinity gains greater than the control for all four weeks (Table 4.2A, Fig. 4.3A). Alkalinity increased significantly in the N treatment during the first week ($p=0.05$) and was marginally greater during the third week ($p=0.07$). Over the entire experiment, the control, N, NA, NAP, and P treatments generated an average of 50, 122, 212, 216, and 24 $\mu eq\ l^{-1}$ of alkalinity, respectively. After four weeks, N enclosures had a mean alkalinity of 212 $\mu eq\ l^{-1}$, while the control and P treatments had 125 $\mu eq\ l^{-1}$. NA and NAP treatments averaged only 29 $\mu eq\ l^{-1}$.

In The Loch, NA and NAP treatments gained more alkalinity each week than the control (Table 4.2B, Fig. 4.3B). Over the two-week experiment, the control, N, NA, NAP, and P enclosures gained averages of 30, 34, 80, 97, and 33 $\mu eq\ l^{-1}$ of alkalinity, respectively. The low alkalinity gain in the N treatment was mainly due to the replicate in block one (N1) since the second replicate gained 60 $\mu eq\ l^{-1}$ of alkalinity. After two weeks, the N treatment had a similar alkalinity concentration as control and P, and these six enclosures averaged 76 $\mu eq\ l^{-1}$. The NA and NAP enclosures had a mean concentration of only 35 $\mu eq\ l^{-1}$ by comparison.

Nitrate loss was not adequate to account for all the alkalinity gain. Exchange of H^+ with calcium ions (Ca^{2+}) and bacterial SO_4 reduction in the sediment also produce alkalinity. We predicted alkalinity generation based on NO_3 loss + Ca gain + SO_4 loss +

PO_4 loss + NH_4 gain. Ca gain was the largest contributor to predicted alkalinity generation besides NO_3 loss (Fig 4.4). Predicted alkalinity generation was similar to measured alkalinity gain with the exception of one outlier for each lake where measured alkalinity gain was less than predicted (Fig. 4.5).

Ca gain did not differ significantly among treatments over the duration of either experiment. In Shelf Lake, the NA and NAP treatments were marginally greater than the control, however (p=0.06 and 0.07, respectively). Ca concentrations increased 65 and 63 $\mu eq\ l^{-1}$ for the NA and NAP treatments, but only 24, 23, and 20 $\mu eq\ l^{-1}$ for the control, N, and P enclosures, respectively. In The Loch, block one enclosures had greater Ca gain than block two enclosures (30 vs. 6 $\mu eq\ l^{-1}$, p=0.002), but no significant differences were detected among treatments. The block effect may have been due to the rockier substrate under block two.

4.4.6 Effects of treatments on N cycling

Denitrification

In Shelf Lake, there was no detectable denitrification in the control and P sediment core incubations. Denitrification rate (standardized to 1 mg^{-1} NO_3-N in the water column) in the N, NA, and NAP sediment cores averaged 550, 480, and 423 $\mu eq\ m^{-2}\ d^{-1}$, respectively, over three incubations (Fig. 4.6A). Significant differences were detected among incubation dates (p=0.02) and with the interaction of incubation date*treatment (p=0.02). Denitrification was detectable in all sediment core incubations in The Loch, and averaged 541, 563 and 937 $\mu eq\ m^{-2}\ d^{-1}$, respectively for N, NA, and NAP treatments over two incubations (Fig. 4.6B). There were marginally significant

differences between incubation dates (p=0.09) and treatments (p=0.12). Because of these possible differences, we used individual treatment means at different incubation dates to estimate weekly denitrification in both Shelf Lake and Loch enclosures.

Estimated denitrification in Shelf Lake was 0 mg N per enclosure per week for control and P enclosures and averaged 28 mg N per enclosure per week for treatments with added N. There were no significant differences in denitrification among Shelf Lake's N treatments (Fig. 4.6C). For The Loch, we estimated 2 mg N denitrified per enclosure per week in the control and P treatments and 20, 19, and 33 mg N per enclosure per week for the N, NA, and NAP treatments, respectively (Fig. 4.6D). Treatments with added N had greater denitrification than both control and P ($p<.0001$), although denitrification for NAP was also greater than N and NA.

Nitrate uptake

Recovery of added NO_3 removed from the water column and found in the phytoplankton, on tiles, and in surface and deeper sediment in Shelf Lake and The Loch averaged 32% and 30% after two weeks, respectively. If denitrified N was included, recovery increased to about 45% and 42 % for Shelf Lake and The Loch, respectively. Algae growing on plastic assimilated some of the added NO_3. Using N:chl a ratios from tiles, we estimated the amount of N contained on the plastic after two weeks. Nitrate recovery increased to 48% and 50% after two weeks if plastic is included. Recovery decreased to 13% after four weeks in Shelf Lake, and this increased to 29% if both denitrification and plastic were included.

In Shelf Lake, recovery of added NO_3 was similar to total NO_3 uptake because ambient NO_3 levels were near detection limit. In The Loch, however, ambient NO_3 concentrations were high and total NO_3 uptake was greater than recovery of added NO_3. Thus, The Loch's control and P enclosures had higher NO_3 uptake than those in Shelf Lake (Fig. 4.7, $p<.0001$). Retention of added NO_3 (i.e. *additional* NO_3 taken up in N treatments beyond that taken up by controls) was marginally greater for Shelf Lake ($p=0.10$), but did not differ among lakes after correcting for greater experimental NO_3 loading to Shelf Lake enclosures ($p=0.63$).

The deeper sediment dominated NO_3 uptake in both lakes, and accounted for an average of 43% and 63% of total NO_3 uptake + denitrification in the N treatments after two weeks in Shelf Lake and The Loch, respectively (Fig. 4.7, Table 4.3). Denitrification and surface sediment were responsible for an average of 21% and 23% of total uptake + denitrification in Shelf Lake N treatments and 17% and 13% in The Loch. Phytoplankton accounted for an average of 11% and 6% across N treatments in Shelf Lake and The Loch, respectively. Even in Shelf Lake's control and P treatments, phytoplankton only accounted for 20% and 26% of total uptake + denitrification after two weeks and 7% and 20% after four weeks. Phytoplankton in The Loch's control and P treatments were responsible for 7% and 20% of total uptake + denitrification. Nitrate uptake on tiles was only 1-2% of total in both lakes. After four weeks in Shelf Lake, uptake by deeper sediment and denitrification were similar and accounted for an average of 34% and 38% of NO_3 uptake + denitrification in N treatments (Table 4.3C). Surface sediment, phytoplankton, and tiles took up a mean of 19%, 9%, and 1% of NO_3 uptake + denitrification, respectively.

In Shelf Lake, all the N treatments had significantly greater NO_3 uptake than the control for deeper sediment ($p=0.0020$), surface sediment ($p=0.0002$), phytoplankton ($p<.0001$), and tiles ($p=0.0001$) over time. There were no significant differences among all the N treatments for benthic uptake. For phytoplankton uptake, NAP was greater than N for week two and greater than NA for week three, in a pattern similar to that of phytoplankton chl *a* and photosynthesis (Fig. 4.1A).

In The Loch, there were no significant differences in NO_3 uptake among treatments for surface sediment and tiles after two weeks. Nitrate uptake by deeper sediment varied among treatments ($p=0.004$), however, and was greater for all treatments compared to the control. NA had the greatest uptake by deeper sediment, followed by N, which had greater uptake than both NAP and P. Nitrate uptake by phytoplankton also varied among treatments ($p=0.001$). N, NA, and NAP were greater than control and P, and NAP was also greater than N and NA.

Ammonium and DON

Over the course of the experiment in Shelf Lake, NH_4 decreased slightly in all treatments except for NAP, which gained an average of 10.5 $\mu eq\ l^{-1}$ (71 mg NH_4-N per enclosure). Ammonium concentrations differed among treatments ($p=0.02$) after the second week. NAP had higher NH_4 levels than all other treatments beginning in week two. The N and NA treatments had lower concentrations than NAP, but greater levels than the control for week three and four. All treatments gained DON over the course of the experiment except NAP, which showed a loss. DON gains were similar among the control, P, N, and NA treatments. In The Loch, NH_4 decreased by only 0.68 $\mu eq\ l^{-1}$ in all

treatments over time. In contrast, all treatments except NA gained DON, although gains were greatest for the control and P.

4.4.7 Relationships between NO_3 uptake and algal growth

The logarithm of phytoplankton photosynthetic rate was positively related to and significantly explained 87% and 69% ($p<.0001$ for both) of the variability in phytoplankton NO_3 uptake for Shelf Lake and The Loch respectively (Fig. 4.8A). Similarly, the logarithm of phytoplankton chl *a* was positively related to and explained 64% ($p=0.003$) and 69% ($p<.0001$) of the variability in phytoplankton NO_3 uptake for Shelf Lake and The Loch respectively (Fig. 4.8B). Tile chl *a*, however, explained only 39% ($p=0.06$) of the variability in tile NO_3 uptake in The Loch and an insignificant amount in Shelf Lake (Fig. 4.8C). There was no relationship between surface sediment chl *a* and NO_3 uptake for either lake (Fig. 4.8D). Chl *a* was not measured below 0.5 cm. Carbon and organic matter in deeper sediment were positively related to and explained 68% and 78% ($p=0.001$ and $p=0.02$) of the variability in NO_3 uptake in deeper sediment across both lakes combined (Fig. 4.8E and F). Deep sediment in The Loch had higher initial carbon (16.6 versus 8.7 mg cm^{-3}, $p=0.0003$) and organic matter, (41.3 versus 17.0 mg cm^{-3}, $p<.0001$) than Shelf Lake, and also had greater NO_3 uptake for N treatments (170 versus 100 mg N $enclosure^{-1}$ 2 wks^{-1}, $p=0.0479$).

4.5 Discussion

We could only recover about half of the added NO_3 removed from the water column after two weeks and even less after four weeks. Low percent recovery is a

common problem in ^{15}N tracer studies, however, and recoveries are not often reported. While not measured as a pool for transformed NO_3, DON is very low in high elevation lakes (Baron and Campbell 1997) and generally did not increase beyond changes observed in our control enclosures. We did not trace NO_3 into zooplankton either, but Axler and Reuter (1996) found both DON and zooplankton to be insignificant sinks for NO_3 in N-limited Castle Lake, California. Some of the N could have been lost with emerging macroinvertebrates, especially midges, which were abundant. Insect emergence accounted for 5% of annual N inputs from oligotrophic Mirror Lake, New Hampshire (Likens 1985). Pelagic and tile bacteria, which passed through our filters, may have taken up some of the missing NO_3. We also suspect that some of the non-recovered N was lost via downwelling. Rocks were often found at greater than 12 cm depths below the sediment and could have provided channels for NH_4 loss. This possibility is further supported by Shelf Lake's decreased recovery and increased NH_4 concentrations at week four compared to week two. Leakage is only suspected for Shelf Lake's N2 enclosure, which lost noticeably more NO_3 from the water column than the other N-addition treatments.

4.5.1 Phytoplankton

Phytoplankton were clearly the best indicators of nutrient and acid stress in the study lakes. Chl *a* and productivity increased in response to addition of deficient nutrients and community composition was altered by both nutrients and acidity (*see* Lafrançois et al. 2002). These effects are well documented in other studies (Blumenshine et al. 1997, Cattaneo 1987, Findlay et al. 1999, Hansson 1992, O'Brien et al. 1992,

Schindler et al. 1985). Our results go further and link water column eutrophication with enhanced NO_3 uptake. Phytoplankton were poorer competitors for NO_3 under N-rich conditions compared to the benthos, however, and competitive ability increased only slightly with the addition of P. Despite tight links between nutrient concentrations, water column productivity, and NO_3 uptake, phytoplankton were not a dominant sink for NO_3 and had limited influence on ecosystem NO_3 uptake. Axler and Reuter (1996) similarly found that only a small portion of NO_3-N was recovered in phytoplankton.

4.5.2 Benthos

The biomass of algae growing on sediment rarely increases following nutrient enrichment (Blumenshine et al. 1997, Vinebrook and Leavitt 1998, 1999) and actually may decrease due to indirect effects such as reduced light and DIC availability (Hansson 1992; Turner et al. 1991, 1994; Vadeboncoeur et al. 2001). Nutrients enhance the biomass and alter the community structure of algae growing on tiles and plastic (Blumenshine et al. 1997, Vinebrook et al. 1998). Epilithic community composition was sensitive to recovery from acidification (Vinebrooke and Graham 1997). In this study, there were no effects of nutrients and acidity on the biomass and community composition of benthic algae growing on tiles and sediment (*see* Lafrançois et al. 2002 for epilithic community results). Having only two replicates certainly limited our statistical power, but earlier experiments where three replicates were used still yielded only one instance of significant response to nutrients (Nydick et al. 2002a). Overall, benthic algae proved to be a poor indicator of nutrient and acid stress in the study lakes, and this was probably

due to the spatial heterogeneity characteristic of benthic environments combined with indirect feedbacks such as shading and DIC limitation.

Not only did benthic algal biomass fail to respond significantly to nutrient addition in the study lakes, but benthic NO_3 uptake for both tiles and sediment was either weakly coupled or not coupled at all to algal biomass. The benthos dominated NO_3 uptake, however, and in most cases more NO_3 was recovered in subsurface sediment than in surface sediment. Nitrate uptake in this non-photosynthetic subsurface layer was related to carbon and organic matter content. In soils, organic C regulates microbial immobilization of N (Barrett and Burke 2000, Delgado et al. 1996, Nadelhoffer et al. 1995). In aquatic ecosystems, organic C is an important regulator of bacterial abundance (Schallenburg and Kalff 1993). This suggests that microbial activity, and not algal assimilation, was responsible for most of NO_3 uptake in the benthos of our study lakes. This is a significant finding because direct uptake of NO_3 into the sediments is not often considered in studies of N retention (Jansson et al. 1994, Saunders and Kalff 2000) or in descriptions of the aquatic N cycle (Wetzel 1983, Horne and Goldman 1994).

Since organic carbon can stabilize inorganic N in several ways and microbial activity was not directly measured, we cannot determine the specific mechanisms for sediment NO_3 uptake in our enclosures. Studies in terrestrial environments have demonstrated that if soil organic matter C:N ratios are wide, microbial immoblization often dominates; but if NO_3 availability is high, dissimilatory nitrate reduction to ammonium (DNRA) becomes more substantial (Barrett and Burke 2000, Silver et al. 2001, Vitousek and Matson 1988). DNRA was also shown to be a key NO_3 pathway in estuarine sediments, and more NO_3 was reduced to NH_4 than was denitrified (An and

Gardner 2002). In our study, only about 20% of benthic NO_3 uptake + denitrification in the N treatments was actually denitrified within two weeks, although this doubled after four weeks. We suspect that a portion of the NO_3 in our enclosures was transformed to NH_4 via DNRA, and that some of this pore water NH_4 was later lost via coupled nitrification-denitrification. Accumulation of NH_4 in porewater may be enhanced under acidic conditions since nitrification is blocked at low pHs (Rudd et al. 1988), although this was not reflected in water column NH_4 concentrations in our study. Clearly, NO_3 uptake pathways in aquatic sediment require further study to determine the relative importance of immobilization versus DNRA and denitrification.

4.5.3 Whole Ecosystem

We hypothesized that ecosystem NO_3 uptake would be limited by high ambient NO_3 concentrations in The Loch compared to Shelf Lake because algal N requirements would be exceeded. In contrast to our predictions, The Loch took up and denitrified more NO_3 than Shelf Lake. This uptake consisted of existing and added NO_3. Ecosystem uptake and denitrification of *added* NO_3 was similar between The Loch and Shelf Lake. The Loch had higher sediment C and organic matter content, however, which likely was indicative of greater microbial activity. Since adding P did not increase ecosystem NO_3 uptake, it is unlikely that these benthic microbial pathways were P deficient, and high NO_3 levels may not necessarily indicate N saturation as predicted for algae. Other factors such as organic carbon substrate and low temperatures may limit uptake and denitrification rates. Acidity had little effect on ecosystem NO_3 uptake and denitrification.

Alkalinity generation generally paralleled ecosystem NO_3 uptake + denitrification, but N treatments unexpectedly gained less alkalinity than the combined nutrient and acid treatments. This partially could be explained in Shelf Lake by slightly greater sediment Ca exchange in the acid treatments, which was also observed in an experimentally acidified lake (Schindler et al. 1986). In addition, for each lake there was one N enclosure that gained less alkalinity than expected from ion balances and comparison with alkalinity gain in other treatments receiving excess N. Shelf's N outlier also had unusually large NO_3 losses, weak algal responses, and low NO_3 recovery compared to the other replicate; we infer leakage as the cause of these atypical measurements. The N enclosure with low alkalinity generation in The Loch did not have unusual algal responses or low NO_3 recovery, however, and leakage is not suspected. Consumption of chloride was a significant contributor to acid neutralization in an enclosure experiment conducted in a Canadian Shield Lake (Schiff and Andersen 1987), and could account for up to 10% of the alkalinity gain in our acid treatments.

Alkalinity generation, like ecosystem NO_3 uptake, was not enhanced in our study lakes by adding PO_4, in addition to NO_3 and acid, despite greater phytoplankton biomass and productivity. In contrast, Davidson et al. (1995) and Findlay et al. (1999) found that both alkalinity and phytoplankton biomass increased in response to whole-lake PO_4 fetilization in acidified, high NO_3 lakes. These oligotrophic lakes were deep, however, and phytoplankton would be expected to play a more integral role in alkalinity generation than in our shallow study lakes (Lodge et al. 1998, Vadeboncoeur et al. 2002).

4.6 Conclusion

During this and previous enclosure experiments, phytoplankton in shallow oligotrophic study lakes readily responded to limiting nutrients with increased chl *a* and photosynthetic rate and phytoplankton also reacted to acid and nutrient amendments by distinct changes in community composition (Nydick et al. 2002a, Lafrançois et al. 2002). These responses have low variability among replicates, and statistical comparisons usually yield significant results. Benthic algae are more spatially heterogeneous and responses are much harder to tease out from background variability. Sediment nutrient supply and indirect effects of added nutrients (i.e. shading and DIC limitation) can dampen benthic response. Phytoplankton chl *a* and productivity can be useful indicators of increased nutrient enrichment, while phytoplankton community composition can indicate acid stress to mountain lakes.

Despite the easily observable changes in the phytoplankton, the processes regulating ecosystem NO_3 retention and alkalinity generation mostly occur in the benthos of shallow lakes. Our results further suggest that microbial processes dominate benthic NO_3 uptake, and that like soils, sediment C content is related to the amount of NO_3 uptake. Microbial processes have long been considered important in benthic nutrient cycling, but are not often linked to direct NO_3 uptake from the water column. This study suggests that benthic microbial activity regulates ecosystem NO_3 uptake and biological alkalinity generation in shallow mountain lakes.

4.7 Acknowledgements

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Table 4.1. Mean lake and control measurements in Shelf Lake and The Loch taken weekly or every two weeks during enclosure experiments from July 17 – Aug 16 (Shelf Lake) and July 19-31 (The Loch), 2001. Lake enclosures were not replicated and consisted of a frame only (i.e. no polyethylene plastic). Control measurements were means of two replicates per lake.

VARIABLE	UNIT	SHELF LAKE		THE LOCH	
		LAKE	CONTROL	LAKE	CONTROL
Temperature	°C	14.7	14.8	11.5	11.7
pH		7.0	7.2	6.7	6.8
Conductivity	$\mu S\ cm^{-1}$	13.1	14.9	10.5	12.2
Alkalinity	$\mu eq\ l^{-1}$	93.3	107.3	38.4	52.9
SiO_2	$mg\ l^{-1}$	2.72	2.07	1.02	0.81
DOC	$mg\ l^{-1}$	2.0	2.7	0.8	1.4
NO_3	$\mu eq\ l^{-1}$	0.17	0.19	15.59	5.20
NH_4	$\mu eq\ l^{-1}$	0.36	0.33	0.60	0.39
PO_4	$\mu eq\ l^{-1}$	0.45	0.34	0.16	0.21
TP	$\mu g\ l^{-1}$	22	26	9	28
DIN:TP	by mass	0.32	0.31	25.1	2.82
Phytoplankton Chl *a*	$mg\ m^{-3}$	1.79	2.99	1.74	4.07
Tile Chl *a*	$mg\ m^{-2}$	4.37	2.92	5.70	4.44
Surface Sediment Chl *a* (0-0.5 cm)	$mg\ m^{-2}$	204.5	107.1	121.8	137.1
Plastic Chl *a* (after four weeks)	$mg\ m^{-2}$	--	0.42	--	1.94
Phytoplankton photosynthetic rate	$mg\ C\ m^{-3}\ h^{-1}$	8.34	24.7	5.23	18.8
Tile photosynthetic rate	$mg\ C\ m^{-2}\ h^{-1}$	28.5	19.0	10.8	6.70
Zooplankton biomass (after four weeks)	$mg\ m^{-3}$	88.5	9.2	0.44	--

Table 4.2. Effect of treatments in A) Shelf Lake and B) The Loch. p-values are shown for the treatment (trt) and time main effects and for the interaction (trt*time). Significant differences from control and among treatments (determined from least squared means, α=0.05) are shown for each week. Arrow represent direction of change and "<" or ">" indicates if a significant treatment effect is less than or greater than another treatment effect. N = NO_3, NA = NO_3 + acid, P = PO_4, and NAP = NO_3 + acid + PO_4. Ø = no significant differences, -- no data, phyto = phytoplankton, part = particulate.

A) Shelf Lake

	Trt effect	Time effect	Trt*time	Week 0	Week 1	Week 2	Week 3	Week 4
Alkalinity Gain	0.0118	0.0234 ↑	0.6141	--	N, NA, NAP ↑	NA, NAP ↑ P ↓	NA, NAP ↑	NA, NAP ↑
Phyto Chl *a*	0.0013	<.0001 ↑	0.1320	--	Ø	NA, NAP ↑	N, NA, NAP ↑	NA, NAP ↑
Tile Chl *a*	0.2196	0.0047 ↑	0.3466	--	Ø	Ø	Ø	Ø
Surface Sediment Chl *a*	0.3527	0.0359 ↑	0.4259	Ø	--	Ø	--	Ø
Plastic Chl *a*	0.5365	--	--	--	--	--	--	Ø

B) The Loch

	Trt effect	Time effect	Trt*time	Week 0	Week 1	Week 2
Alkalinity Gain	0.0118	0.0234 ↑	0.6141	--	NA, NAP ↑	NA, NAP ↑
Phyto Chl *a*	0.1702	<.0001 ↑	0.0051	Ø	Ø	NAP ↑
Tile Chl *a*	0.1422	0.1464	0.4911	Ø	Ø	Ø
Surface Sediment Chl *a*	0.3111	0.0840 ↑	0.7072	Ø	--	Ø
Plastic Chl *a*	0.7681	--	--	--	--	Ø at week 4

Table 4.3. Percent of total NO_3 uptake and denitrification for algal and sediment compartments in enclosures after A) two weeks in The Loch, B) two weeks in Shelf Lake, and C) four weeks in Shelf Lakes. The last column is total mg N taken up and denitrified per enclosure within the time period. N = NO_3, NA = NO_3 + acid, and NAP = NO_3 + acid + PO_4.

A) The Loch – Two Weeks

	Phyto-plankton	Tile	Surface Sediment	Deeper Sediment	Denitrif-cation	Total (mg N)
Control	5	1	16	75	3	125
N	4	2	15	64	14	274
NA	1	1	10	75	13	303
NAP	11	2	14	48	24	279
P	5	1	10	82	1	162

B) Shelf Lake – Two Weeks

	Phyto-plankton	Tile	Surface Sediment	Deeper Sediment	Denitrif-cation	Total (mg N)
Control	20	0	7	73	0	2
N	8	2	21	49	21	226
NA	12	1	34	22	30	185
NAP	13	1	14	59	13	254
P	26	2	25	47	0	1

C) Shelf Lake – Four Weeks

	Phyto-plankton	Tile	Surface Sediment	Deeper Sediment	Denitrif-cation	Total (mg N)
Control	7	1	12	79	0	2
N	7	1	28	35	29	349
NA	7	0	16	33	44	293
NAP	13	1	12	35	39	271
P	20	2	44	34	0	1

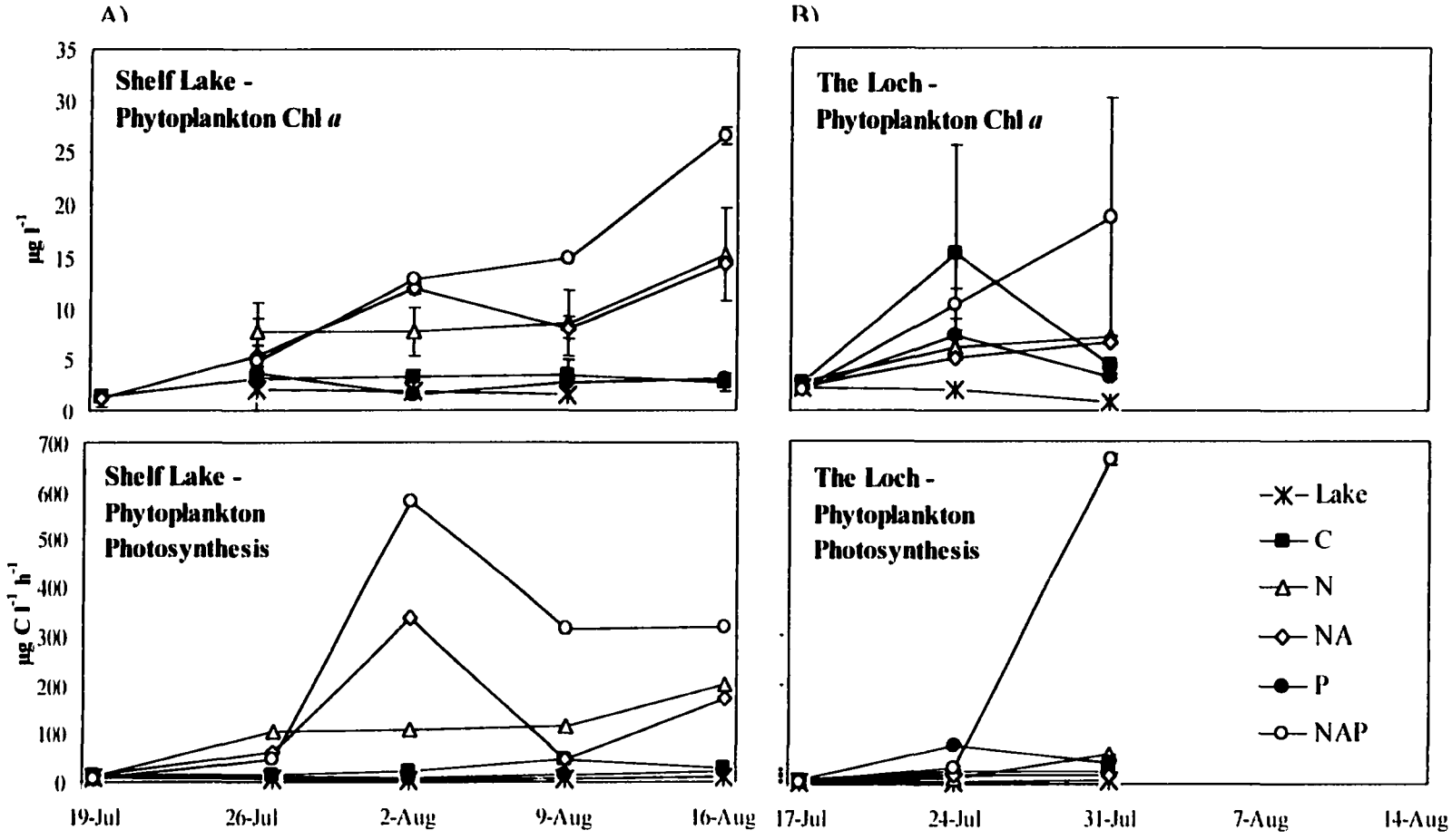

Figure 4.1. Phytoplankton chl *a* and photosynthetic rate for enclosure experiments conducted in A) Shelf Lake and B) The Loch during summer 2001. Error bars represent ± 1 standard error. C = control, N = NO_3, NA = NO_3 + acid, P = PO_4, and NAP = NO_3 + acid + PO_4.

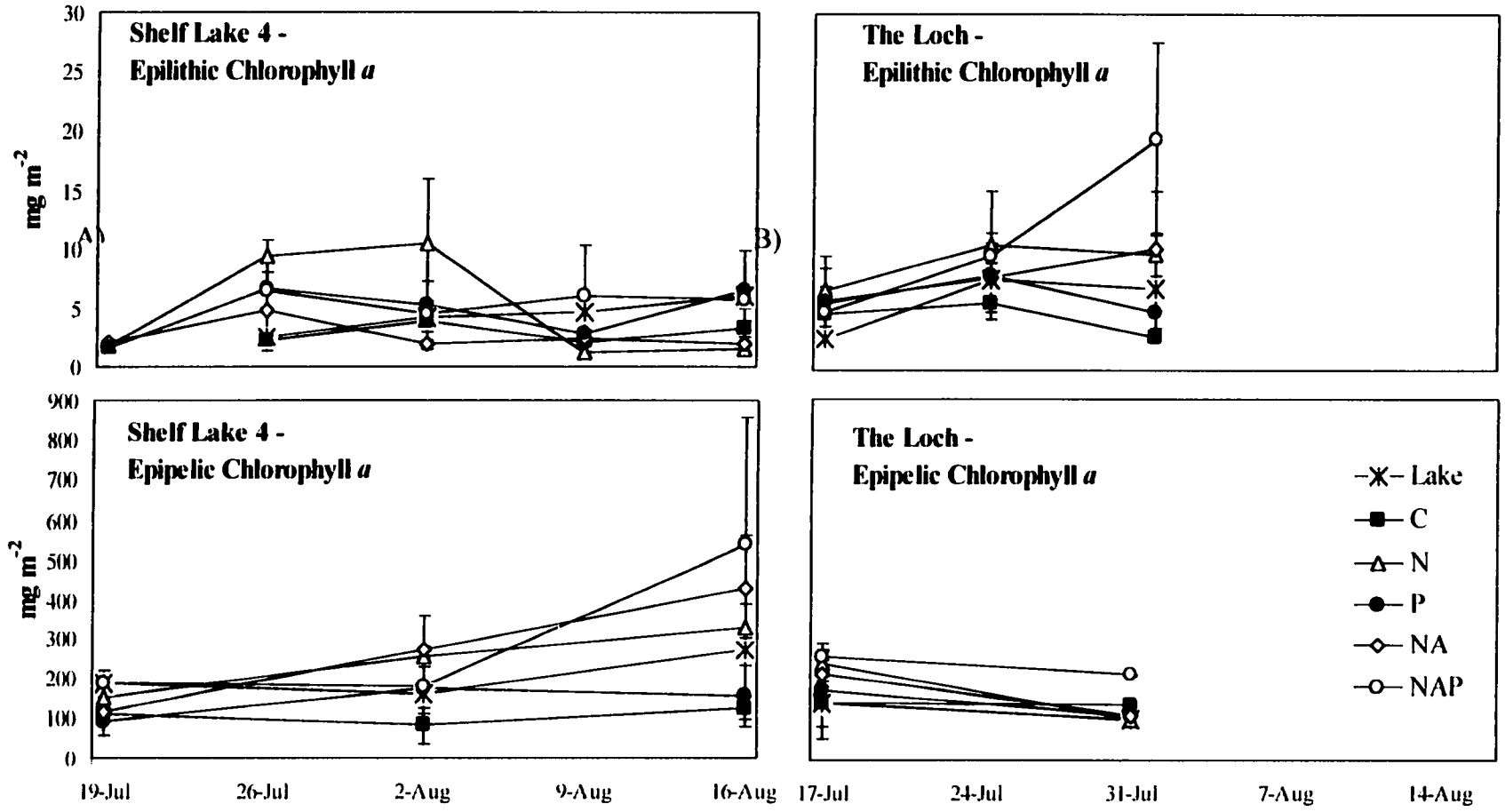

Figure 4.2. Tile and surface sediment (top 0.5 cm) chl *a* for enclosure experiments conducted in A) Shelf Lake and B) The Loch. Error bars represent ± 1 standard error. C = control, N = NO_3, NA = NO_3 + acid, P = PO_4, and NAP = NO_3 + acid + PO_4.

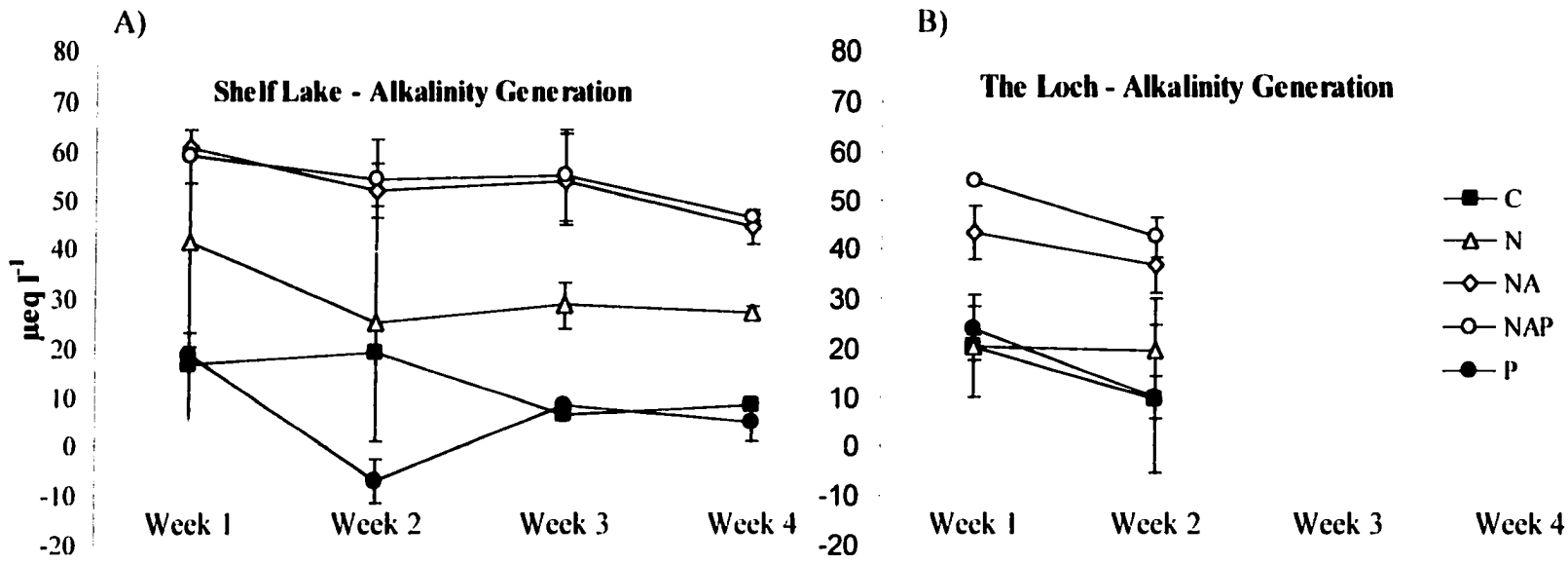

Figure 3. Weekly alkalinity generation for enclosure experiments conducted in A) Shelf Lake and B) The Loch. Error bars represent ± 1 standard error. C = control, N = NO_3, NA = NO_3 + acid, P = PO_4, and NAP = NO_3 + acid + PO_4.

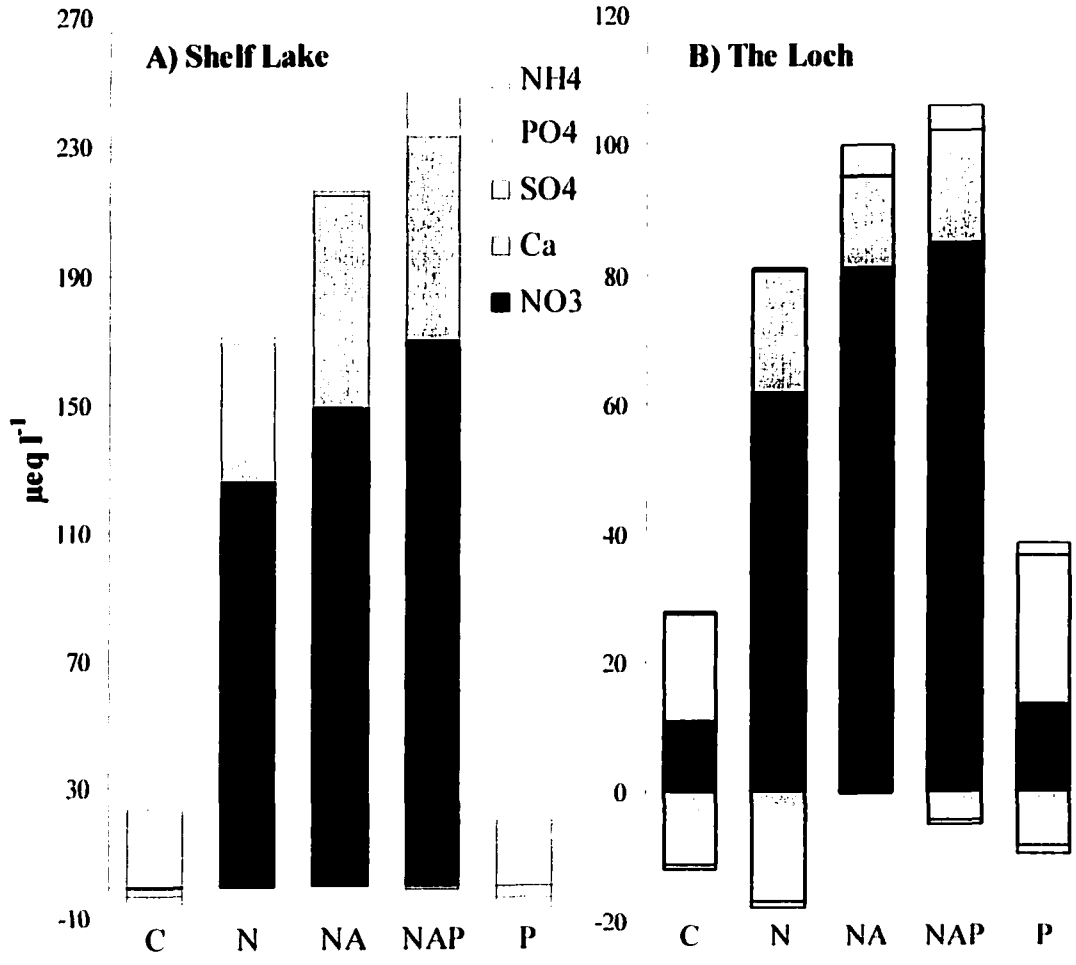

Figure 4.4. Predicted alkalinity generation based on ion balances for enclosure experiments conducted in A) Shelf Lake and B) The Loch.

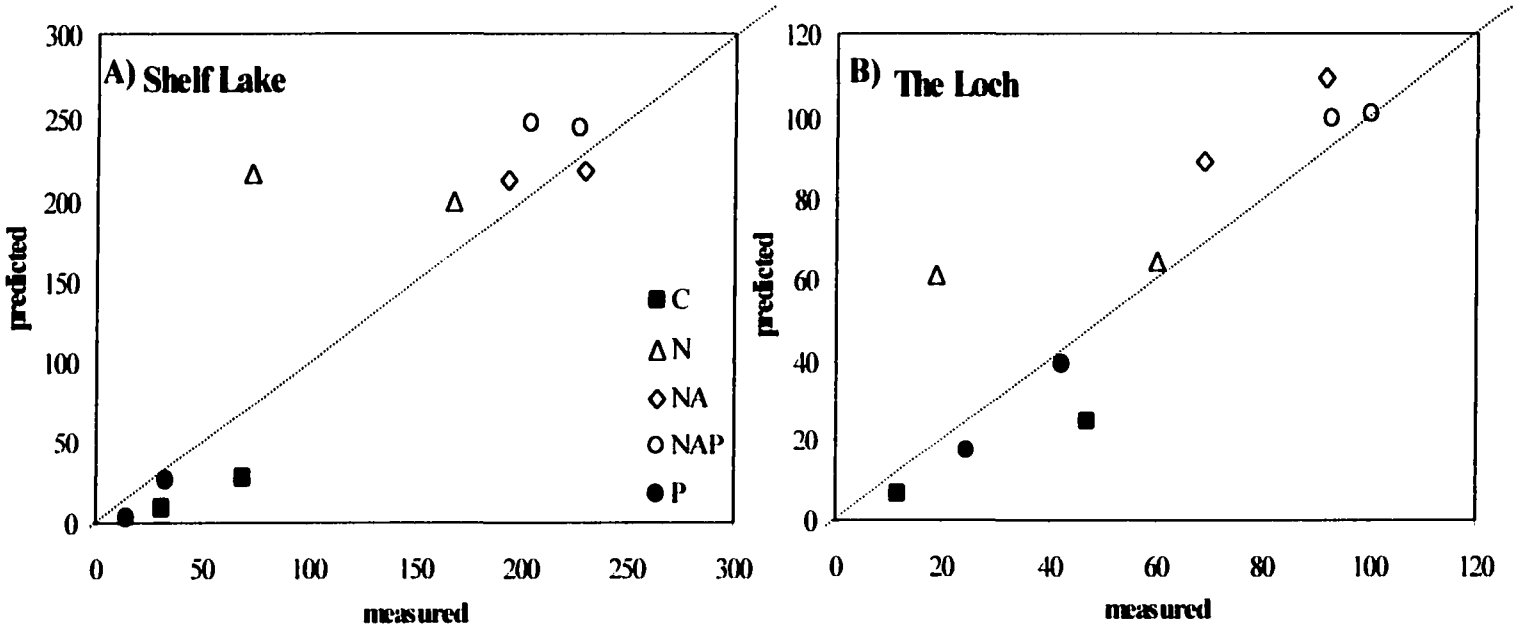

Figure 4.5. Measured versus predicted alkalinity generation for enclosure experiments conducted in A) The Loch (two weeks) and B) Shelf Lake (four weeks). Alkalinity generation was predicted from NO_3 loss + Ca gain + SO_4 loss + PO_4 loss + NH_4 gain, and is expressed as $\mu eq\ l^{-1}$. Dashed line is 1:1.

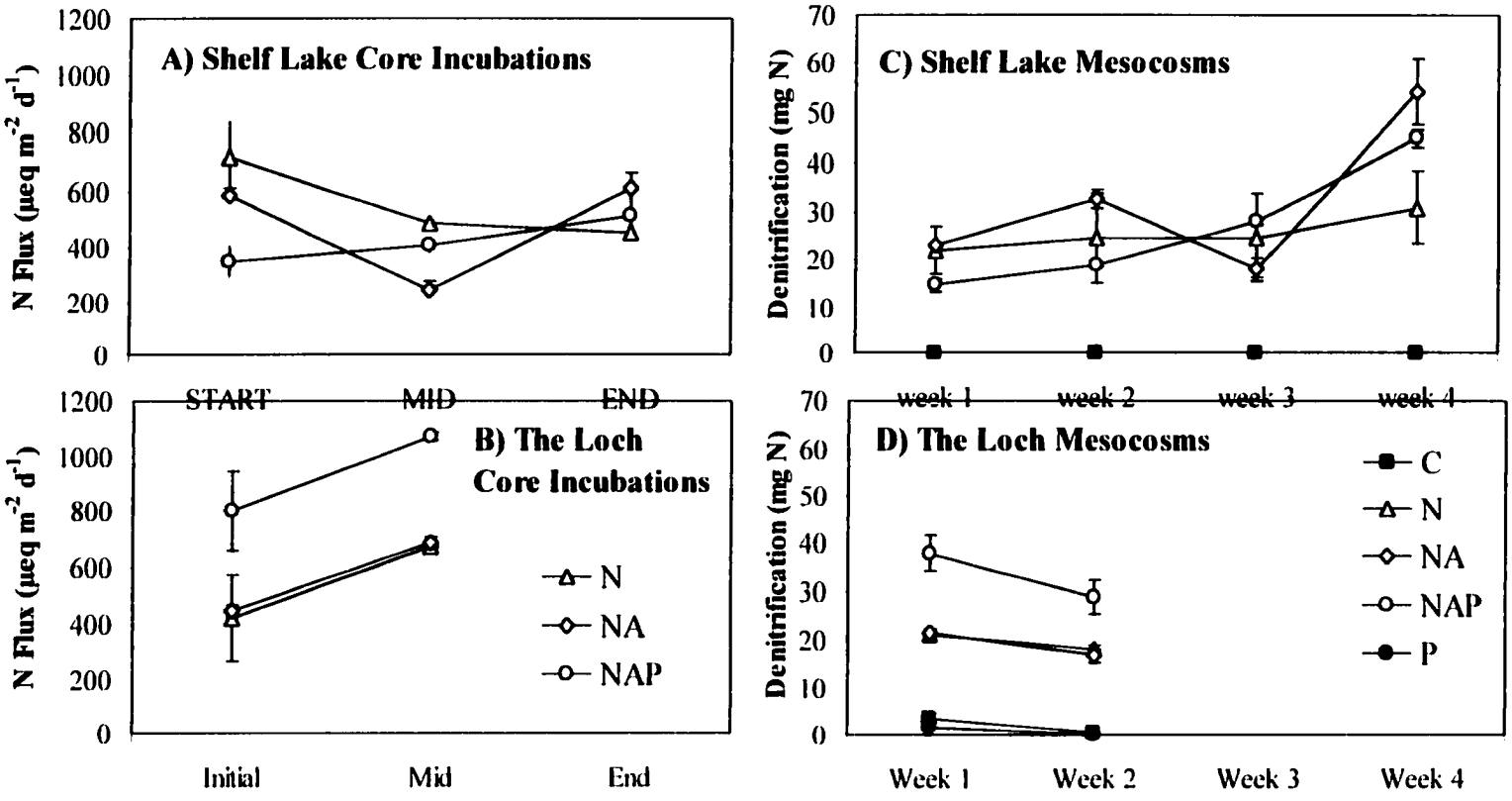

Figure 6.6. Denitrification in A) Shelf Lake core incubations, B) Loch core incubations, C) Shelf Lake enclosures, and D) Loch enclosures. Denitrification rates in core incubations are standardized to 1 mg l^{-1} NO_3-N in the water column. Denitrification for enclosures is mg N denitrified per enclosure per week based on core incubations. Error bars represent ± 1 standard error. C = control, N = NO_3, NA = NO_3 + acid, P = PO_4, and NAP = NO_3 + acid + PO_4.

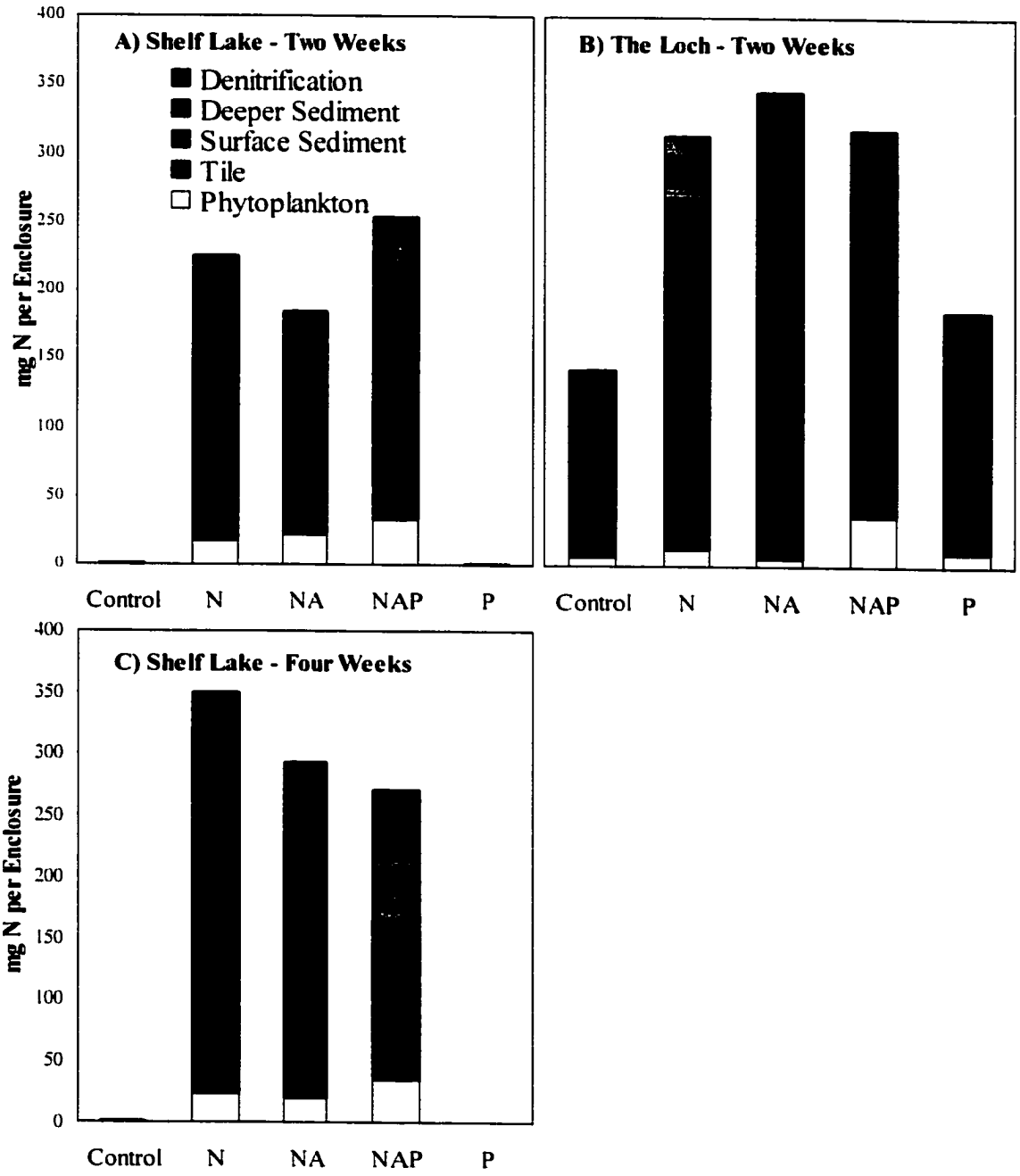

Figure 4.7. Nitrate uptake by denitrification, deep sediment, surface sediment, tiles, and phytoplankton after two weeks for enclosure experiments conducted in A) Shelf Lake, B) The Loch, and after four weeks in C) Shelf Lake. C = control, N = NO_3, NA = NO_3 + acid, P = PO_4, and NAP = NO_3 + acid + PO_4.

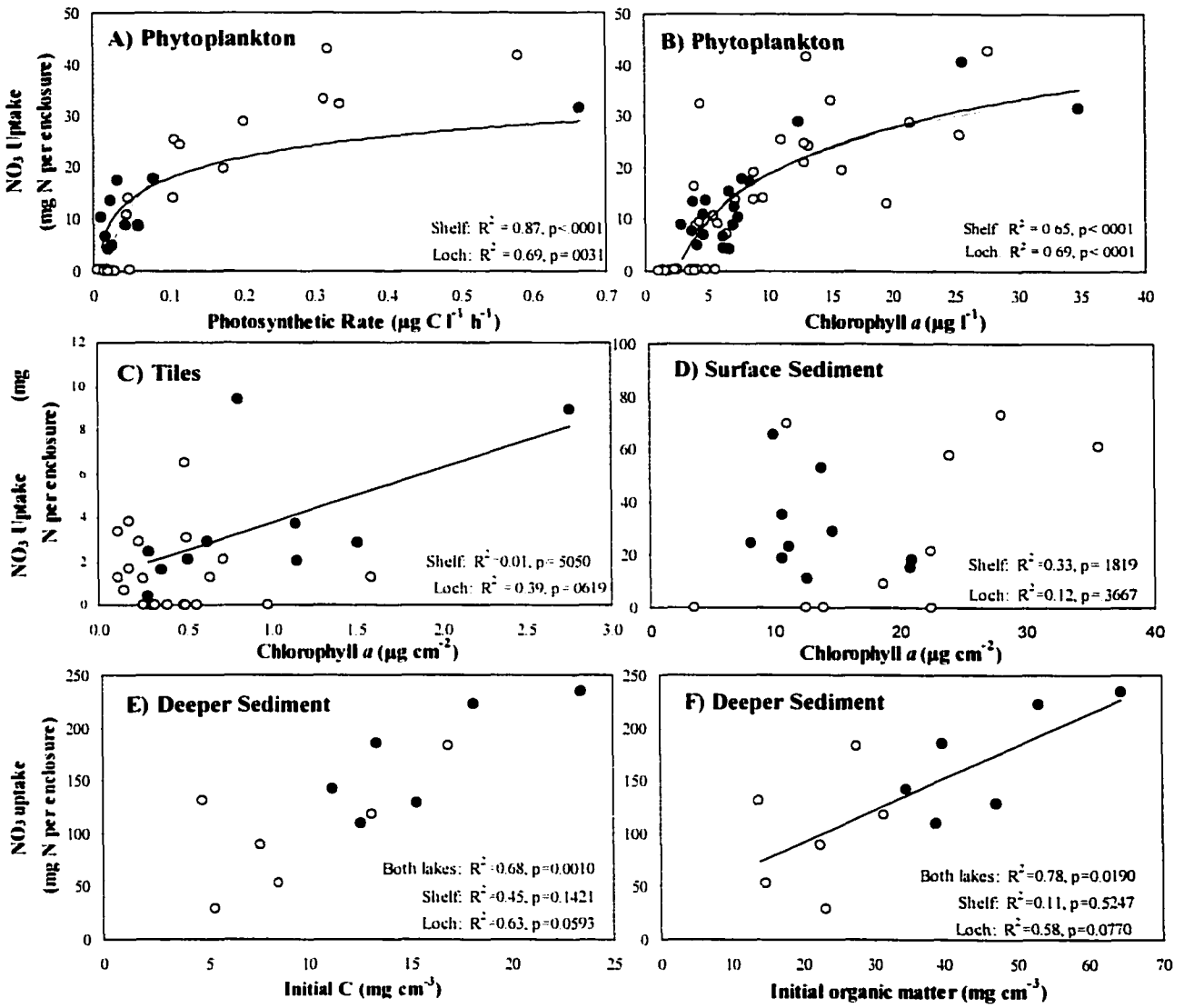

Figure 4.8. Variability in NO_3 uptake explained by A) phytoplankton chl *a*, B) phytoplankton photosynthetic rate. C) tile chl *a*. D) surface sediment chl *a*, E) deeper sediment carbon, and F) deeper sediment organic matter. Open circles represent Shelf Lake data points, while closed circles show data points for The Loch.

5. Assessing the vulnerability of western mountain lakes (USA) to eutrophication and acidification from rising atmospheric nitrogen deposition

5.1 Abstract

Long-term lake monitoring and paleolimnolgical studies of lake sediment cores provide evidence than even low levels of atmospheric nitrogen (N) deposition can alter sensitive high elevation lake chemistry and induce eutrophication. These data are convincing, but the studies are limited in geographic scope. We used values from the Western Lake Survey, an extensive and statistically robust dataset of lake physical and chemical measurements, to evaluate potential effects of N deposition on western mountain lake ecosystems. A quarter of western mountain lakes were primarily N limited and will likely show some degree of eutrophication should N inputs increase. Nitrogen limitation was especially common in the Pacific Northwest. Twenty-one percent of the lakes were acid sensitive due to alkalinity of 50 $\mu eq\ l^{-1}$ or less, and 12% were even more acid sensitive based on both measured alkalinity and potential biological alkalinity generation. The Sierra Nevada had the highest proportion of acid sensitive lakes. Nitrogen deposition was significantly, although somewhat weakly, positively related to lake NO_3 levels in the Rocky Mountain regions. The Southern Rockies Region, which includes the Colorado Front Range, had the most lakes with elevated NO_3 levels ($>2\mu eq\ l^{-1}$), while the Pacific Northwest had the least. We conclude that N deposition has already raised NO_3 levels and caused eutrophication in many lakes of the Southern Rockies. High elevation

lakes in the Pacific Northwest still have low NO_3 levels and show a high incidence of N limitation, making them vulnerable to atmospheric driven eutrophication.

5.2 Introduction

The likelihood of eutrophication or acidification of surface waters resulting from air pollution depends on the magnitude of atmospheric deposition, the uptake capacity of the watershed, and the sensitivity of the water body. In the western United States, atmospheric deposition is lower than levels encountered in the East, but water resources are also more vulnerable. Few eastern lakes can be characterized as alpine or even subalpine, while about 16,000 high-elevation lakes exist in the western states (Bahls 1992). These mountain lakes are especially sensitive to the effects of atmospheric deposition; high-elevation watersheds have limited ability to immobilize nutrients and buffer acidity due to steep slopes, sparse vegetation, shallow soils, a short growing season, snowmelt dominated hydrology, and a high occurrence of slow-weathering crystalline bedrock (Campbell et al. 2000, Omernik and Griffith 1986, Williams et al. 1996). For example, the proportion of bare rock and talus in mountain watersheds is known to be an important control on surface water NO_3 levels (Clow and Sueker 2000, Kopacek et al. 2000). Furthermore, mountain lakes are generally oligotrophic, so even the slight introduction of a limiting nutrient can lead to marked change. Many western mountain regions, including the Colorado Front Range, the Sierra Nevada, the Uintah Mountains, and portions of the Cascade Range, are located in close proximity to urban and agricultural emission sources.

Sulfate concentrations in deposition have decreased following Title IV of the Clean Air Act Amendments, but nitrogen (N) levels have been rising in many parts of the western U.S. (Lynch et al. 1996, Nilles and Conley 2001). Because of these trends, N is often the air pollutant of concern in the mountain west (Peterson et al. 1998). Nitrogen's importance as a limiting nutrient in aquatic systems has often been overlooked despite evidence that N limitation of algal growth is somewhat common (Elser et al. 1990, Guilford and Hecky 2000, Lafrançois et al. In Prep. Morris and Lewis 1988) and has been documented by experimental enrichments in many western regions including Yellowstone National Park, WY (Interlandi and Kilham 1998); the Sawtooth Mountains, ID (Wurtbaugh et al. 1997); the Sierra Nevada Mountains, CA (Axler et al. 1982, Goldman et al. 1989); boreal forests of northern Minnesota (Axler et al. 1994), Wyoming's Snowy Range (Nydick et al. 2002a), and montane forests on the western slope of the Colorado Front Range (Morris and Lewis 1988). Effects of even moderately elevated N deposition have already been observed in the Colorado Front Range (Baron et al. 2000) and for Lake Tahoe in the Sierra Nevada, California-Nevada (Goldman 1988, Jassby et al. 1994). These effects include increased algal biomass and primary productivity, loss of water clarity, elevated NO_3 concentrations, and algal species shifts (Baron et al. 2000, Golman 1988, Wolfe et al. 2001).

Acid levels in deposition have also been increasing in certain western locales, including the Colorado Front Range (Baron et al. 2000, Lynch et al. 1996). Previous assessments of western lake chemistry have focused on geochemical alkalinity as an indicator of acidification potential (Eilers et al. 1989, Landers et al. 1987), but have not considered in-lake biological alkalinity generation due to reduction of NO_3. Studies of

temperate lakes in eastern North America and southern Norway have shown biological processes to be important sources of alkalinity in dilute lakes (Kelly et al. 1982, Rudd et al. 1986, Schindler et al. 1986). Enhanced uptake of NO_3 by phytoplankton has led to measurable alkalinity gains in deep lakes (Davidson et al. 1995, Findlay et al. 1999). In shallow lakes, however, the majority of NO_3 transformation and storage occurs in the benthos (Axler and Reuter 1996, Nydick et al. 2002b). Nitrate removal efficiencies based on measured denitrification rates ranged from 7-99% of NO_3 loading in temperate, oligotrophic lakes (Kelly et al. 1987).

Given rising N levels in western deposition and increases in acidity in some locales, we evaluated the sensitivity of western mountain lakes to increased NO_3 and acid inputs. In this paper, our objectives are to assess: 1) the sensitivity of western mountain lakes to N-induced eutrophication and 2) the efficiency of biological NO_3 transformations in buffering acid inputs to western mountain lakes. In doing so, we locate regions with high eutrophication and acidification potential and investigate spatial patterns in lake NO_3 concentrations.

5.3 Methods

5.3.1 Dataset

The Western Lake Survey (WLS; U.S. EPA NHEERL Western Ecology Division 1988) was designed to provide a statistically robust and geographically extensive database of lake physical and chemical properties representing areas of the western United States expected to contain lakes with alkalinities less than 400 $\mu eq\ l^{-1}$ (Landers et al. 1987). The estimated target population size was 10,393 lakes. Although this survey is

dated and changes in lake chemistry may have occurred since sampling was conducted in autumn 1985, no other database exists that is nearly as extensive or robust. The regions surveyed included California, the Pacific Northwest, the Northern Rockies, the Central Rockies, and the Southern Rockies. We eliminated from the dataset lakes with disturbances such as roads, dams, livestock, mining, wells, and fire, and lakes located below 1,000 m in order to reduce confounding influences on lake chemistry. Our subset contained 597 of the original 751 WLS lakes.

5.3.2 Sensitivity to eutrophication

In order to assess sensitivity to eutrophication from increased N loading, we estimated the nutrient limitation status of each WLS subset lake by using the dissolved inorganic nitrogen:total phosphorus (DIN:TP) ratio. This ratio had 84% and 89% accuracy predicting nutrients limiting to phytoplankton growth as determined with bioassays conducted in Northern Colorado montane and Sierra Nevada alpine lakes, respectively (Morris and Lewis 1988, Sickman 2001). The DIN:TP index best represents N and P available to the phytoplankton from both internal and external sources (Morris and Lewis 1988). Other indices such as TN:TP rely solely on seston composition ratios that may include detritus as well as live cells, or ratios of external nutrient supply (i.e. DIN:SRP) which do not include internal P stores.

5.3.3 Resistance to acidification

Predicting the efficiency of NO_3 transformation, and, hence, biological alkalinity generation, is difficult because many processes such as denitrification, immobilization in

the sediment, and algal assimilation are involved and the importance of each process varies depending on lake characteristics. Factors that regulate water contact time with the sediment such as lake depth and hydraulic residence time influence benthic NO_3 uptake and denitrification (Kelly et al. 1987, Saunders and Kalff 2001, Windolf et al. 1996). Phytoplankton NO_3 uptake, in contrast, is dependant on nutrient limitation, but will only be significant to whole lake alkalinity generation in deep lakes (Nydick et al. 2002b).

We used a mass balance model by Kelly et al. (1987) to estimate NO_3 removal due to denitrification in WLS subset lakes. The model accurately predicted NO_3 removal coefficients for five temperate lakes located in eastern North America and southern Norway. These lakes had oxic hypolimnia, low primary productivity, and low alkalinity. Modeled removal efficiencies were calculated with mass transfer coefficients (S_N) obtained from chemical budgets and measured rates of denitrification. We used the mean S_N of 8.3±0.9 in our estimates of WSL subset NO_3 removal. The model also included hydraulic residence time and mean depth. The WLS dataset only provided estimates of hydraulic residence time for drainage lakes, so we assumed a residence time of 0.7 y^{-1} for seepage lakes based on the average for drainage lakes.

Lakes most sensitive to acidification from N deposition are those with both low geochemical alkalinity and low NO_3 removal efficiency (i.e. low potential biological alkalinity generation). We categorized lakes with alkalinity $\leq$50 $\mu eq\ l^{-1}$ as "acid sensitive" and those with both alkalinity $\leq$50 $\mu eq\ l^{-1}$ and a NO_3 removal $\leq$50% as "very acid-sensitive".

5.3.4 Relationship between lake NO_3 and N deposition

We hypothesized that N deposition affects lake NO_3 concentrations and developed regression models to explore sources of variability in lake NO_3 levels. Because many factors other than N deposition influence lake NO_3 concentrations, we included available watershed and lake characteristics as potential terms in our models. Lake mean depth, surface area, volume, total phosphorus (TP) concentration, Secchi depth, and hydraulic residence time (available for drainage lakes only), as well as watershed area and precipitation were obtained from the WLS dataset. Vertical distance (i.e. elevation) from timberline was calculated as an index of vegetation cover, since catchment vegetation was not included in the WLS. Lakes were separated into neighborhood groups of no more than 30 lakes each. Timberline elevation for each group was estimated using digital elevation models (USGS DEMs; 1:250,000) overlaid with vegetation cover maps (USGS Gap Analysis Program; Western Region, Montana, Wyoming, Colorado, and New Mexico) in ArcView 3.2.

Nitrogen deposition at each lake was estimated in ArcView 3.2 by extrapolating 1985 data from 54 NADP/NTN stations and extracting point values at each lake (NADP 2002). Although this provides data for each lake, the scarcity of NADP sites at high elevations introduces error based on orographic precipitation patterns and small-scale variability. Finer scale deposition estimates were obtained for lakes in the Rocky Mountains by extracting point values based on maps created from average 1992-1999 NADP concentrations combined with snow survey concentrations and modeled estimates of precipitation (Nanus et al. In Review). These maps had a 1-kilometer resolution, and provide more robust estimates of actual N deposition at individual lakes, but coverage

was available for only two-fifths of the study lakes (referred to as "Rocky Mountain subset lakes") and values may reflect changes that occurred after the WLS was completed.

The best models of lake NO_3 concentrations were selected using Mallows' C_p statistic in SAS 8.2. This statistic considers all possible models, selects the best based on likelihoods, and gives a penalty for additional terms. Response surface analysis was first performed to select interactions and squared terms to add to the set of possible explanatory variables. Only variables with p-values less than 0.05 were included in the best models. Nitrate concentrations were log-transformed to meet the assumptions of normality and homogeneity of variance. A best model was selected for each of four datasets: A) All WLS subset lakes (n=597), using 1985 N deposition from NADP data as a potential explanatory variable, B) WLS subset drainage lakes only (n=476), but including residence time and mean areal outflow (i.e. mean depth/residence time) as potential explanatory variables, and using 1985 N deposition from NADP data, C) Rocky Mountain subset lakes only (n=291), using 1992-1999 N deposition from integrated snow sampling and NADP data (Nanua at al. In Review), and D) Rocky Mountain subset drainage lakes only (n=225), but including residence time and mean areal outflow, and using 1992-1999 N deposition.

5.4 Results

5.4.1 Lake Chemistry

The WLS subset of lakes had a mean NO_3 concentration (± 1 standard error) of 1.68 ± 0.14 μeq l^{-1}, with values ranging from 0.00 to 32.14 μeq l^{-1}. Almost 80% of the

lakes had NO_3 levels ≤ 2 μeq l^{-1} (Figure 5.1). The Pacific Northwest had the greatest percentage of low NO_3 lakes and also had the lowest mean (0.36 μeq l^{-1}). There were fewer low NO_3 lakes in the Southern and Central Rockies where the mean was 2.91 and 2.68 μeq l^{-1}, respectively. Lakes with high NO_3 levels were mostly located in the Front Range (Colorado), Uintah Mountains (Utah), and Beartooth and Absaroka Ranges (Montana/Wyoming; Figure 5.2).

Ratios of DIN:TP for WLS subset lakes suggest that 24% of non-disturbed western mountain lakes were primarily N limited and therefore susceptible to eutrophication from increasing N inputs. Extrapolated to the population of non-disturbed western mountain lakes, this probably represents over two thousand lakes that could experience some degree of N-induced eutrophication. Nitrogen limited lakes were found in all regions and comprised 17-19% of each region except for the Pacific Northwest, which was 34% N limited (Table 5.1, Figure 5.3). Thirty-nine percent of lakes were estimated to be primarily P limited. Phosphorus limitation was most common in California and the Southern Rockies. The remaining lakes (37%) were estimated to be co-limited by both N and P.

Using the mass-balance model developed by Kelly et al. (1987), we calculated that 62% of the lakes could remove 50% of NO_3 inputs and would be expected to neutralize only half of acid inputs from N deposition (Figure 5.4). Eighteen percent would remove less than 20% of NO_3 inputs, while only 6% would remove more than 80% of NO_3 loading. Additional NO_3 could be immobilized in sediments or assimilated by phytoplankton, but these results suggest that a substantial number of lakes will have low biological acid neutralization capacity from denitrification alone.

Of the 597 lakes, 126 (21%) had alkalinity less than or equal to 50 $\mu eq\ l^{-1}$ and were categorized as acid sensitive. California had the greatest incidence of acid sensitivity (37%) within its WLS subset lakes, and the majority of these lakes were in the Sierra Nevada (Table 5.2). Other regions were 8-24% acid sensitive. Seventy-four of WLS subset lakes had both alkalinity less than 50 $\mu eq\ l^{-1}$ and NO_3 removal efficiency less than 50%. Eighteen of these lakes were deep (mean depth > 10m), but only two were estimated to be N limited, which suggests efficient NO_3 removal due to phytoplankton uptake. Thus, we categorized 72 lakes (12%) as very sensitive to acidification should nitric acid inputs increase. Very acid sensitive lakes were located in every region (Table 5.2), but were most common in California (23%), especially in the Sierra Nevada (Figure 5.5). Nine to 15% of lakes in other regions were very acid sensitive.

5.4.2 Relationship between N deposition and lake NO_3

Nitrogen deposition from 1985 was greatest (up to 3 kg wet DIN $ha^{-1}\ y^{-1}$) in eastern and central Colorado, especially near Denver, and around Salt Lake City, Utah (Figure 5.6). Other noticeable peaks in deposition of about 2 kg wet DIN $ha^{-1}\ y^{-1}$ occurred near San Francisco, California and Seattle, Washington. Background levels in areas far from urban centers were $\leq$1 kg wet DIN $ha^{-1}\ y^{-1}$. The highest DIN levels for the 1992-1999 average for the Rocky Mountains were 4-5 kg DIN $ha^{-1}\ y^{-1}$, and were found in the Northern Front Range (Colorado-Wyoming), near Salt Lake City, Utah, and in Northwest Wyoming just south of Yellowstone National Park (Nanus et al. In Review).

High lake NO_3 levels were found in the highest N deposition areas (Colorado Front Range west of Denver and Utah's Uintah Mountains southeast of Salt Lake City),

but high concentrations were also found in areas with background deposition (Beartooth and Absaroka Ranges, Montana and Wyoming). Some areas with moderate N deposition had mostly low NO_3 lakes (Sierra Nevada near San Francisco, California and the Cascade Range near Seattle, Washington).

The regression models explained 19.5 to 24% of the variability in lake NO_3 concentrations (Table 5.3). Nitrogen deposition or its interaction with another variable was a significant term in three of the four models. Deposition from 1992-99 x distance to timberline was the N deposition term with the highest partial R^2 and explained 14.4% of the variability in lake NO_3 for the Rocky Mountain subset (n=291; Table 5.3A). Nitrogen deposition alone contributed another 4.2% of variance in this model. The model for the Rocky Mountain subset was similar to the model for Rocky Mountain subset drainage lakes only (n=224), but N deposition terms had more explanatory power in the former. Nitrogen deposition extrapolated from 1985 NADP data had little explanatory power. Mean areal outflow x 1985 deposition was a significant N deposition term, but explained only 1.9% of NO_3 variability (Table 5.5D).

5.5 Discussion

In our analysis of non-disturbed western mountain lakes, about a quarter of the lakes are expected to experience some degree of eutrophication should N inputs rise. In some areas, N deposition is already elevated and eutrophication has already been observed, although examples are rare. Few long-term monitoring programs existed before the 1980's, and many programs were designed to assess acidification and did not include measurements of algal biomass and productivity necessary to document

eutrophication. An exception is Lake Tahoe, a very deep subalpine lake located in the Sierra Nevada Mountains. Since the 1960's, Secchi disk transparency decreased by 7 m, annual primary productivity doubled, NO_3 content of the lake increased, phytoplankton shifted from N and P co-limitation to consistent P limitation, and the eutrophic to mesotrophic diatom species *Fragilaria crotonensis* became more dominant (Goldman 1988). High N:P ratios in atmospheric deposition compared to runoff provide strong evidence that deposition has been driving these changes (Jassby et al. 1994).

In the Colorado Front Range, monitoring data do not exist to link N deposition to eutrophication. Surveys conducted in the late 1990's, however, showed that lakes located east of the Continental Divide and closer to N emission sources had higher NO_3 concentrations than those on the west side (Baron et al. 2000). Furthermore, fossil remains in sediment cores from two lakes on the eastern side of the Divide show that diatom biovolumes increased while *F. crotonensis* and another mesotrophic diatom, *Asterionella formosa*, became dominant at about 1950-1979 when N emissions began to increase rapidly (Wolfe et al. 2001). An independent analysis of a sediment core from a nearby high NO_3 lake showed similar eutrophication responses beginning in the mid-1900's (Waters et al. 2002). Abundances of both *F. crotonensis* and *A. formosa* were positively correlated to TN:TP ratios in lakes of the Greater Yellowstone Ecosystem and *F. crotonensis* was favored by high NO_3 concentrations in laboratory bioassays, giving support to the notion that increases in atmospheric N were responsible for the species shifts observed in these Colorado Front Range lakes (Interlandi and Kilham 1998, Interland et al. 1999).

Effects of elevated NO_3 inputs have been studied with micro- and meso-scale experiments in shallow lakes in the southern Rocky Mountains (Lafrançois et al. 2002a, Nydick et al. 2002a, Nydick et al. 2002c). Experimental NO_3 additions did not cause eutrophication responses in lakes with high NO_3 concentrations, but productivity was enhanced with the addition of P, another often-limiting nutrient. Nitrate additions to low NO_3 lakes enhanced phytoplankton productivity and biomass, however, and caused shifts in species dominance from chrysophytes toward chlorophytes, cyanophytes, and diatoms. The taxa observed following NO_3 addition were the same as those inhabiting lakes with the highest NO_3 concentrations in the surrounding area (Lafrançois et al. 2002b). Benthic algae, both on artificial tiles and on sediment, rarely showed detectable response to nutrient enrichment in southern Rocky Mountain lakes (Lafrançois et al. 2002a, Nydick et al. 2002a). Nutrient additions in very shallow (<1 m) boreal ponds resulted in higher growth of algae on tiles, however (Vinebrook and Leavitt 1999).

It might be expected that increases in primary productivity resulting from enhanced nutrient input would amplify zooplankton abundance, leading to negligible gains in algal biomass. Such has been the case in many fertilized lakes (Cottingham et al. 1997, Paul et al. 1995, Vanni 1987), but this scenario may not occur as often in oligotrophic systems where zooplankton assemblages are sparse and often dominated by copepods and rotifers, as opposed to *Daphnia* sp. which are known to be efficient grazers (Berquist et al. 1985, Carpenter et al. 2001, Cottingham and Schindler 2000) We have found that neither zooplankton abundance nor biomass increased in response to four to eleven-fold gains in phytoplankton chlorophyll *a* and primary productivity resulting from NO_3 amendments to small alpine lakes in the southern Rocky Mountains (Nydick et al.

2002a). Zooplankton biomass actually decreased following NO_3 enrichment due to predominance of inedible algae (Lafrançois et al. 2002a). Similarly, fertilization experiments in Lake Tahoe and lakes of the Sawtooth Mountains, ID resulted in substantial phytoplankton gains, but negligible grazing impacts (Elser and Goldman 1991).

In addition to N, atmospheric deposition can provide substantial amounts of P to mountain ecosystems and contributed significantly to P loading in Lake Tahoe (Jassby et al. 1994, Lewis et al. 1985). Phosphorus deposition may be responsible for increasing TP levels in Sierra Nevada lakes adjacent to the heavily fertilized San Joaquin Valley (Sickman 2001). Increased lake TP concentrations were also detected in five of seven National Parks in a comparison of synoptic surveys conducted in 1985 and 1999 (Clow et al. In Review). Responses due to combined N and P fertilizations are often greater and more prolonged than reactions to N alone, even in N limited lakes (elser et al. 1990, Lafrançois et al 2002a, Nydick et al. 2002a). Thus, enhanced P deposition is a concern in terms of both the percentage of lakes affected and the degree of eutrophication.

Twenty-one percent of non-disturbed western mountain lakes were acid sensitive due to alkalinities below 50 $\mu eq\ l^{-1}$. Twelve percent were very acid sensitive based on low alkalinity combined with low NO_3 denitrification removal efficiencies and low potential for alkalinity generation via phytoplankton NO_3 uptake. Chronic acidification has not been documented in mountain lakes in the western U.S., but N deposition has contributed significantly to the acidification of lakes in the northeastern U.S. and Europe (Brimblecombe and Stedman 1982, Grennfelt and Hutberg 1986), and artificial acidification by nitric acid had been documented (Rudd et al. 1990). Episodic

acidification during early snowmelt has been observed in headwater streams of the Colorado Front Range (Williams and Tonnesen 2000), southern Wyoming (Vertucci and Corn 1996), and Sierra Nevada (Williams and Melack 1991), albeit rarely.

We found a significant, although somewhat weak, relationship between N deposition and NO_3 concentrations for Rocky Mountain lakes. Many factors interact to affect lake NO_3 concentrations. Variation in N deposition due to emissions depends on the quantity and type of emissions, distance from source, and wind trajectories. Nitrogen input to lakes not only depends on the amount and timing of N deposition, but is also related to many watershed characteristics such as the ratio of watershed:lake area, vegetation coverage, and topography. Lake NO_3 concentrations are further controlled by N retention, which is in turn regulated by factors such as hydraulic residence time, lake depth, and sediment characteristics. Given the complexity of these relationships and the errors associated with extrapolating N deposition to points in between sampling stations, we might not expect to detect an effect of N deposition on lake NO_3 concentrations. Such was the case when using 1985 NADP data extrapolated to the entire subset of 597 WLS subset lakes. Most of the NADP sites were not located in mountainous regions and likely underestimated N deposition to higher elevations in regions with elevated N deposition. Furthermore, sampling locations were relatively few considering the size of the study area. When N deposition was extrapolated from high resolution maps created from NADP data combined with snowpack surveys and orographic precipitation modeling in the Rocky Mountains, however, N deposition and the interaction of N deposition with distance from timberline explained almost 20% of NO_3 variability. Our results suggest that the combination of high N deposition, low catchment vegetation

cover, and a large watershed area: lake area ratio increases the likelihood of high lake NO_3 concentrations. We also found that high quality N deposition data is necessary to elucidate large-scale relationships between N deposition and lake chemistry.

Inconsistencies between lake NO_3 concentrations and N deposition did occur, however, and were most notable in the Absaroka and Beartooth Ranges, where high NO_3 lakes occurred despite low N deposition, and on the west coast of the USA, where mostly low NO_3 lakes were found despite centers of moderate N deposition around San Francisco and Seattle. Although evidence is scant, high NO_3 levels in Absaroka and Beartooth Mountain lakes may be due to the abundance of mineral deposits. The absence of many high NO_3 lakes on the West Coast is due to wind and precipitation patterns, which limit N deposition and subsequent effects to lower elevations (Fenn et al. 2002). In contrast, the Colorado Front Range experiences upslope summer storms, which bring polluted air up from the Denver urban/agricultural (Baron and Denning 1993, Bossert 1990). With continuing urbanization, increasing agriculture and livestock operations, and the possibility of trans-Pacific N transport from Asia (Wilkening et al. 2000), it is likely that N deposition and its effects on lakes will increase in Pacific as well as mid-continental mountain ranges in the next several decades.

5.6 Conclusion

Algal growth in a quarter of western mountain lakes likely will increase in response to higher N loadings from atmospheric deposition. The proportion of N limited lakes was greatest in the Pacific Northwest, which also had the lowest lake NO_3 concentrations. Nitrate concentrations were highest in the Rocky Mountains where N

deposition was identified as a significant source of variability in lake NO_3 levels. The Colorado Front Range had the highest levels of N deposition and some of the highest lake NO_3 concentrations in this analysis; other studies have linked both chemical and biological changes to N deposition in this area. Over 20% of lakes had alkalinity of 50 $\mu eq\ l^{-1}$ or less, and more than 60% of lakes were unlikely to biological neutralize half of acid inputs associated with N deposition. Acidification potential, determined by both measured alkalinity and potential biological alkalinity generation, was greatest in the Sierra Nevada. Thus, past effects of N deposition seem to be greatest in the Rocky Mountains, especially the Colorado Front Range, while future vulnerability is greatest for Pacific mountain ranges.

5.7 Acknowledgements

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Table 5.1. Number of lakes included for each region and the percent of lakes in each that are N, NP co-, and P limited.

Region	Total # of Lakes	% N limited	% NP co-limited	% P limited
California	108	17	31	52
Pacific Northwest	111	34	31	29
Northern Rockies	128	19	44	38
Central Rockies	130	18	35	37
Southern Rockies	120	18	37	45
TOTAL # or %	597	24	37	39

Table 5.2. Number of WLS subset lakes for each region and the percent thai were classified as acid sensitive (alkalinity≤ 50 μeq l^{-1}) and very acid sensitive (alkalinity≤ 50 μeq l^{-1} and NO_3 removal<50%).

Region	Total # of lakes	% Acid Sensitive	% Very Acid Sensitive
California	108	37	21
Pacific Northwest	111	24	13
Northern Rockies	128	22	10
Central Rockies	130	8	7
Southern Rockies	120	18	13
TOTAL	597	21	12

Table 5.3. Best models to explain variability in lake NO_3 concentrations. A) All lakes, using 1985 N deposition from NADP data. B) Drainage lakes only, but including residence time and mean areal outflow (i.e. mean depth/residence time), and using 1985 N deposition from NADP data. C) Rocky Mountain lake subset, using 1992-99 N deposition from integrated snow sampling and NADP data (Nanus et al. In Review). D) Rocky Mountain lake subset, drainage lakes only, but including residence time and mean areal outflow, and using 1992-99 N deposition from integrating snow sampling and NADP data (Nanus et al. In Review).

A) All Lakes, 1985 N deposition, Adjusted R^2=0.2397, n=597

VARIABLE	T	P-VALUE	PARTIAL R^2
Watershed Area:Lake Area	6.62	<.0001	0.0689
Elevation	6.30	<.0001	0.0630
Distance from Timberline x Elevation	6.24	<.0001	0.0618
Watershed Area x Watershed Area	-4.99	<.0001	0.0405
Distance from Timberline	-4.37	<.0001	0.0313

B) Drainage Lakes Only, 1985 N deposition, Adjusted R^2=0.2299, n=476

VARIABLE	T	P-VALUE	PARTIAL R^2
Distance from Timberline x Elevation	5.46	<.0001	0.0606
Distance from Timberline	-3.18	0.0020	0.0304
Elevation	3.10	0.0020	0.0203
Mean Areal Outflow x 1985 Deposition	**3.00**	**0.0028**	**0.0191**
Watershed Area:Lake Area	2.80	0.0053	0.0167
Precipitation	-2.73	0.0065	0.0159
Mean Areal Outflow	-2.65	0.0083	0.0149
Residence Time	-2.46	0.0143	0.0129
Mean Depth	2.17	0.0305	0.0101

C) Rocky Mountain Subset, 1999 N deposition, Adjusted R^2=0.1950, n=291

VARIABLE	T	P-VALUE	PARTIAL R^2
1999 N Deposition x Distance to Timberline	**6.95**	**<.0001**	**0.1440**
Watershed Area:Lake Area	3.90	0.0001	0.0503
1999 N Deposition	**3.55**	**0.0005**	**0.0420**

D) Rocky Mountain Subset, Drainage Lakes Only, 1999 N deposition, Adjusted R^2=0.2096, n=225

VARIABLE	T	P-VALUE	PARTIAL R^2
1999 N Deposition x Distance to Timberline	**5.65**	**<.0001**	**0.1271**
Watershed Area:Lake Area	2.76	0.0063	0.0336
1999 N Deposition	**2.48**	**0.1380**	**0.0274**
Mean Depth	2.26	0.0249	0.0228
Hydraulic Residence Time	-2.23	0.0265	0.0223

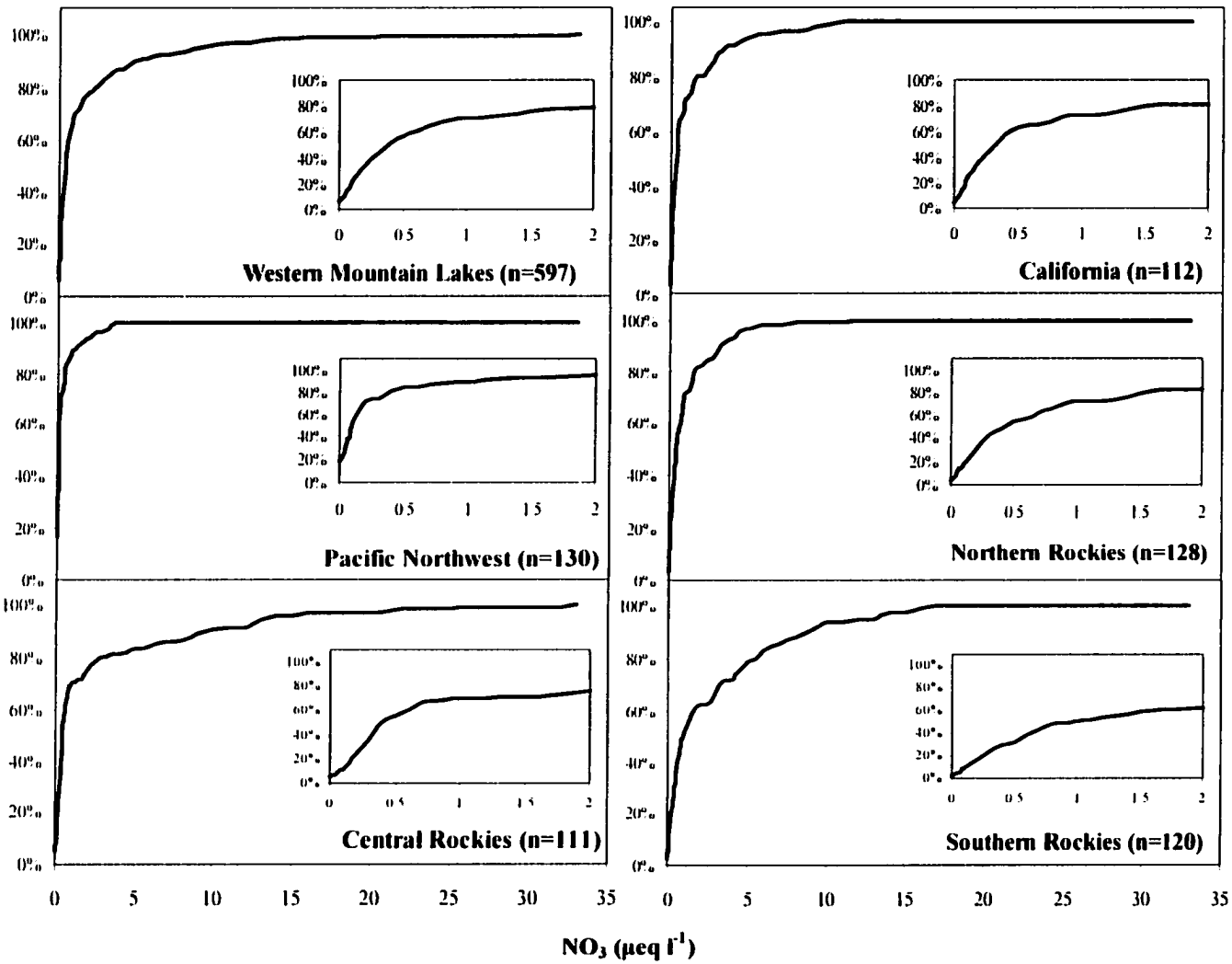

Figure 5.1. Cumulative frequency distributions of WLS subset lake NO_3 concentrations. Inset plots show the cumulative frequency distribution for a subset of lakes with low NO_3 (up to 2 μeq l^{-1}).

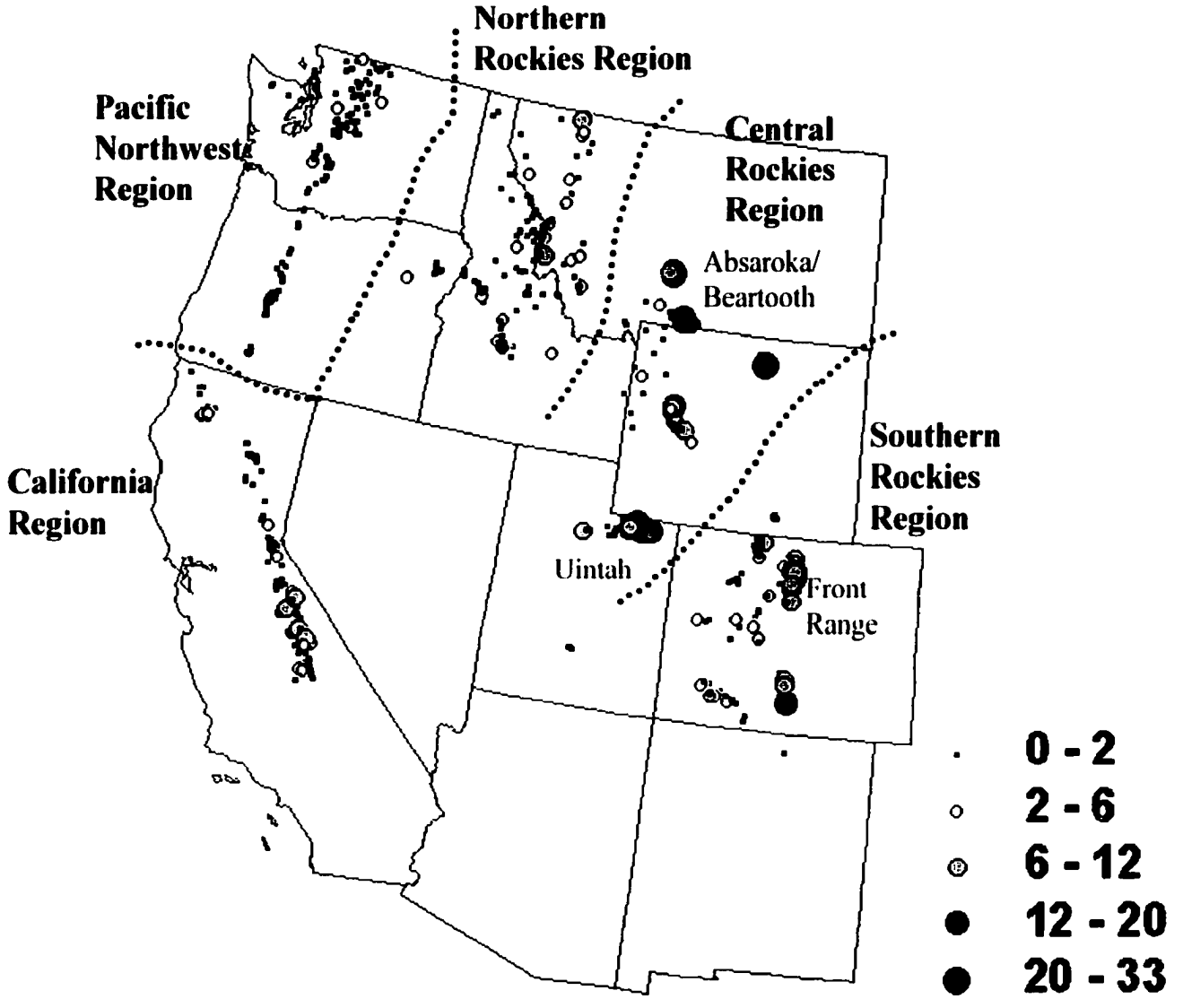

Figure 5.2.
WLS subset lake NO_3 concentrations ($\mu eq\ l^{-1}$). Extent of regions used in analyis are shown with dashed lines. Hotspots of high NO_3 levels were mostly located in the Beartooth-Absoroka (MT/WY), Uintah (UT), and Front Range (CO) mountains.

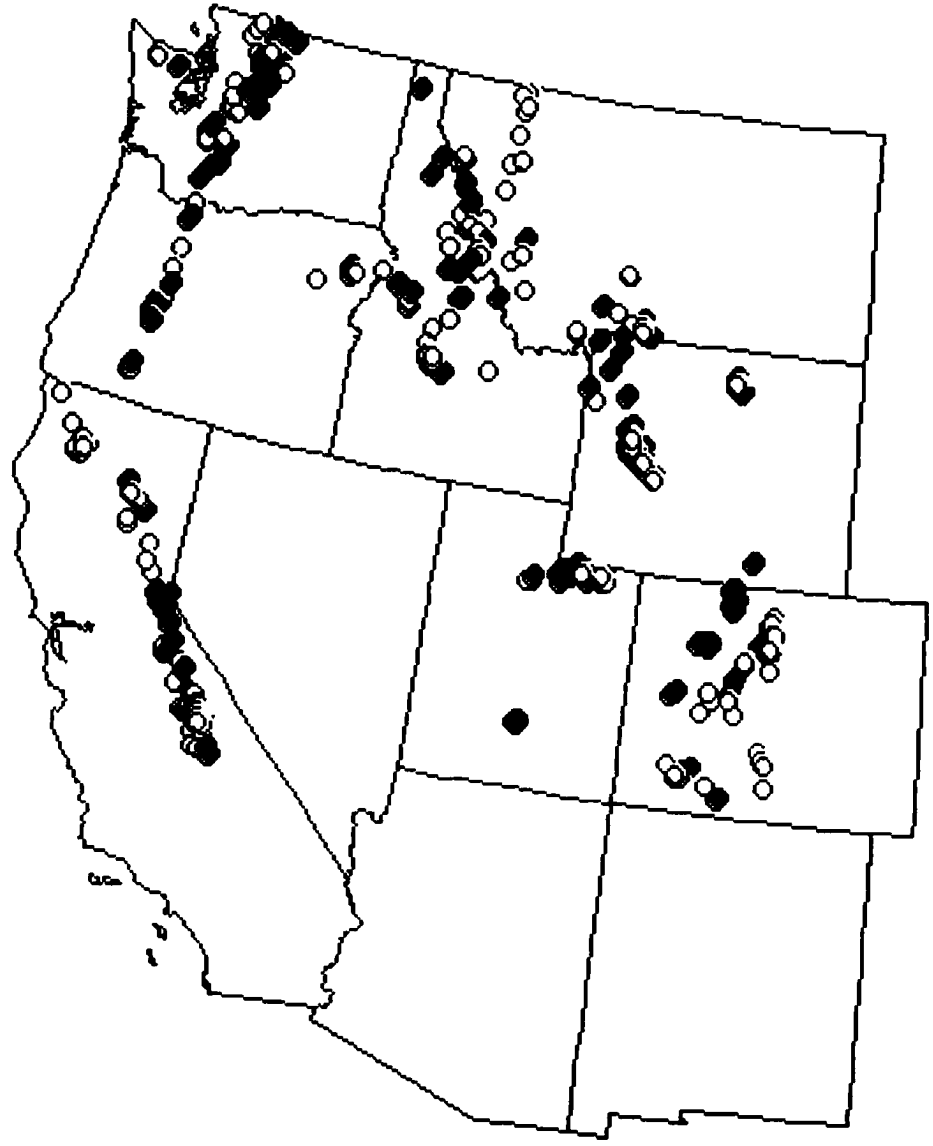

Figure 5.3. Nutrient limitation status of WLS subset lakes as estimated by DIN:TP ratio. Dark circles indicate N limitation (24% of lakes), while open circles show P limitation (39% of lakes). Lakes with N and P co-limitation are not shown.

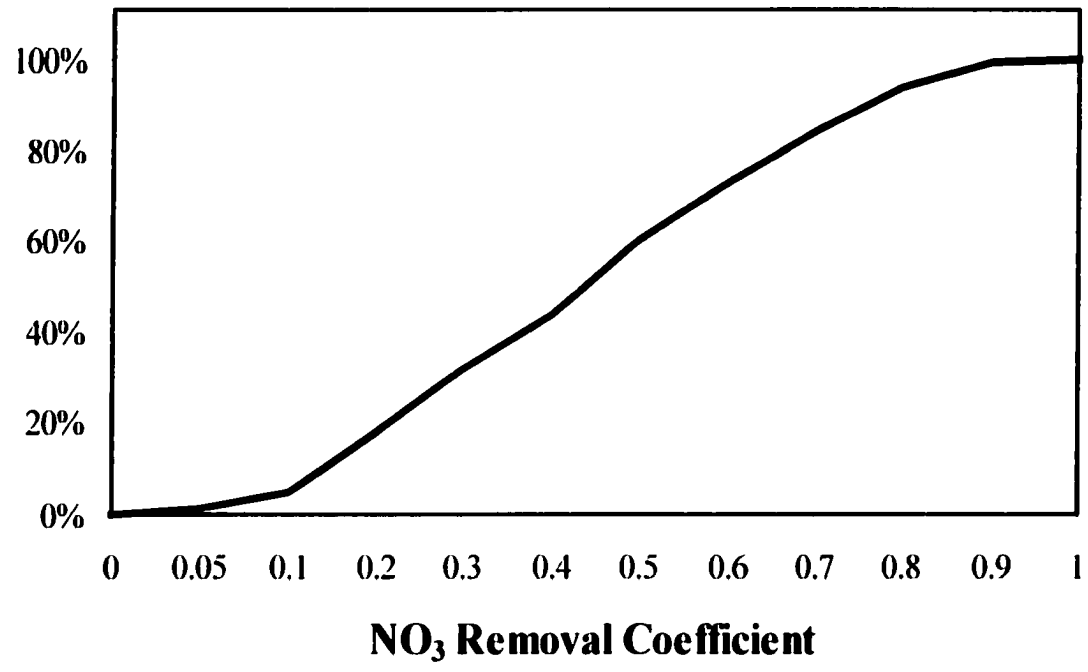

Figure 5.4. Cumulative frequency distribution of lake NO_3 removal coefficients for WLS subset lakes (n=597), determined following Kelly et al. (1987).

Figure 5.6. Location of WLS subset lakes classified as "acid-sensitive" (squares; alkalinity≤50 μeq l^{-1}) and "very acid sensitive" (circles; alkalinity≤50 μeq l^{-1} and NO_3 removal ≤50%). Crosses represent lakes with alkalinity greater than 50 μeq l^{-1}.

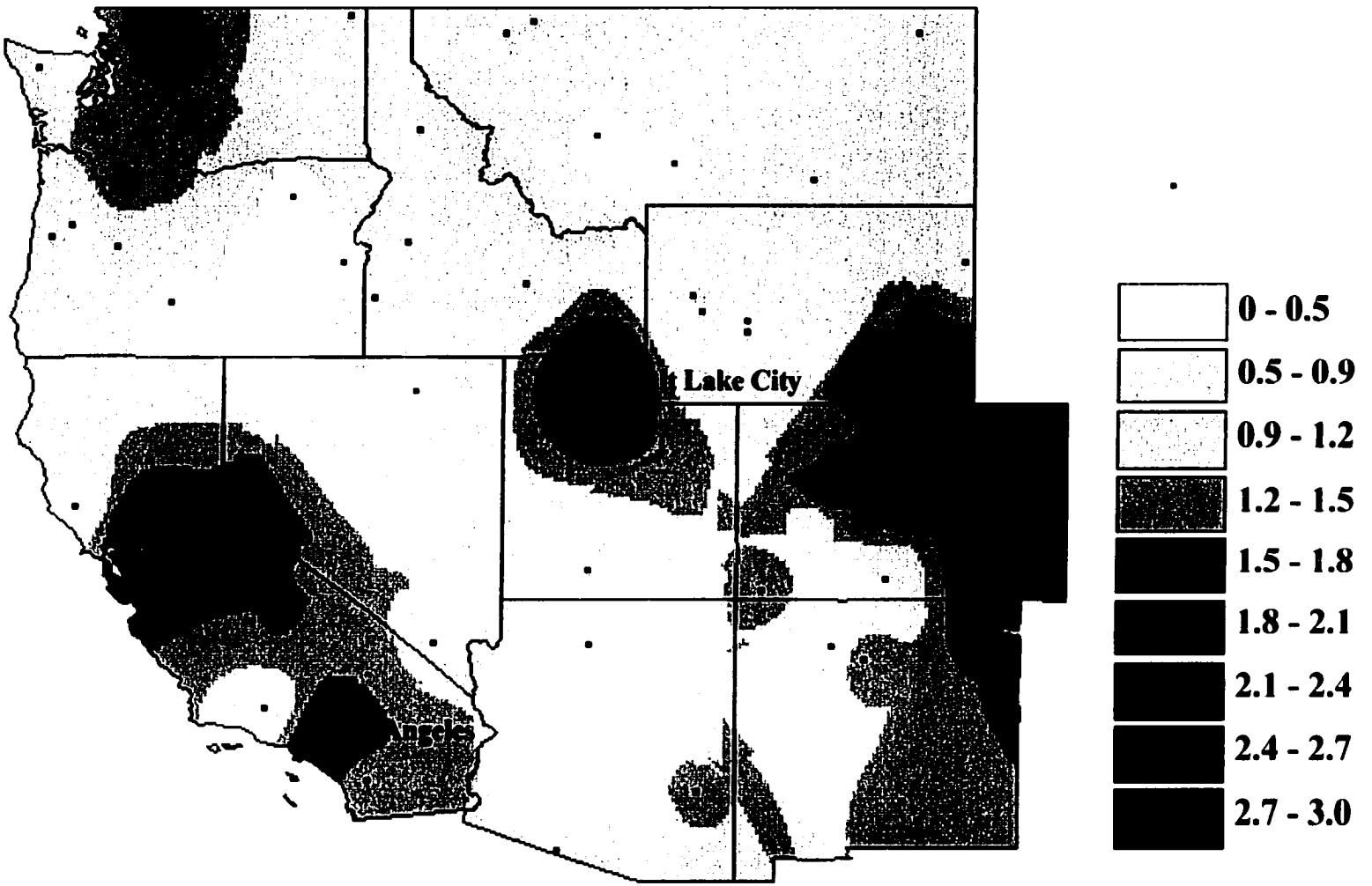

Figure 5.6. Atmospheric N deposition for the western USA, determined by extrapolating values from NADP sites for water year 1985. Deposition is given as mg wet DIN (NO_3 + NH_4) ha^{-1} y^{-1}. Dots represent NADP sites.