RATES, IMPORTANCE, AND CONTROLS OF NITROGEN FIXATION IN OLIGOTROPHIC ARCTIC LAKES, TOOLIK, ALASKA

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RATES, IMPORTANCE, AND CONTROLS OF NITROGEN FIXATION IN OLIGOTROPHIC ARCTIC LAKES, TOOLIK, ALASKA

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Biological nitrogen (N) fixation of atmospheric N_2 by free-living cyanobacteria in aquatic environments is common, and in many ecosystems, it can account for a significant portion of the biologically available N inputs. Although N fixation can compensate for N limitation, N limitation is maintained over relatively long time scales in many oligotrophic lake ecosystems. This dissertation examines the importance of benthic and pelagic N fixation in the N economies of oligotrophic lakes in arctic Alaska (Chapter 1) and examines nutrient, light, and grazer controls on benthic N fixation (Chapters 2 and 3). Both benthic and pelagic N fixation are prevalent in many lakes across the Alaskan arctic landscape, ranging from 0.12 – 1.5 mg N m^{-2} day^{-1} and 0 – 2.56 mg N m^{-2} day^{-1} respectively. Pelagic N fixation is much higher than has been reported elsewhere for oligotrophic lakes, and is more important than previously thought, comprising ~ 75% of N inputs to one lake. Benthic N fixation is lower than has been reported for other oligotrophic systems, and is roughly equivalent to N inputs from atmospheric deposition on an areal basis (~25 mg N m^{-2} $year^{-1}$). On the landscape scale, N fixation in lakes roughly equal that in terrestrial ecosystems in this Arctic region. Benthic N fixation generally appears to have a saturating response to light availability within individual lakes, but light does not explain variation in benthic N fixation across lakes or years. Whole-lake fertilization

and laboratory experiments indicate that P input stimulates benthic N fixation while N input suppresses N fixation when N is added either alone or in conjunction with P in Redfield proportion. Snails at ambient density cause a small decline in benthic N fixation (0.85 – 1.8% reduction over the summer). These patterns are corroborated in the landscape: lakes on younger surfaces have higher P, more snails, and higher rates of N-fixation than lakes on older surfaces.

BIOGRAPHICAL SKETCH

Gretchen Maria Gettel grew up in Vermont where beautiful surroundings, interested family members, and inspired teachers fostered her native interest in science. One of the many activities that Gretchen's father, Courtland D. Gettel, had her do was to sit down in a meadow with a magnifying glass and a sketch book with the instructions to draw what she saw. Gretchen's grandmother, Mary Dickinson "Dickie" Gettel, sent her hunting for geodes with a rock hammer from Dickie's own undergraduate career in geology. Gretchen's grandfather, William Dabney Gettel, showed Gretchen how he recorded daily temperature and precipitation, calculated monthly averages, and graphed the data by hand. Gretchen's mother, Karen Tryon Bell, responded to Gretchen's first proclamation of, "I want to be a marine biologist" in the 3rd grade, with the encouragement: "You can be whatever you want."

Gretchen graduated from Mill River Union High School in North Clarendon, Vermont in 1989. Along the way, she encountered some remarkable women. Mrs. Carolyn Raiford taught 8th, 10th, and 11th grade life science though AP biology. Through years of creative science projects, well-designed laboratory assignments and a broad curriculum, she instilled the fundamentals of the scientific method and the discipline, skills, and joy associated with independent learning. A family friend, Ms. Elizabeth Ryder, gave Gretchen a microscope and an idea for a science project examining the effect of Vitamin C on lymphocytes. That project won a silver medal at the Vermont State Science Fair, and then she continued to gravitate towards the natural sciences. Mrs. Margie McCouch met Gretchen when she was 84 years old and retired as a biological oceanographer from the Woods Hole Oceanographic Institution. For the next ten years, Margie shared a correspondence which comprised gifts of books (including one that she herself wrote), photographs, and ocean-floor maps.

Although she had lost her sight, Margie continued to provide Gretchen with illustrations of science as an academic profession through Gretchen's early college years.

Gretchen attended college at Boston University where she majored in Biology with specialization in Marine Science. When she was a sophomore, she met Dr. Kate Lajtha in general ecology class. Kate taught Gretchen about the role of chemistry in understanding how natural systems work and opened the possibilities of a career in ecology when she sent Gretchen to Woods Hole to work in Dr. Ivan Valiela's laboratory. There, Gretchen learned about the role of nutrients in causing eutrophication in Waquoit Bay on Cape Cod, and Dr. Ken Foreman taught her how to do nutrient analyses (and how to drive a stick shift). It was the following summer in 1992 that Gretchen was hired by the Ecosystems Center in Woods Hole to go to the Arctic to search for nutrients in oligotrophic waters of northern Alaska. After completing a senior thesis project on the age and growth of arctic grayling with Dr. Linda Deegan, Gretchen graduated from Boston University in 1993.

Gretchen worked for two years at the International Pacific Halibut Commission in Seattle before starting a Masters of Science in the Water Resources Program at University of Minnesota with Dr. Anne Hershey in 1995. Her Master's thesis research was also conducted in the Arctic at Toolik Field Station. Her work examined patterns in fish distribution and food web structure in lakes in three river valleys on the North Slope. Anne and one committee member in particular, Dr. John Pastor, taught Gretchen to think about how processes operating at large spatial and temporal scales can affect patterns in ecosystem structure and function at very small scales. As part of the work she did with Anne, she organized 8 – 10 member survey trips to 80+ lakes on the North Slope, riding in (and getting sick in) a helicopter for the first time. The more she saw, the more Gretchen really fell in love with the arctic

landscape. Continuing to work in the Arctic was a very easy thing for her to decision to make; imposing marine biological interests in the form of SCUBA diving in the Arctic was not so easy.

Gretchen completed her Master's work in 1998 and started graduate school with Dr. Robert Howarth at Cornell University that fall. As a member of the Biogeochemistry Program, she was able to merge her interests in biogeochemistry, ecology, and landscape processes by combining survey work with field experiments to examine the controls of nitrogen fixation on the bottom of lakes. This work entailed more helicopters and lots of time spent at 4^{o}C in dry suits. After 120+ dives, Gretchen likes to say that diving in the Arctic is cool to say you did and stupid to keep doing – and so she decided to finish her dissertation, available here in just over a page per dive, if you're curious.

Gretchen continues to work in biogeochemistry, and she is currently pursuing research ideas strongly influenced by work in the Howarth laboratory, discussions among students in the Biogeochemistry Program, and by researchers at Toolik Field Station and Ecosystems Center in Woods Hole. She is very happy in a post-doctoral position with a new mentor, Dr. William (Bill) McDowell, at the University of New Hampshire, where she is learning about how carbon quality affects nitrogen cycling at the watershed scales in southeastern New Hampshire. And she loves being back in the beautiful surroundings of New England.

I dedicate this work to my grandparents, Mary Tryon Gortner, Clayton Gortner, Mary Dickinson Gettel, and William Dabney Gettel, who encouraged me to follow my own path and to keep on going once I was on it.

ACKNOWLEDGMENTS

There are so many people to thank. My committee chair, Dr. Robert Howarth, strongly influenced my world view on nitrogen cycling in ways that will steer the rest of my scientific career. Bob has an ability to distill a scientific problem and give great advice in about 10 minutes. Dr. Anne Giblin's commitment to me and this project went above and beyond. Anne helped design sediment chambers, and she was an extraordinary dive buddy, mentor in the lab, and a voice of reason (to which I should have listened more often). Anne and her husband, Dr. Ken Foreman, and their two kids, Mike and Nick, housed, fed, and encouraged me for 3 months while I wrote my dissertation in Woods Hole. Most importantly, Anne helped me see the value of this work in a broad scientific context. Dr. Alex Flecker was generous with ideas and resources at his field site in Rio Las Marias, VZ for a side project to my dissertation, and he gave outstanding feedback on my writing. Dr. Nelson Hairston's critical approach to science greatly improved the quality of my work and thought.

The students I was fortunate to mentor — Marissa Weiss, Corey Lawerence, Lyndon Valicenti — all worked long hours into the Arctic white nights, not only for their own projects, but for mine. Lyndon Valicenti was particularly instrumental to this work; she collected water column data and counted algae cells, and maintained a positive outlook especially when the going got tough. Dan Steinburg was not only my dive buddy and laboratory assistant, but also an engaged, interested student capable of helping me make decisions in the lab and in the field, all while maintaining a laid-back demeanor and sense of humor. Marissa Weiss lapped me by publishing her nitrogen fixation work before I even completed my dissertation, and I remember fondly her help, humor, and conversations over long stretches of shooting samples into the GC. I hope they learned as much from me as I did from them.

Marcus Gay, Sam Kelsey, and Ian Washbourne were also dependable dive buddies, and their help in the laboratory was critical to the completion of this project. Drs. Roxanne Marino and Dennis Swaney helped think through laboratory methods and sediment diffusion corrections. Dr. Sandy Tartowski not only taught me everything she knew about running a gas chromatograph, but also shaped my intellectual and professional development early in graduate school. Other members of the Howarth Lab including Drs. Francis Chan, Brian Roberts, and Beth Boyer helped through numerous conversations about this work. Drs. Francoise Vermeylen and Andrew Cooper cheerfully assisted me with statistical analysis, even though I required multiple explanations with the same datasets and the same code. All the scientists at the Ecosystems Center in Woods Hole and associated with the Arctic LTER supported and helped me with this work in ways too numerous to list here.

The Institute of Arctic Biology and VECO Polar Resources provided logistical support without which this work would not have been possible. In particular, Naomi Whitty organized helicopter logistics and was the model of professionalism in balancing our work needs with safety. Pilots Ed, Butch, and Landis allowed soaking-wet, muddy divers into their helicopters and sometimes made fast flights in questionable weather to make sure we weren't stranded in the snow after diving in 4^{o}C water. Jay Burnside from VECO Polar Resources worked closely with Neil Bettez and me on the construction of the Incubation Facility at Toolik Field Station, which was the single most important facility that allowed me to conduct this research.

The social and professional opportunities provided by the Biogeochemistry Program were by far my favorite part of being a graduate student at Cornell University. The interactions at seminars were outstanding, and the graduate students became my best friends, colleagues, and mentors. I would particularly like to thank those in my cohort: Karin Rebel, Kathy Bailey, Peter Weishampel, Rich Phillips, and

Noel Gurwick. I really want to list everyone else, but I'm afraid I'm going to forget someone, and it would make for an awfully long acknowledgement section – I trust that you know who you are! My officemates, Bryon Daley, Becky Doyle, Jeanne Robertson, Robert Harris, and Pete McIntyre were often the best reasons to show up in Corson each day. To those who considered staying at 107 Miller Street a pre-requisite for graduation, isn't it nice to know that it finally worked for me? Thank you all for all the good food and libation, and above all – the stimulating, fun, and supportive conversations.

My family never wavered in their support of this endeavor, never stopped having faith in me, and never asked that dreaded question, "When are you going to finish?" My brother, Gregory Gettel, was and still is unconditionally proud of me even through late -night phone calls and pep talks, for which I am deeply grateful. Lastly, Neil Bettez applied his outstanding talents in doing science to my work in ways too many to list, and was my very best friend and biggest fan throughout. (I wouldn't have made it without you). Thank you.

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CHAPTER 1

THE RATES AND IMPORTANCE OF BENTHIC AND WATER-COLUMN NITROGEN FIXATION IN OLIGOTROPHIC ARCTIC LAKES

ABSTRACT.

N fixation in the water-column and benthic environments is prevalent across many lakes and landscape types in arctic Alaska. Benthic N fixation rates are low (0.12—1.5 mg N $m^{-2}day^{-1}$) relative to other measured rates in oligotrophic systems, but areal rates of water-column N fixation are much higher (up to 2.56 mg N $m^{-2}day^{-1}$) than rates reported in most other oligotrophic lakes and are similar to rates measured in oligotrophic oceanic systems. Benthic and water-column N fixation in these lakes accounts for up to 0.6—7% of autotrophic N demand; however, N fixation represents a new input that supports new production and supplies N that is then available for recycling many times. Benthic N fixation accounts for up to 30% of the recycled N flux from sediments to the water column and is equal to or greater than the nitrogen input from precipitation. Benthic N fixation comprises a significant portion (6 – 100%) of depth-integrated water-column N fixation in the epilimnion; however, water-column N fixation comprised a significant portion (75%) of N inputs to Lake Fog 2. This result indicates that water-column N fixation is more important to the N economy of these oligotrophic lakes than was previously thought. Finally, N fixation in lake ecosystems appears to equal or exceed that of terrestrial ecosystems; thus, N fixation in lake ecosystems is important to N inputs across the arctic landscape.

INTRODUCTION.

In the Arctic, net primary production in terrestrial and aquatic habitats is commonly limited by nitrogen (N) (Shaver et al. 1986; Levine and Whalen 2001).

Nitrogen deposition rates are low (Galloway et al. 2004), and primary producers in both terrestrial and aquatic ecosystems depend on a high degree of N recycling to satisfy their N demand (Galloway et al. 2004; Chapin and Bledsoe 1992). Biological N fixation may be an important source of new N that can support new production, contribute to the N pool available for short-term cycling, or make up for losses (Alexander 1974; Barsdate and Alexander 1975; Alexander et al. 1978; Chapin and Bledsoe 1992).

Though N fixation has been known to be important to the N budgets of terrestrial ecosystems in the Arctic (Alexander 1974; Barsdate and Alexander 1975; Alexander et al. 1978; Chapin and Bledsoe 1992; Weiss et al. 2005; Hobara et al. In press), much less is known about the role of N fixation in aquatic ecosystems. However, lakes in the Arctic are commonly N-limited, either alone or in conjunction with phosphorus (Levine and Whalen 2001), and N limitation may be in part maintained by slow rates of N fixation (Howarth et al. 1988).

In oligotrophic lakes, benthic processes are often important. Because water-column production is low, light penetration is deep; as a result, littoral zones can comprise a significant portion of lake area (Ramlal et al. 1994). Benthic algae can contribute significantly to whole-lake productivity (Wetzel 1964; Vadeboncoeur et al. 2001), and N-fixing filamentous cyanobacteria are common on sediment surfaces (Moeller and Roskoski 1978; Loeb and Reuter 1981). Thus, inputs of new N through benthic autotrophic N fixation may be important to whole-lake N budgets. For example, Bergman and Welsch (1990) found that benthic N fixation could account for 16% of the N inputs to an oligotrophic, arctic pond in the Northwest Territories, Canada. In Lake Tahoe, benthic N fixation accounts for up to 32% of total N input to the lake (fixation data from Reuter et al. 1986; loading data from Rast and Lee 1978, as reported in Howarth et al. 1988), and benthic N fixation contributes up to 30 – 93%

of inorganic N uptake by the periphyton community (Reuter et al. 1986). Higgins et al. (2001) found that epilithic N fixation in Lake Malawi accounted for 36% of total N inputs, nearly equaling inputs from atmospheric and riverine sources; furthermore, epilithic N fixation exceeded N input from water-column N fixation.

In contrast with benthic environments, studies in water-column environments in oligotrophic lakes have generally indicated that N-fixation rates are rather low and comprise a small portion of total N inputs (Howarth et al. 1988). However, water-column measurements of N fixation in oligotrophic lakes are rare, and several lines of evidence suggest that N fixation may be more prevalent than was previously thought. For example, MacGregor et al. (2001) showed that ^{15}N was close to the atmospheric signature, indicating inputs from N fixation at 15 m depth in Lake Michigan; and they also demonstrated that the *nifH* gene, which encodes the Fe protein component for the nitrogen-fixing enzyme, nitrogenase, is prevalent through the water column. Although presence of the *nifH* gene is not conclusive evidence for N fixation, Zani et al. (2000) showed using reverse-transcriptase polymerase chain reaction (RT-PCR) that the expression of the *nifH* gene was prevalent in Lake George, a mesotrophic lake in upstate New York. In oligotrophic oceans, isotopic ^{15}N signatures in nitrate and particulate N pools indicate significant inputs of depleted ^{15}N, presumably from N fixation (e.g., Montoya et al. 2004), and unicellular cyanobacteria have also been shown to contribute significantly to oceanic N budgets (Montoya et al. 2004; Zehr et al. 2001), even though they were previously thought to be unimportant. Despite these insights, however, measured rates of N fixation in oligotrophic lakes remain sparse.

The conclusions reached in these studies contrast with previous work in oligotrophic lakes in the Alaskan Arctic, which indicated that N-fixation rates were too low to be important to lake N budgets. Alexander et al. (1989) concluded that *in situ* N fixation is a minor source of nitrogen to the thaw ponds near Barrow, Alaska,

when compared with the role of N fixation in terrestrial budgets. Alexander et al. (1989) further hypothesized that neither benthic nor water-column N fixation was important to the N budget of Toolik Lake, an oligotrophic lake in Arctic Alaska (Arctic Long Term Ecological Research Site). It appears that N fixation is potentially important in other oligotrophic systems, but contradictory conclusions resulting from very few studies in northern latitudes indicate that more work is needed.

Here I present the results of survey work in which rates of benthic and water-column N fixation were compared in lakes across a variety of landscape types present in the vicinity of Toolik Field Station (Arctic LTER database http://ecosystems.mbl.edu/arc/default.htm), North Slope Alaska. I also evaluate the importance of N fixation in the context of other N inputs and outputs by constructing an N budget for one lake. This budget uses values published from the literature in conjunction with seasonal measurements of benthic and water-column N fixation during one field season. Finally, I compare my measured N fixation rates with other rates from terrestrial and aquatic ecosystems in the Arctic.

METHODS.

Site Description.

Toolik Field Station is located in the northern foothills of the Brooks Mountain Range in arctic Alaska (68°37′N, 149°35′W) about 150 miles north of the Arctic Circle (Figure 1.1). The area is underlain by continuous permafrost, and lakes in the region are generally shallow (3 – 15 m) glacial kettles. Maximum epilimnetic temperatures range from 13 – 18 °C, and summertime depth to thermocline is about 5 m. Lakes are typically dimictic, and ice-free season occurs mid-June to mid-September (Artic LTER database Miller et al. 1986). The most important feature of lakes in this landscape is that they are ultra-oligotrophic (Miller et al. 1986), and water-column

concentrations of ammonium, nitrate, and phosphate are near detection limit, with concentrations below 0.1 μM (Artic LTER database). Water-column ^{14}C-primary production is low, ranging from 12 – 16 g C $m^{-2}year^{-1}$ (Miller et al. 1986). Because water column production is low, bottom O_2 concentrations remain high throughout the summer (7 – 8 mg l^{-1}). Secchi depth ranges 6 – 10 m in the summer, and the 1% light level occurs as deep as 16 m in some lakes (Arctic LTER database).

In the vicinity of Toolik Field Station, Pleistocene valley glaciations have occurred for periods of time between 12,000 to 800,000 years before the present (Hamilton 2003). Three dominant advances and retreats occurred in nearby drainages, exposing landscapes at different times. The oldest landscape, the Sagavanirktok, was exposed 250,000 – 800,000 years ago; the intermediate landscape resulted from Itkillik II glaciation and was exposed 25,000 – 50,000 years ago; and the youngest landscape resulted from the Itkillik I glaciation and was exposed 10,000 – 12,000 years ago.

I conducted a survey across these different landscapes to measure benthic and water-column N fixation and benthic productivity. I also performed more extensive sampling of a lake on a younger surface in 2003 in order to assess the importance of N fixation to primary production and overall N budget in more detail. The locations of these lakes are shown in Figure A1.1.

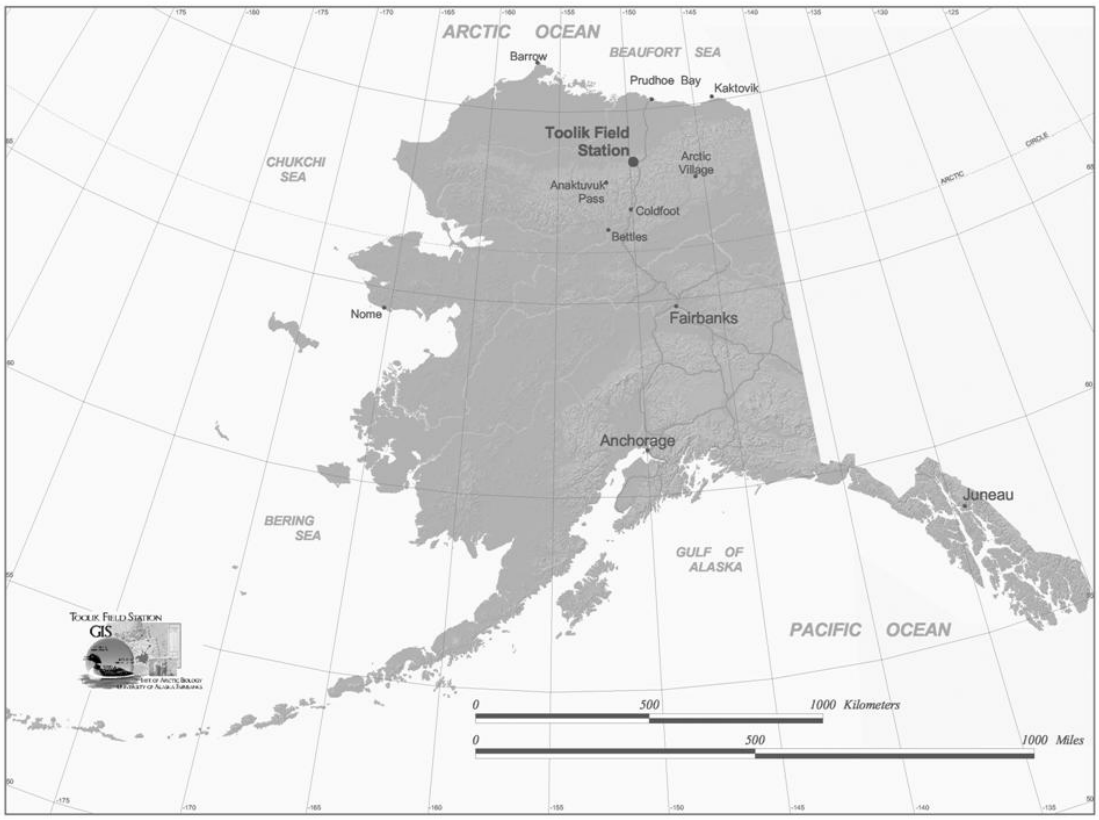

Figure 1.1. Map of Alaska showing the location of Toolik Field Station, Arctic LTER (68°37′N, 149°35′W)

Surveys. Surveys of benthic and water-column N fixation were conducted in mid-summer 2002 in which three lakes on the three glacial surfaces were visited via helicopter and sampled once during the summer sampling season (total of 9 lakes). To this data set, I added information from subsequent years or from other lakes that were also visited in mid-July in 2002 or 2003. Some of these included regularly sampled lakes as part of the Arctic LTER. To compare LTER lakes with survey lakes, I used only one-time sampling in July even if lakes were more regularly sampled. These lakes ranged in size from 1,785 m^2 to 1,446,485 m^2 and in depth from 3 – 22 m. The lakes also had variable light extinction coefficients, ranging from 0.5 – 1.7 (Table 1.1). Lakes on the older surfaces were generally more dilute, with specific conductivities

ranging from 6 – 7 μS cm^{-1} ^{o}C, whereas lakes on the younger surfaces ranged from 133 – 333 μS cm^{-1} ^{o}C (Table 1.1).

Five replicate sediment cores that had an intact mud–water interface were collected using SCUBA at 3 m in each lake for benthic N fixation and production measurements (described below). In addition to sediment cores, five replicate water-column samples were collected from a raft at the epilimnion (1 m depth), the metalimnion (at the thermocline; typically 3 – 5 m) and the hypolimnion (1 m from the bottom of the lake). Other water-column parameters, including chlorophyll a, NH_4^+, NO_3^- and soluble reactive phosphorus (SRP), were characterized according to methods described in the Artic LTER lake protocols.

Table. 1.1. Site characteristics for survey lakes.

Lake	Surface	Lake Area (m^2)	Watershed Area (m^2)	Depth (m)	Conductivity ($\mu S\ cm^{-1}\ ^oC$)	Vertical light extinction coefficient
NE-12	Itkillik I	69980	1167776	14	145.0	0.9
Fog 2	Itkillik I	60151	456764	16	135.3	0.5
Fog 4	Itkillik I	20624	260987	3	175.0	1.3
S-6	Itkillik I	6673	60717	7	168.3	0.7
NE-9B	Itkillik I	1785	138255	7	335.0	1.5
GTH 33	Itkillik II	26880	716257	11	19.7	1.5
GTH 61	Itkillik II	127065	870131	4	63.4	0.7
GTH 74	Itkillik II	50557	1067605	6	25.3	1.2
GTH 83	Sagavanirktok	13060	219331	6	16.7	1.2
GTH 85	Sagavanirktok	37359	3606810	7	8.7	1.4
GTH 86	Sagavanirktok	31260	1436577	9	7.0	1.7
Toolik	Itkillik 1 and II	1446485	5584768	22	40.0	1.0

Rates of N fixation. N fixation in mud cores was measured by the acetylene reduction assay (ARA), which quantifies the reduction of acetylene (C_2H_2) to ethylene (C_2H_4) by the nitrogenase enzyme (Hardy et al. 1968). Mud cores were about 10 cm deep, and they were collected in 30 cm tall core tubes 9.5 cm in diameter. Core tubes were placed between two 1 cm thick clear polycarbonate rectangular plates, which were held together by nylon-threaded rod. The top plate had a bulkhead-style septum port to allow sampling of the headspace. Mud cores had about 1 L overlying lake water with a 100 ml gas headspace. The water–gas interface had an externally operated magnetic stirring apparatus to maintain the water and gas phases in equilibrium, and the magnet was high enough above the mud surface that the sediments were not disturbed by the gentle stirring. Since the volume of gas and water phases are needed to calculate gas concentration, the volume of the water phase was determined by measuring the height of the water from the mud surface to the surface of the water to the nearest mm, and the volume of the gas headspace was determined when the incubation was completed by weighing the core on a 2 kg scale, then filling the tube to the top with water and re-weighing it. The total amount of ethylene produced present in the gas and water phase was determined using Henry's Law, and ethylene solubility was determined according to a temperature relationship presented in Sander (1999).

Acetylene was introduced as saturated water, which reduces contamination by ethylene that is ubiquitous in both acetylene tanks and carbide. Saturation was achieved by bubbling acetylene through water for about 25 minutes while on a stir plate to ensure that the water is saturated and in equilibrium with the atmosphere (see Marino et al. 2003 for details). The acetylene was also contaminated with ammonia, which when added to core incubations can cause a reduction in N-fixation rates. To address this problem, the acetylene was first bubbled through a 10% solution of

sulfuric acid to trap ammonium in solution, resulting in ammonium concentrations that were similar to lake-water concentrations near detection. Incubations lasted 4 – 6 hours, over which ethylene production was linear. Ethylene samples were analyzed on a Shimadzu GC 8-A Flame Ion Detector (FID) using a Porapak N column, mesh size 80/100.

Soils and sediments can contain heterotrophic bacteria known to consume ethylene (Elsgaard and Andersen 1998; Jackel et al. 2004), whereas diatoms and higher plants can produce ethylene (Abeles et al. 1992; Lee and Baker 1992), so I collected cores to apply corrections for ethylene production and consumption. Ethylene was rarely produced without the presence of acetylene, but ethylene consumption was linear and ranged from 5 – 15 % over the course of the incubations. Corrections were minor and did not change qualitative results.

Moles of ethylene produced were converted to moles of N_2 fixed by assuming a 3:1 conversion factor. Although the relationship between moles of ethylene produced and moles of N_2 fixed is not always fixed at the assumed 3:1 value (Graham et al. 1980; Seitzinger and Garber 1987), other measured conversion factors for cyanobacteria from benthic environments are reasonably constrained, ranging from 1.9 to 5.4 (Howarth et al. 1988). Using a 3:1 conversion ratio also allows us to compare with the vast majority of other studies that also use this ratio to report and compare among measurements (e.g., Howarth et al. 1988; Alexander et al. 1989; Grimm and Petrone 1997; Higgins et al. 2001).

N fixation in water samples was measured according to methods described in Marino et al. (2003). Briefly, water samples were collected and filtered through fine mesh zooplankton net (335 micron) to remove large grazers. Ninety ml of sample were poured into 110 ml glass serum bottles, and 10 ml of acetylene-saturated water were added. Bottles were immediately capped and placed in the incubation tank.

Ethylene consumption and production blanks were also included, but no ethylene production was ever measured, and ethylene consumption was likely a result of analytical error rather than consumption processes. After 4 – 6 hr, samples were equilibrated by shaking for 2 minutes according to Flett et al. (1976), and samples were withdrawn and analyzed using a gas chromatograph as above. Although conversion factors for water-column production are more variable than for benthic environments (Howarth et al. 1998), ethylene production was also converted to nitrogen fixation rates using the same 3:1 conversion factor for moles of ethylene produced to moles of N_2 fixed.

Gross Primary Production. Measures of benthic metabolism were made using a chamber design similar to the ARA. Core tubes were again placed between two 1 cm thick clear polycarbonate rectangular plates, which were held together by four nylon-threaded rods. The top plate had a water-filled port to allow the insertion of a data-logging WTW Oxi 340i O_2 probe while preventing the chamber from experiencing air exchange with the atmosphere. Changes in oxygen consumption and production were recorded every 15 minutes over periods of dark and ambient lake light levels, each lasting approximately 12 hours. The probe was approximately 15 cm above the surface of the sediment and had a magnetic stirrer attached to its tip that kept the membrane from consuming oxygen. This stirrer and the floating stirrer were operated by the external magnetic stirrer as described above. Incubations were done at ambient lake light and temperature. Gross Primary Production (GPP) was calculated as the rate of O_2 production during the light period of the incubation and multiplied by 24 hours (there are 24 hours of daylight during summer months in the Arctic) and summed with estimates of respiration, which was calculated as the rate of O_2 consumption during the dark period and multiplied over 24 hours. This method assumes that respiration that occurs in the daylight is equal to nighttime respiration

(Wetzel and Likens 1991; Strickland and Parsons 1972). However, recent work has shown that daytime respiration is likely higher than dark respiration due to mitochondrial respiration, photorespiration, and the Mehler Reaction (Roberts 2004). Therefore, this method likely underestimates GPP, although it is commonly used (Howarth and Michaels 2000).

In order to assess the proportion of autotrophic N demand met by N fixation in benthic habitats, I assumed that the algal C:N molar ratio is 7 based on an analysis by Hillebrand and Sommer (1999) for benthic algae. NPP was calculated as the proportion of GPP ranging from 25 – 75% because determining actual NPP from GPP is not possible (Falkowski and Raven 1997). To compare the importance of water-column N fixation to primary production, I used ^{14}C-production measurements published in Miller et al. (1986), assuming that ^{14}C production was an approximate measure of NPP and that phytoplankton have a molecular C:N ratio = 106:16 (Redfield 1958).

Site description and intensive sampling, Lake Fog 2. Fog 2 is a headwater lake on the youngest surface (12,000 years old). It has no inlet stream, and inputs of water are a result of sheet flow. Inputs of cations and anions are probably high as Fog 2 has a relatively high conductivity (150 $\mu S\ cm^{-1}$). Water-column chlorophyll a is 1.8 $\mu g\ L^{-1}$, and water-column nutrients are near the analytical detection limit. Fog 2 has a surface area of 5.6 ha, a maximum depth of 20 m, and an average depth of 7.8 m, and the watershed-to-lake area ratio is high at 7.6. Incubation experiments of water-column production show strongest responses to N addition followed by N & P addition, indicating primary N limitation and secondary P limitation (Gross et al. unpublished data). Fog 2 has relatively high rates of benthic production (up to 300 mg $C\ m^{-2}\ day^{-1}$).

Benthic and water-column N fixation were measured 3 times throughout the summer of 2003 to evaluate the importance of N fixation to other sources of N inputs. Benthic N fixation was measured at 3 – 5 m and 5 – 8 m depths in a similar manner as described for survey lakes above. Water-column fixation measurements were also made 3 times throughout the season at depth intervals including 0, 1, 3, 5, 8, 12, and 15 m.

Construction of N budget for Lake Fog 2.

Rates of N fixation. Previous work indicates that there is no clear seasonal pattern in benthic or water-column N fixation rates throughout the summer (Chapter 2; Appendix 2), so estimates of N fixation were averaged to obtain an average daily N-fixation rate and multiplied by the length of the growing season, considered to be 60 days from 15 June – 15 August. Biological activity is assumed to be low or nonexistent in winter months, so estimates of growing-season rates are taken to be representative of annual rates. This is probably an underestimate of the annual rates because significant microbial activity has been shown to occur under the ice and during spring runoff events (O'Brien et al. 1997; Crump et al. 2003); in addition, I did not sample through ice-up, which typically occurs in early- to mid-September (Arctic LTER database.) However, this assumption allowed a comparison of this work with previous work conducted at the Toolik Lake field site (e.g., Whalen and Cornwell 1985; Alexander et al. 1978).

The annual estimate of N fixation between 3 – 5 and 5 – 8 meters was achieved by multiplying the seasonal estimate by the area of the lake bottom between 3 – 5 and 5 – 8 m depth profiles. The estimate below 8 m in Fog 2 was based on extrapolations of a N fixation–Irradiance (NI) curve generated for Fog 2 from 7 m (Gettel, Chapter 2). The curve was used to extend measurements down to the 2% light level in Fog 2,

which is on average at 12 m. Since this NI curve was developed on an areal basis rather than a biomass basis, using this curve may overestimate N fixation if cyanobacteria are less abundant at deeper depths; however, NI curves showed a similar alpha (initial response to light) at 3 and 7 m, indicating that this assumption may be reasonable (Chapter 2).

Runoff. Lake Fog 2 does not have an incoming stream, so inputs to the watershed are a result of sheet flow. Because inputs due to sheet flow are difficult to measure, a range of estimates were determined from other published studies conducted in nearby watersheds. Areal estimates of runoff from the Kuparuk River watershed (100 mg N m^{-2} $year^{-1}$; Peterson et al. 1992) and the Toolik Lake watershed (3,966 mg N m^{-2} lake $area^{-1}$ $year^{-1}$; Whalen and Cornwell 1985) were scaled to the area of the Fog 2 watershed and the area of the lake (units of input from runoff are in mg N m^{-2} of the lake). For the third estimate, I used estimates of N losses from various vegetation types from the Imnaviat watershed from Shaver et al. (1990). The dominant vegetation type in the Fog 2 watershed is dry acidic heath and moist acidic tundra (Walker et al. 1994), and in the Shaver et al. (1990) study, N losses from dry acidic heath and moist acidic tundra were 7 mg N $m^{-2}year^{-1}$ and 18.6 mg N $m^{-2}year^{-1}$, respectively. These losses were calculated from empirical measurements of N concentrations in soil water and estimates of water loss from the entire watershed, which was based on the difference between measured precipitation inputs and evapotranspiration (75 L m^{-2} $year^{-1}$). I considered this estimate of N inputs to Fog 2 via runoff to be the "best guess" since estimates from the Kuparuk River and Toolik Lake watersheds integrate losses over landscapes of different geologic surfaces and vegetation types that do not exist in the Fog 2 watershed (Walker et al. 1994; Hamilton 2003). Toolik and Kuparuk watersheds also have lake chains in their headwaters, which could affect

nutrient output (Kling et al. 2000). The value calculated from this best guess is represented in parentheses in the budget table.

N return from sediments and losses of N by burial. Loss of N in Fog 2 due to burial was estimated from Toolik Lake measurements, which was estimated as 294 mg N $m^{-2}year^{-1}$ using estimates developed by Whalen and Cornell (1985). Sediment budgets were constructed in which inputs were calculated from data from sediment traps that were deployed for summer months and extrapolated year-round and compared with sedimentation rates determined using ^{210}Pb sediment profiles and aluminum : nutrient ratios (Whalen and Cornwell 1985). Measurements of N accumulation under the ice during the winter and from depth-sediment profiles were used to estimate net N mineralization as 154 – 308 mg N m^{-2} $year^{-1}$ (Whalen and Cornwell 1985; Alexander et al. 1989). Because data on annual N flux or sedimentation from Fog 2 are not available, these Toolik Lake estimates were used. Lake Fog 2 and Toolik Lakes are similar in some important respects; both are deep oligotrophic lakes; much of the watershed in Toolik is also on the young (12,000 year) surface; both lakes have similar water-column chlorophyll a concentrations (~1.5 μg L^{-1}), particulate carbon concentrations (25 – 30 μM), and sediment organic content of 12 – 18%.

Losses due to denitrification and stream outflow. Gaseous losses due to denitrification are difficult to assess in oligotrophic systems because measurements are sparse and extremely variable. There is one study available for Toolik Lake (Klingensmith and Alexander 1983), but this study probably reports underestimates of denitrification because the acetylene block method, which inhibits nitrification and therefore the supply of nitrate, was used. Therefore, I also considered two other studies from oligotrophic lakes: central Ontario lakes (Molot and Dillon 1993), and Lake Otrasket in northern Sweden (Jonsson and Jansson 1997) which reported ranges

in losses from 1 – 1268 mg N $m^{-2}year^{-1}$, both using sediment-budget techniques. I used this range to calculate gaseous losses, but because Fog 2 and Toolik Lake are more similar to one another than are the lakes in these studies, I considered the Toolik measurement (318 mg N $m^{-2}year^{-1}$) to be the best-guess estimate.

Losses from one outlet stream required information on discharge and N concentration. Since stream-discharge information is not available, I used estimates of runoff and evapotranspiration from Shaver et al. (1990) and assumed that lake volume remains constant on a yearly time scale. Shaver et al. (1990) estimated runoff to be 75 L m^{-2} $year^{-1}$ (34,257 m^3 of runoff for Fog 2) and evapotranspiration to be 76 L m^{-2} $year^{-1}$ (4298 m^{-3} $year^{-1}$ from the surface of Fog 2). The balance of runoff and evaporation leaves 29,998 m^3 of water per year exiting the lake through stream flow. Since the small outlet stream is frozen solid over the winter, I assume that most of the discharge occurs in the summer. The output of N from stream flow was estimated by using depth-profile measurements of summertime TDN and particulate N (PN) from Lake Fog 2 as described above (Arctic LTER database). Because concentrations did not vary with depth, the summertime average was calculated by averaging the depth profile information over three dates in 2003.

RESULTS AND DISCUSSION.

Prevalence of N fixation across the landscape. Benthic N fixation was detected in all survey lakes and ranged from 0.2 – 1.5 mg N m^{-2} day^{-1} and averaged 0.53 mg N m^{-2} day^{-1} (Figure 1.2, Table 1.2). Water-column N fixation was detected in all but one survey (lake S-6) and ranged from 0.37 – 1.62 μg L^{-1} (See Appendix 2 for depth-profile information). On an areal basis, water-column fixation integrated over 3 m ranged from 0 – 2.56 mg N $m^{-2}day^{-1}$ and averaged 1.6 mg N m^{-2} day^{-1} (Figure 1.2, Table 1.2). Integrated over 3 m depth, total N fixation ranged from 21 to 276 mg N

m^{-2} $year^{-1}$. The portion that benthic N fixation contributed to the total integrated rate (benthic + water column) at 3 m was variable from lake to lake, ranging from 6 to 100%, and averaged 28%. In one case (lake S-6), no water-column fixation was detected, and benthic N fixation accounted for 100% of the integrated areal rate. In some lakes, benthic N fixation was nearly equal to water-column rates (GTH 85, GTH 74). In most other lakes, benthic N fixation was 10 – 20% of the total integrated rate at 3m.

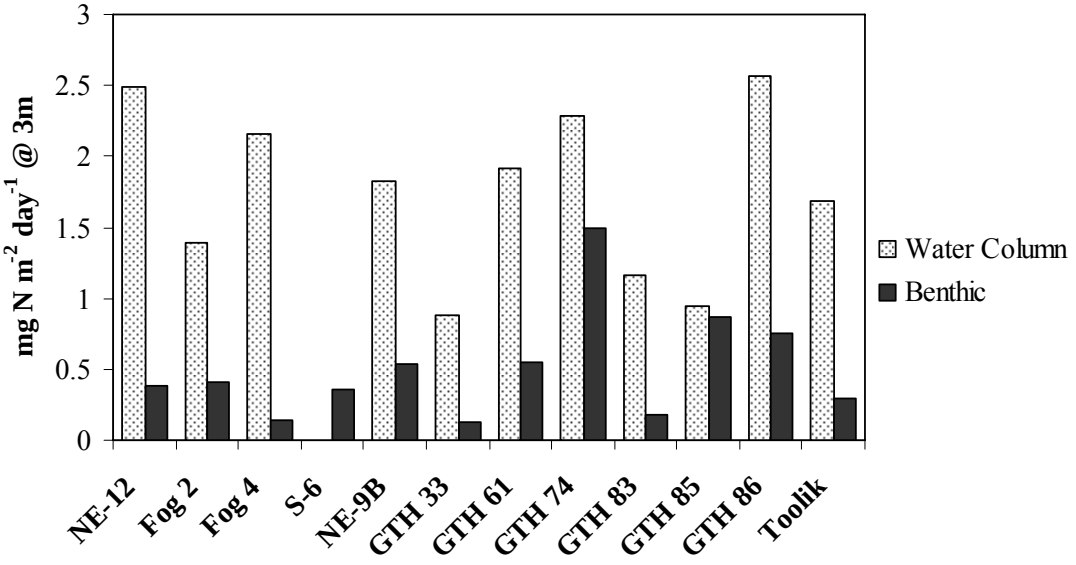

Figure 1.2. Benthic N fixation (black bars) and water-column N fixation (stippled bars) in survey lakes conducted in the vicinity of Toolik Field Station, Alaska.

Table 1.2. Rates of benthic and water-column fixation for lakes in the vicinity of Toolik Field Station. The annual rate assumes a 60-day growing season (see text).

Lake	Benthic N fixation at 3m (mg N m^{-2} day^{-1})	Water-column N fixation integrated over 3m (mg N m^{-2} day^{-1})	Total annual N fixation at 3m (mg N m^{-2} $year^{-1}$)	% Benthic N fixation of total integrated at 3m	Water-column N fixation integrated over entire water column (mg N m^{-2} day^{-1})
NE-12	0.38	2.5	173	13	7.62
Fog 2	0.4	1.39	107	23	6.69
Fog 4	0.14	2.15	137	6	2.15
S-6	0.36	0	22	100	0
NE-9B	0.54	1.83	142	23	3.22
GTH 33	0.12	0.88	60	12	2.72
GTH 61	0.55	1.91	148	22	2.54
GTH 74	1.5	2.29	227	40	3.62
GTH 83	0.18	1.16	80	14	3.14
GTH 85	0.86	0.94	108	48	1.91
GTH 86	0.76	2.56	199	23	4.27
Toolik	0.29	1.69	119	15	10.44

The results from the survey show that both benthic and water-column N fixation are present in lakes across many different landscape types in arctic Alaska. Benthic N fixation comprised a significant portion of the integrated areal rates of N fixation; however, water-column N fixation was surprisingly important, contributing more to the areal rates of N fixation than has been documented for oligotrophic lakes elsewhere (e.g., Howarth et al. 1988 review). The contribution of water-column N fixation to the total integrated areal rate is underestimated because data from the survey lakes integrated only in the epilimnion up to 3 m. When water-column N fixation rates are integrated over the depth of the entire lake (depths listed in Table 1.1; data in Table 1.2), the areal rates are up to as much as 6 times higher. These results indicate that N input via fixation in both the benthos and water column is a significant N input in lakes across the arctic landscape.

Rates of benthic N fixation measured in this study are generally lower than, but of the same magnitude as, rates reported for other oligotrophic lakes sediments in high altitudes, (0.2 – 1.5 mg N $m^{-2}day^{-1}$ in this study vs. 2 – 11 mg N m^{-2} day^{-1} in others [Table 1.3]). Compared to benthic N fixation in oligotrophic systems in lower latitudes, however, arctic rates are generally lower by two orders of magnitude (e.g., 1 – 180 mg N $m^{-2}day^{-1}$ in lower latitudes) except in a few instances where tropical salt-water systems are also very low, exhibiting fixation rates similar to those found in this study. The difference in these rates may be due to a number of environmental factors, including low light and temperature regimes, but more research is needed to understand why benthic rates in the Arctic appear to be generally lower than those in other oligotrophic systems.

Table 1.3. Benthic N fixation rates from ecosystems from the Tropics to the Arctic.

Habitat	Original reported Units	mg N m^{-2} day^{-1}	Reference	Notes
Algal Mats and Periphyton, Tropical and Subtropical				
Marine				
algal turf - Caribbean coral reef (Tague Bay, St. Croix)	nmol C_2H_4 cm^{-2} hr^{-1}	8	Williams and Carpenter 1997	a
algal turf - (Britomart Reef, Great Barrier Reef, Australia)	nmol C_2H_4 cm^{-2} hr^{-1}	81	Wilkinson and Sammarco 1983	a
algal turf (Eneewetak Atoll, Marshall Islands)	nmole N cm^{-2} hr^{-1}	180	Wiebe et al. 1975	a
coral reef - (Great Barrier Reef, Australia)	nmol C_2H_4 cm^{-2} hr^{-1}	3	Wilkinson et al. 1984	b
cyanobacterial mat - high saline coastal lagoon (Storr's Lake, Bahamas)	μmol C_2H_4 m^{-2} hr^{-1}	0.0	Pinckney et al. 1995	a
cyanobacterial mat - low saline coastal lagoon (Pigeon Creek Lake, Bahamas)	μmol C_2H_4 m^{-2} hr^{-1}	1	Pinckney et al. 1995	a
Freshwater				
mixed cyanobacterial mat - desert stream (Sycamore Creek, Arizona)	mg N m^{-2} day^{-1}	83	Grimm and Petrone 1997	c
Anabeana - desert stream (Sycamore Creek, Arizona)	mg N m^{-2} day^{-1}	144	Grimm and Petrone 1997	c
epilithic algae - desert stream (Sycamore Creek, Arizona)	mg N m^{-2} day^{-1}	42	Grimm and Petrone 1997	c
periphyton - Amazonian floodplain lake (Lake Calado, Brazil)	mmol N m^{-2} day^{-1}	32	Doyle and Fisher 1994	d
periphyton - Amazonian floodplain lake (Lake Batata, Brazil)	nmol N dm^{-2} hr^{-1}	0.5	Enrich-Prast and Esteves 1998	e
periphyton- tropical lake (Lake Malawi)	mg N m^{-2} day^{-1}	13	Higgins et al. 2001	f

Notes: [a]Daytime hourly rate X 12 hours (tropics); [b]average of outer reef, inner reef, and middle-shelf reefs, used daytime hourly rate X12 hours (tropics); [c]daytime incubation, chamber with flow incubation; [d]calibrated with ^{15}N, periphyton growing on macrophytes, number is integrated over year-round sampling and light and dark incubations, estimate is the mean between 1989 and 1990 N-fixation rates; [e]one-day experiment during July flood phase, periphyton associated with culms of a macrophyte, *Oryza glumaepatula* Steud; [f]hourly rate from averaged mean rates form 2 m depth at 5 sites, conversion factor is 4:1; [g]no activity was observed late autumn through spring, so assumed 175 days of activity in late spring through mid autumn; [h]integrated over whole year, so converted to daily units by dividing by 365 and converted to areal units by dividing by surface area of pond; calibrated with ^{15}N; [i]Acetylene Reduction (using 3:1) conversion factor, unvegetated sand and mud is a mean of those habitats (n=3), periphyton community is also mean (N=3). Assumed 10 hours for per day; [j]maximum rate [k]maximum hourly rate X 20 hours, daylight (arctic, August); [l]cited from Bergman and Welch (1990), maximum hourly rate x 10 hours daylight (Aug – Sept.)

Table 1.3. (Continued).

Habitat	Original reported Units	mg N m^{-2} day^{-1}	Reference	Notes
Algal Mats and Periphyton, Temperate and mid latitudes				
Freshwater				
Moss and wood substrate - Coniferous forest stream (A.J. Andrews Experimental Forest, Oregon)	g N m^{-2} yr^{-1}	4	Triska et al. 1984	g
Nostoc - oligotrophic pond sediment (Mare's Egg Spring, Oregon)	moles N yr^{-1}	11	Dodds and Castenholz 1987	h
Unvegetated sand and mud – (Mirror Lake, NH)	μgm N m^{-2} hr^{-1}	0.2	Moeller and Roskoski 1977	i
Periphyton, including *Nostoc* and *Anabeana* colonies (Mirror Lake, NH)	μgm N m^{-2} hr^{-1}	3	Moeller and Roskoski 1978	i
Algal Mats and Periphyton, Alpine and Arctic				
Freshwater				
Nostoc sp.- seasonal mountain stream (Rocky Creek, California)	mg N m^{-2} yr^{-1}	11	Horne and Carmiggelt 1975	j
periphyton - arctic lake (Spring Lake, NWT, Canada)	mg N m^{-2} hr^{-1}	11	Bergmann and Welch 1990	k
periphyton - mountain lake (Crater Lake, CA)	mg N $m^{-2}hr^{-1}$	2	Loeb and Reuter 1981	l
periphyton - mountain lake *(*Lake Tahoe, *CA)*	mg N $m^{-2}hr^{-1}$	3	Loeb and Rueter 1981	l
periphyton - mountain lake (Fallen Leaf, CA)	mg N $m^{-2}hr^{-1}$	1	Loeb and Rueter 1981	l
periphyton - mountain lake (Donner, CA)	mg N $m^{-2}hr^{-1}$	2	Loeb and Rueter 1981	l
periphyton - mountain lake (Castle, CA)	mg N $m^{-2}hr^{-1}$	2	Loeb and Rueter 1981	l

The water-column rates presented here show very different results from the benthic N fixation data when compared with measured rates in other oligotrophic systems. Water-column N fixation rates are 2 – 3 orders of magnitude higher than water-column N fixation rates in oligotrophic lakes in Ontario and the Great Lakes, which range from 0 – 0.3 mg N m^{-2} $year^{-1}$ as reported by Howarth et al. (1988). In fact, the maximum water-column rate in our study approaches the minimum reported rates for eutrophic lakes in the Howarth et al. (1988) review, which ranged from 200 – 9,200 mg N m^{-2} $year^{-1}$. The rates in these lakes are the same order of magnitude as many studies reporting areal rates of N fixation in oligotrophic oceanic regions, which range from 0.03 – 55.37 mg N $m^{-2}day^{-1}$ from the North Pacific, Arabian Sea, North Atlantic, and Caribbean as summarized in Capone et al. (2005) and Montoya et al. (2004). The results in this study add to the growing body of evidence in both freshwater and oceanic systems that N fixation in oligotrophic systems may be more prevalent than was previously thought.

In one of the only other studies that has published data on N fixation in Toolik-area lakes, Alexander et al. (1989) used the same analytical technique (acetylene reduction assay), to measure water-column and benthic N fixation in Toolik Lake in 1976, 1979, and 1980. They detected nitrogenase activity in every sample during 1976 and found that water-column rates were similar to data presented here (2.2 – 11.6 mg N m^{-2} day^{-1}). They also presented benthic N-fixation rates that when multiplied over a 5 cm depth to calculate an areal rate show 0.14 mg N m^{-2} day^{-1}, also well within the range of the estimates shown in this study. Although Alexander et al. (1989) found similar rates in their 1976 survey, they did not detect the same spatial and temporal extent of nitrogenase in subsequent surveys in 1979 and 1980 in Toolik Lake. I also found that water-column N fixation was not always detectable (as in Lake S-6 and early in the season in some lakes; Appendix 2). However, water-column N

fixation was detected throughout the season in Lake Fog 2, and in most other lakes most of the time. Water-column N fixation dominated areal rates of total integrated N fixation in most of the lakes sampled for this study. More work is needed to document the seasonal dynamics of water-column N fixation to evaluate its contribution over time.

Importance of N fixation to primary production and N return from sediments. Little is known about how much N fixation contributes to primary production, though it is thought to be rather small even in productive ecosystems because most of the demand is met by recycling (Howarth et al. 1988). Using estimates from Miller et al. (1986) for water-column production in Toolik Lake, water-column N fixation accounts for, on average, 3 – 7% of N demand by primary production. Howarth et al. (1988) also show that N fixation for planktonic systems is generally low (0.3 – 8.9%), even in eutrophic systems when N fixation rates are high. This contribution is also similar to results from benthic habitats where the average contribution of N fixation to NPP in the survey lakes varied from 0.21% – 4.02%. Most of this variation is due to variation in rates of N fixation, and not to the variation in the range of NPP to GPP I assume (25% to 75%; Table 1.4).

Although the contribution of N fixation to primary production is small for both benthic and water-column habitats, N fixation contributes to the recycled pool of available N, which can fuel primary production many times. For example, the N return from the sediments of Toolik Lake is 154 – 308 mg N m^{-2} $year^{-1}$ (Whalen and Cornwell 1985; Alexander et al. 1989) and assumed to be similar for Fog 2 (Table 1.5). Given a 60-day growing season, benthic N fixation at 3m in Toolik Lake is 17.3 mg N m^{-2} $year^{-1}$ and accounts for 5.5 – 11% of this N-flux. Using the more detailed depth-integrated measurement of N fixation from Lake Fog 2, benthic N fixation contributes a similar amount, 8 – 16 % of the flux. Water-column N fixation exceeds

this return from the sediments by a factor of about 2, but water-column N fixation may not be important to this flux if recycling occurs predominately in the water column. These results indicate that N fixation is important to the inorganic N pool in lake sediments and comprises a significant portion of the N flux from sediments on an annual basis. Because N fixation contributes to the pool of N available for recycling, it is also useful to evaluate the importance of N fixation relative to other inputs.

Fog 2 budget and importance of N fixation relative to other N inputs. Although the uncertainty in estimates for inputs and outputs for nitrogen is wide, best-guess estimates show that the budget is nearly balanced, with outputs exceeding inputs by about 2145 g N $year^{-1}$ (37 mg N m^{-2} lake $area^{-1}$ $year^{-1}$), or about 5.7% of the total input (Table 1.5). Using best-guess estimates, the largest losses of N in Fog 2 appear to be burial and denitrification (42 and 45%, respectively), while losses due to stream outflow are low (about 12.5% of total output). N return from sediments in Fog 2 is about 23 – 50% of inputs, indicating that recycling is probably important to the N economy of this lake.

Benthic N fixation was the smallest source of N to the lake at about 1% of the total inputs at 6 mg N m^{-2} $year^{-1}$ (Figure 1.3; Table 1.5). The next largest source was precipitation at 25 mg N m^{-2} $year^{-1}$, or 3% of total inputs. Watershed inputs were the second largest source of N to the lake at 141 mg N m^{-2} $year^{-1}$ (21% of total). Water-column N fixation dominated the N inputs to Lake Fog 2, contributing 491 mg N m^{-2} $year^{-1}$, or 74% of the N inputs to Lake Fog 2 (Figure 1.3; Table 1.5).

Table 1.4. Importance of N fixation to autotrophic N demand. N demand is shown assuming net primary production (NPP) ranges from 25 – 75% of GPP, and the C:N ratio for benthic algae=7 (Hillebrand and Sommer 1999).

Lake	GPP	N Demand (mg N m^{-2} day^{-1})	% N demand met by N fixation
Fog 2	227 ± 42	10.03 - 20.08	1.34 - 4.02
NE-12	388 ± 24	17.11 - 51.33	0.74 - 2.22
S-6	812 ± 25	35.83 - 107.49	0.58 - 1.75
GTH 33	174 ± 14	7.69 - 23.07	0.81 - 1.61
GTH 61	473 ± 28	20.86 - 62.57	0.88 - 2.63
GTH 74	520 ± 21	22.94 - 68.82	2.18 - 6.54
GTH 83	677 ± 82	29.85 - 89.56	0.21 - 0.62
GTH 85	724 ± 65	31.94 - 95.83	0.90 - 2.70
GTH 86	145 ± 51	6.41 - 19.22	0.40 - 1.2

Table 1.5. N budget for Lake Fog 2. The column labeled "Total N" is the amount of N per year entering, leaving, or being recycled in the entire lake. The column labeled Fog 2 is the Total N scaled to areal rates for Fog 2. These numbers were computed by using the data presented in the "measurement" and the "scaling factor" columns. The estimates in parentheses are the best estimates for the budget based on considerations and assumptions explained in the text.

	Measurement (mg N m^{-2} $year^{-1}$)	**Scaling Factor**	**Total N (grams N $year^{-1}$)**	**Fog 2 (mg N m^{-2} $year^{-1}$)**	**Source**
Input:s					
Precipitation	25	Area of Lake = 56,562 m^2	**1,414**	**25**	Arctic LTER database
N fixation, benthic		Depth integrated to 12 m (Total area of Lake = 56,562 m^2)	**362**	**6**	
N fixation, water column,		Volume integrated over 16m (Total Volume = 498,440 m^3)	**27,750**	**491**	This Study
Run-off					
Sagavanirktok Toposequence	6.9 - 18.6*	Area of Fog 2 watershed = 456,764 m^2	3,152 - 8,496 **(7,993)**	**(141)**	Shaver et al. 1990
Total Inputs			**32,678 -38,022 (37,474)**	**578 – 1,330 (663)**	
Recycling:					
Nitrogen release from sediments	154-308	Area of Lake = 56,562 m^2	**8,711 - 17,421**	**154 – 308**	Alexander et al. 1989, Whalen and Cornwell 1985
Output:s					
Burial	294	Area of Lake = 56,562 m^2	16,629	294	Whalen and Cornwell 1985
Denitrification					
Toolik	318	Area of Lake = 56,562 m^2	**17,987**	318	Klingensmith and Alexander 1983
Stream outflow			**5,003**	88	
Total Outputs		Area of Lake = 56,562 m^2	**39,619**	**700**	

*Expressed on a per m^2 of watershed

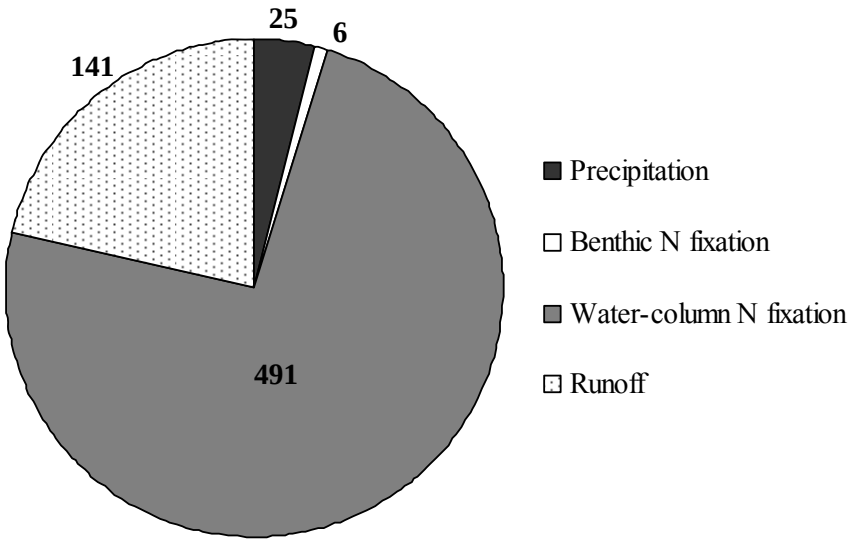

Figure 1.3. Inputs to Fog 2 (mg N $m^{-2}year^{-1}$).

Surprisingly, water-column N fixation contributes more than 75% of the N input lake, while benthic N fixation is responsible the smallest portion of N inputs. It is surprising that water-column N fixation contributed so much to the total N budget of Lake Fog 2, because it contributed only ~0.02% of N in other oligotrophic lakes (summarized in Howarth et al. 1988). In fact, the results for Fog 2 are more similar to what has been reported for eutrophic lakes with water-column N fixation which comprises a much larger portion of total N inputs (5 – 82% ; Howarth et al. 1988). In a notable exception, water-column N fixation contributed 82% to the N inputs for an oligotrophic salt lake (Pyramid Lake), which was considered an outlier by the Howarth et al. (1988) review; perhaps water-column N fixation is more important than was previously thought, especially given the sparse number of measurements made to date.

The Fog 2 budget corroborates the results from the survey work and indicates that N fixation is important to the N economy of this lake. Extrapolating the one-time

measurements of survey work to annual rates assuming a 60-day growing season, benthic N fixation from the survey ranges from 7 – 89 mg N m^{-2} $year^{-1}$, and water-column N fixation ranges from 0 – 452 mg N $m^{-2}year^{-1}$. Data from the Arctic LTER database show that bulk N deposition is 14 mg N m^{-2} in the summer, and nearly doubles during the winter, for a total of 25 mg N m^{-2} $year^{-1}$. Thus, benthic N fixation is about equal to or exceeds atmospheric inputs on an areal basis.

The relatively low contribution of benthic N fixation to the whole-lake N budget is also surprising given that benthic processes are considered important in oligotrophic systems. I may underestimate the importance of benthic N fixation because it was not measured cobble substrate, which is common at the ice-scour depth up to 3 m in depth. This is an important omission to consider because 20% of the area of the bottom in Lake Fog 2 is from 0 – 3 m, and other researchers have found that epilithic algae in oligotrophic lakes supply as much as a third of N inputs (e.g., Lake Malawi – Higgins et al. 2001; Lake Tahoe as reported by Howarth et al. 1988). Studies that have examined algal taxonomy on cobble substrates in Toolik Lake suggest that heterocystic cyanobacteria aren't common (Cuker 1983); however, little is known about the epilithic algal communities in other lakes in the vicinity of Toolik.

The geomorphology of Lake Fog 2 also affects the relative contribution of water-column and benthic N fixation. Fog 2 is deep relative to most other lakes in the vicinity of Toolik (Burkart et al. In prep.) and has a small watershed : lake area ratio (7.6), indicating that N inputs from the watershed are more likely to be relatively small compared with inputs from *in situ* N fixation. Other headwater lakes in the region also have small watersheds and are relatively deep (Arctic LTER database); thus Fog 2 is probably representative of other headwater lakes but is not typical of many other lakes in the area, which are shallower and have larger watersheds. In those lakes, benthic N

fixation is probably more important than in Fog 2, and *in situ* N fixation is likely less important than inputs from the watershed.

Lake Fog 2 is on a young surface and may receive enough phosphorus input from weathering sources to favor N fixation either by reducing the N:P ratio below Redfield ratio or by alleviating P limitation of N fixers (Appendix 1; Chapter 2; Vitousek and Howarth 1991; Vitousek et al. 2002). Losses of N appear to exceed inputs in Fog 2, which may also maintain low N:P ratio favorable for N fixation and may be the reason why Fog 2 appears to remain N-limited despite high inputs from fixation.

Comparison of aquatic and terrestrial N fixation rates in the Arctic. Rates of N fixation in aquatic ecosystems in the Arctic vary from 0.14 – 672 mg N m^{-2} $year^{-1}$ as reported in this and other studies (Table 1.6). The lowest measured rates in Arctic aquatic ecosystems occurred in the Kuparuk River (0.14—0.36 mg N $m^{-2}year^{-1}$); intermediate rates of N fixation occurred in Barrow-area ponds (12—28 mg $N^{-2}year^{-1}$); and the highest rates of N fixation occurred in lakes in the vicinity of Toolik Field Station (22—227 mg N $m^{-2}year^{-1}$). The variation in these habitats may be a result of nutrient limitation status; for example, the Kuparuk River is phosphorus, not N-limited (Peterson et al. 1993), which could explain the low rates of N fixation. Barrow-area ponds may exhibit lower areal rates of N fixation because they are shallower and have smaller depth-integrated rates; it may also be a result of higher latitude, colder temperatures, and shorter growing season. The rates reported for Toolik-area lakes in this study are within the range that has previously been reported (Alexander 1989), but these data suggest that N inputs via fixation is an important input that was previously overlooked.

N fixation in the Toolik-area lakes was also the same order of magnitude as rates of terrestrial N fixation, which ranged from 19—255 mg N $m^{-2}year^{-1}$ for

measurements on tundra ecosystems; Table 1.6). The maximum rate of aquatic N fixation exceeds that the maximum terrestrial rate by a factor of ~2.5 (672 mg N m^{-2} $year^{-1}$). The exception to this generality is a model by Cleveland et al. (1999) that predicts higher (280—940 mg N $m^{-2}year^{-1}$) rates of terrestrial N fixation rates than have been reported from measurements. These patterns suggest that lakes are as important as terrestrial ecosystems as sources of N input to the landscape, and may in some instances be 'hot-spots' for N fixation.

In summary, N fixation in lake ecosystems in the Arctic was previously considered to be unimportant because rates are low relative to other ecosystems. However, N fixation in lake ecosystems is prevalent across the landscape and appears to contribute significantly to their N economies. Inputs of N from benthic environments is equal to or greater than inputs from precipitation on an areal basis; water-column N fixation is much higher than was previously found for other oligotrophic systems, contributing as much 75 % of the total N inputs to one lake. N fixation represents a source of new N that can support new production, and it contributes significantly to the pool of available for recycling. Comparison of aquatic rates of N fixation with those of terrestrial ecosystems shows that N input from benthic and water-column N fixation is also an important pathway of N input across the arctic landscape.

Table 1.6. Rates of N fixation in aquatic and terrestrial habitats in the Arctic.

Habitat	Original Reported Units	mg N m^{-2} $year^{-1}$	Method	Conversion Factor	Calibrated with ^{15}N?	Source
Aquatic						
Lake Fog 2, water column+benthic	mg N $m^{-2}$$year^{-1}$	497	AR	3	No	This study
Toolik Lake Water Column (0-5m)	mmol m^{-2} day^{-1}	118 – 672	AR	3	No	Alexander et al. 1989
Toolik Inlet Stream, Epilithon	mmol m^{-2} day^{-1}	168	AR	3	No	Alexander et al. 1989
Kuparuk River						
Reference (Unfertilized)	mg N m^{-2} day^{-1}	0.14	AR	3	No	Lawerence et al. unpubl.
Fertilized (P)	mg N m^{-2} day^{-1}	0.36	AR	3	No	Lawerence et al. unpubl.
Tundra Pond Sediment (Barrow)	mmol m^{-2} day^{-1}	1.2	AR	3	No	Alexander et al. 1989
Tundra Pond Sediment (Barrow)	mg N $m^{-2}$$year^{-1}$	28	AR	3	Yes	Prentki et al. 1980
Water column+ benthic integrated 3m						
11 lakes in the vicinity of Toolik Lake	mg N m^{-2} day^{-1}	22 – 227	AR	3	No	This study
Water-column integrated to depth						
11 lakes in the vicinity of Toolik Lake	mg N m^{-2} day	0 – 626				This study
Terrestrial						
Tundra (Barrow Region)	mg N $m^{-2}$$year^{-1}$	100 – 143	AR	1.5	Yes	Alexander et al. 1978
Tundra		69.3				Barsdate and Alexander 1975
Immnaviat Watershed (acidic tundra)	mg N $m^{-2}$$year^{-1}$	109 – 133	AR	3	No	Hobara et al. in press
Acidic tundra - lichens *Peltigera aphthosa* and *Peltigera polydactyla*	kg ha^{-1} $year^{-1}$	10 – 25	AR	3.2	Yes	Weiss et al. 2005
Non-acidic tundra - lichens *Peltigera aphthosa* and *Peltigera polydactyla*	kg ha^{-1} $year^{-1}$	1.5 – 3.5	AR	3.2	Yes	Weiss et al. 2005
Tundra (Alaska, Scandanavia, Canada)	mg N $m^{-2}$$year^{-1}$	19 – 255	Lit. Review			Chapin and Bledsoe 1992
Arctic tundra	kg ha^{-1} $year^{-1}$	280 – 940	Model	-	-	Cleveland et al. 1999

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CHAPTER 2

LIGHT AND NUTRIENT CONTROLS OF BENTHIC NITROGEN FIXATION IN OLIGOTROPHIC, ARCTIC LAKES: RESULTS FROM WHOLE-LAKE FERTILIZATIONS AND SEDIMENT-CORE EXPERIMENTS

INTRODUCTION.

Currently, most lakes in arctic Alaska are oligotrophic but are expected to increase in productivity because of higher inputs of nitrogen (N) and phosphorus (P) from human activity and climate warming (Hobbie et al. 1999; Hinzman et al. 2005). In lakes undergoing eutrophication in the temperate zone, excess inputs of P are often considered to be the primary cause because N_2-fixing cyanobacteria compensate for N limitation, and P limitation of net primary production is maintained (Schindler 1977, Flett et al. 1980, Hendzel et al. 1994, Findlay et al. 1994). However, this paradigm doesn't apply for many oligotrophic lakes because cyanobacteria generally do not form blooms, and N limitation is prevalent (Howarth et al. 1988b). Excess N inputs from N deposition and other forms of human development have caused increases in lake productivity and changes in algal community structure in oligotrophic lakes in the Western United States (Goldman 1988, Baron et al. 2000, Nydick et al. 2003), and the role of N in the eutrophication of lakes in the Northern Hemisphere is becoming increasingly appreciated (Bergstrom and Jansson 2006).

Even though oligotrophic lakes are often N-limited, little is known about what prevents N_2 fixation from alleviating N limitation in these environments (Vitousek et al. 2002; Howarth et al. 1988a). N_2 fixation itself may be limited by P availability because N_2 fixers have a higher P requirement than many other autotrophs (Vitousek and Howarth 1991; Vitousek et al. 2002). As N and P inputs to arctic lakes gradually increase, productivity will likely increase (Hobbie et al. 1999; O'Brien et al. 2005), but

the role of N_2 fixation in this process is unknown. Will increases in nutrients stimulate N_2 fixation and cause lakes to be P-limited? Or, will increased nutrient inputs cause a compensatory decline in N_2 fixation and maintain them in N-limited status?

In contrast with their more productive counterparts, oligotrophic systems have low water-column algal biomass, their light penetration is high, and lake bottoms are often productive (Ramlal et al. 1994; Vadeboncoeur et al. 2003). N_2-fixing cyanobacteria are common in oligotrophic lake sediments, and some researchers have hypothesized that benthic N_2 fixation in oligotrophic systems may be important to their N budgets and to their benthic primary producers (Moeller and Roskoski 1978; Reuter et al. 1986; Bergmann and Welch 1990; Higgins et al. 2001). Understanding how benthic N_2 fixation and primary production are controlled by nutrients is important to understanding how oligotrophic lakes respond to environmental changes that result in increased nutrient inputs.

There are very few studies that examine the response of benthic N_2 fixation to N and P supply and eutrophication (but see Bergman and Welsh 1990 for an exception). Sediment-based processes, however, may change the relative availability of these nutrients differently from expected in the water column (Howarth et al. 1988a). For example, sediments tend to be low-oxygen environments, so P availability may be increased as a result of the chemical reduction and solubility of Fe–P binding complexes (Wetzel 2001). In addition, mineralization in the sediments may increase N availability relative to the water column, which may suppress N_2 fixation (Capone 1988). In arctic, oligotrophic lakes, sediments tend to be less reducing than their more productive temperate counterparts, and iron and manganese oxides are abundant (Cornwell and Kipphut 1992), which may in turn reduce the bioavailability of P (O'Brien et al. 2005). In addition, mineralization rates in

oligotrophic lake systems are low, while N demand by primary producers is high (Alexander et al. 1989), which may maintain low levels of N availability in sediments.

Light may also control N_2 fixation in benthic environments, especially in oligotrophic systems where benthic N_2 fixers are predominately autotrophic. Autotrophs fix N_2 using energy captured by photosynthesis, but oxygen created during photosynthesis can damage the nitrogenase enzyme (Paerl 1990). N_2 fixers have different strategies to deal with this constraint. They may rely on stored carbon to fix N_2 during periods of low light; or, they may rely on recently synthesized carbon at high light levels to fuel N_2 fixation in heterocysts that protect the nitrogenase enzyme from oxygen. The responses of N_2 fixation to increasing light levels in different environments are variable, ranging from saturating, linear, and inhibitory (Higgins et al. 2001, Grimm and Petrone 1997, Lewis and Levine 1984). Light may be an important factor affecting N_2 fixation in arctic lake sediments because summertime daylight occurs 24 hours a day, and water clarity is high.

The purpose of this study is to examine nutrient and light effects on benthic N_2 fixation and primary production in oligotrophic, arctic lakes. I compare the responses to low-level fertilization of a deep and shallow lake with two reference lakes and examine the mechanisms behind these responses in intact mud core incubations in which nutrients and light are manipulated in controlled laboratory conditions. Low-level fertilization is designed to reflect gradual increases in nutrient inputs that will likely occur as a result of warming climate and increased human activity in arctic regions, and specifically to examine the role of the benthos in whole-lake responses to increased nutrient inputs as lakes undergo eutrophication.

METHODS.

Site description.

Toolik Field Station is located in arctic Alaska (68°37′N, 149°35′W), about 240 km above the Arctic Circle in the northern foothills of the Brooks Mountain Range (Figure 2.1). The landscape is rolling tundra terrain underlain by continuous permafrost, and lakes in this region are dimictic and commonly shallow (3 – 15 m) glacial kettles. The arctic summer growing season is defined by the period when lake surfaces are ice-free and lasts from mid-June to mid-September, and maximum epilimnetic temperatures range from 13 – 18 °C during summer months. Lakes in the region are considered ultra-oligotrophic (Miller et al. 1986), and water-column concentrations of ammonium, nitrate, and phosphate are near detection limit, with concentrations below 0.1 μM (Arctic LTER database (http://ecosystems.mbl.edu/arc/default.htm).

Water-column ^{14}C-primary production measurements range from 12 – 16 g C m^{-2} $year^{-1}$ (Miller et al. 1986). Secchi depth ranges 6 – 10 m in the summer, and areal rates of benthic production equal that of water-column production in sediments above the thermocline, which is about 5 m in the summer (Artic LTER database). Lake bottoms are generally composed of extremely fine-grained, unconsolidated sediment. The N_2-fixing cyanobacteria *Nostoc sp.* forms colonies as large as 2 cm in diameter and is visibly prevalent on sediment surfaces in many lakes, including the lakes in this study (personal observation).

Overall experimental design. To examine nutrient and light controls on benthic N_2 fixation and primary production, I evaluate results from on-going whole-lake fertilizations of a deep and shallow lake and compare these results to pre-fertilization conditions and to unfertilized reference lakes. Treatment and reference lakes were sampled before the start of fertilization in 2000, and treatment lakes were

fertilized with N and P in the summer growing seasons in starting in 2001. This paper presents data from 2000 – 2003. Data from the whole-lake fertilization experiment were analyzed using repeated measures ANOVA in Proc Mixed in SAS v. 9.1 (2002).

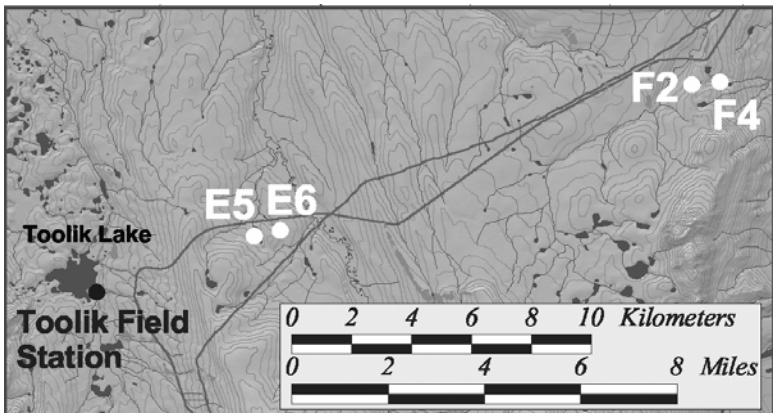

Figure 2.1. Map showing the location of fertilization lakes (E – 5, and E – 6) and reference lakes (Fog 2 and Fog 4) relative to Toolik Field Station on the North Slope of Alaska, 68°37′N, 149°35′W. Refer to Figure 1.1 in Chapter 1 for map of Alaska showing the location of Toolik Field Station.

In order to examine the mechanisms causing observed patterns in the whole-lake fertilization experiment, I also present data from two separate controlled laboratory experiments. The first experiment ("light response curve") examined the response of N_2 fixation to various light levels using intact sediment cores collected from shallow and deep stations from both fertilization and both reference lakes. N_2-fixation–Irradiance curves ("NI Curves") were fit to data in a manner analogous to Photosynthesis–Irradiance curves (Webb et al. 1974). The second experiment ("core nutrient addition experiment") was a one-way ANOVA design in which N and P were added alone and together in Redfield proportion to intact sediment cores collected from the deep reference lake. The details of each experiment are described below.

Whole-lake fertilization. A relatively deep lake (E-5) and a shallow lake (Lake E-6) were fertilized with N and P and were paired with deep (Fog 2) and shallow (Fog 4) reference lakes that were not fertilized. Lakes E-5 and E-6 are both on the oldest

glacial surface in the vicinity of Toolik originating from the Sagavanirktok River glaciation (250,000 – 800,000 ybp). The larger lake, E-5, is 1.3 ha in size and has a maximum depth of 12 m; E-6 is 0.2 ha and has a maximum depth of 3 m. Both fertilization lakes have a low conductivity of 7 – 12 μS sec^{-1} and relatively low rates of benthic primary production (60 – 110 mg C m^{-2} day^{-1}). Both lakes have one outlet stream; E-6 had no inlet stream, and E-5 has two permanent inlet streams. Lake E-6 comprises two shallow basins divided by a shallow, rocky shoal. Maximum depth in the smallest basin is 1.5 m, and maximum depth in the larger basin is 3 m. Lakes Fog 2 and Fog 4 are on a different geologic surface resulting from the advance of the Itkillik Phase II glaciation (12,000 – 25,000 ybp; Hamilton 2003)). Lake Fog 4 is similar in size to lake E-6 (0.19 ha) but has a simple basin with a similar maximum depth of 3 meters. Fog 4 has a much higher conductivity than E-6 (90 – 120 μS sec^{-1}) and has one inlet stream connecting it to two upstream lakes and one outlet stream. Fog 2 is larger and deeper than E-5 with a surface area of 5.6 ha and a maximum depth of 20 m. Fog 2 also has a relatively high conductivity (150 μS cm^{-1}), indicating that watershed inputs of cations and anions from weathering are high. Fog 2 has no inlet stream and one outlet stream. These lake characteristics are summarized in Table 2.1.

In years 2001 – 2003, Lakes E-5 and E-6 were fertilized with N and P in Redfield proportion (16:1) at 4 μmol N m^{-3} $year^{-1}$ and 0.25 μmol P m^{-3} $year^{-1}$, or approximately 4 times the ambient N loading to Toolik Lake (Whalen and Cornwell 1985). In lake E-5, the fertilization was applied on the basis of the volume of the epilimnion, which is considered to be the volume of water above 4 m and equal to about 75% of total lake volume. Lake E-6 is too shallow to stratify, so fertilization was applied on the basis of its total volume. Nitrogen as NH_4NO_3 and P was added as H_3PO_4 to the surface using a solar-powered peristaltic pump which pumped fertilizer from a reservoir in a continuous drip from a raft anchored in the center of each lake.

Rhodamine dye studies confirmed that mixing occurred before the fertilizer was washed out through outlet streams (George Kling pers. comm.) The fertilizer was added for 42 days from early July to mid August each summer from 2001 – 2003.

Table 2.1 Characteristics of fertilization and reference lakes.

	Fertilization Lakes		**Reference Lakes**	
	E5 (deep)	E6 (shallow)	Fog 2 (deep)	Fog 4 (shallow)
Maximum Depth (m)	12	3	20	3
Surface area (ha)	1.3	0.2	5.6	0.19
Conductivity ($\mu S\ sec^{-1}$)	7-12	7-12	150	90-120

Benthic parameters including N_2 fixation, primary production, respiration, and chlorophyll were measured three times per season in all lakes except the pre-fertilization year (2000), in which benthic processes were characterized once in mid-July. Measurements were made at 3 m in lakes E-5 and Fog 2 because the substrate is large rock cobble from 0 – 3 m due to ice-scour. In lakes E-6 and Fog 4, measurements were made at 1.5 m and 2.5 m, respectively, because these lakes had mud bottoms similar in texture to E-5 and Fog 2 and peat substrate at shallower depths. In years 2002 and 2003, "deep" stations in E-5, E-6, and Fog 2 were also sampled to assess how within-lake light variation affects N_2 fixation. In E-5 and Fog 2, deep stations were 6 – 7 m and in E-6, the deep station was at 2.5 m. No "deep" station was added in Lake Fog 4 because the geomorphology of the lake basin is simple, and the maximum depth in that lake was already being sampled. The deep stations were not included in statistical analyses to test for treatment effects because there were no data for the pre-treatment year.

Water-column profiles of inorganic nutrients, light, chlorophyll and primary production were measured weekly at 1 m intervals in the fertilization lakes. In the

reference lakes, water-column sampling was conducted three times per season because previous sampling showed that the seasonal variability in these lakes was very low (Arctic LTER database). Light profiles were conducted using a LI-COR LI-192 underwater quantum sensor, which was corrected for ambient light using a LI-COR LI-190 quantum deck sensor. Light extinction coefficient in the fertilized and reference lakes was calculated for each sampling date and used to determine the seasonal average according to the following equation:

$$I_z = I_o e^{-kz}$$

where I_z is the light in μE m^{-2} sec^{-1} at depth, z; I_o is the light at the surface, and k is the extinction coefficient (Wetzel and Likens 1991). Average photosynthetically active radiation (PAR) reaching the sediments in each summer was calculated by using average daily measurements from the weather station on Toolik Lake over the course of the sampling period in each year. PAR was measured every 10 minutes and averaged every hour using Li-Cor, Model LI-190SB and a Campbell 21x datalogger. These results were related to N_2-fixation rates by a random coefficient regression analysis (described below).

Rates of N_2 fixation: Three intact mud cores for N_2-fixation measurements were taken at each station on each sampling date in each lake and were incubated in the Toolik Field Station incubation facility at ambient lake temperature and light conditions, which ranged from 6 to 13 ^oC and from 2 – 150 μE $m^{-2}sec^{-1}$, respectively. N_2-fixation measurements were performed using the acetylene reduction assay (ARA), which quantifies the reduction of acetylene (C_2H_2) to ethylene (C_2H_4) by the nitrogenase enzyme (Hardy et al. 1968). Details of N_2-fixation measurements are given elsewhere (Chapter 3). Briefly, SCUBA was used to collect intact mud cores approximately 10 cm in length that had an undisturbed sediment-water interface. Cores tubes were 30 cm tall and 9.75 cm in diameter, resulting in 72 cm^2 of sediment

with about 1 L overlying water in the headspace. Cores were transported via helicopter in dark coolers with lake water in the bottom that both prevented cores from sloshing in transport and kept them at ambient temperature. Core tubes containing the mud and the overlying water were placed between 1 cm-thick clear polycarbonate rectangular plates held together by nylon-threaded rod that had a groove and O-ring to prevent leaking. The top plate had a bulkhead-style septum port to allow sampling of a gas headspace overlying the water. Acetylene was introduced to the core as saturated water, which reduces contamination by ethylene common in many acetylene tanks (see Marino et al. 2003 for details). In addition to ethylene contamination, the acetylene was also contaminated with ammonia, which when added to core incubations may cause a reduction in N_2-fixation rates. To address this problem, the acetylene was first bubbled through a 10% solution of sulfuric acid to trap ammonium in solution. This procedure resulted in ammonium concentrations in the acetylene water that were similar to lake-water concentrations near the analytical detection limit. The gas headspace was about 100 ml, resulting in an optimal 10:1 liquid:gas phase volume ratio for acetylene reduction assays (Flett et al. 1976). The water–gas interface in the core tubes had an externally operated magnetic stirring apparatus to maintain the water and gas phases in equilibrium throughout the incubation period. The magnet was about 15 cm above the mud surface, which prevented the sediments from being disturbed by the gentle stirring. Measurements of N_2 fixation in the overlying water proved negligible.

Because sediments can contain heterotrophic bacteria known to consume ethylene (Jackel et al. 2004; Elsgaard and Andersen 1998), and diatoms can produce ethylene (Lee and Baker 1992; Abeles et al. 1992), two additional cores were collected in order to apply corrections for ethylene production and consumption. Ethylene was rarely produced without the presence of acetylene, but ethylene consumption ranged

from 5 – 15 % over the course of the incubations. Ethylene loss rate was linear over the course of the incubations, and the correction for each treatment was applied accordingly. Corrections were minor and did not change qualitative results.

The total amount of ethylene present in the gas and water phase was determined using Henry's Law (Flett et al. 1976), and ethylene solubility was determined according a temperature–solubility relationship presented in Sander (1999). Ethylene samples were analyzed on a Shimadzu GC 8-A Flame Ion Detector (FID) using a Porapak N column, mesh size 80/100. Ethylene produced was converted to moles N_2 fixed assuming a 3:1 conversion ratio, which is reasonably constrained for sediments (Howarth et al. 1988b)

In situ determination of benthic primary production. Benthic primary production and respiration was estimated *in situ* by documenting the rate of oxygen consumption and production in opaque and transparent benthic chambers over 3 – 5 days. Measurements were made in each fertilization lake three times per season at the same depths as the N_2-fixation measurements, but only once per season in the reference lakes. Data for benthic primary production and respiration from the "deep" stations are not presented in this paper. Benthic chambers were cube-shaped and made of Lexan®, and had a volume of approximately 40 L. When deployed, they sank into the mud bottom and enclosed 0.15 m^2 of sediment. Tygon tubes connected chambers to the surface of the lake and allowed the water overlying the sediment to be sampled for oxygen as well as nutrients and gasses. Oxygen samples were collected in BOD bottles according to Arctic LTER protocols. Briefly, the bottle was overfilled by twice its volume by carefully inserting a piece of tygon tubing from the sample syringe and slowly expelling the water so as not to introduce atmospheric O_2. The bottles were fixed in the field by adding $MnCl_2$ and NaI, stoppered, wrapped in Parafilm, and transported to the lab in a container that kept them upright and safe from

jostling. O_2 concentration was measured using Winkler titration according to Arctic LTER protocols.

Community respiration (CR) was calculated as the rate of O_2 consumption in the dark chamber over the 3 – 5 day deployment and expressed as a daily rate. Gross primary production (GPP) was also expressed as a daily rate and calculated as the rate of O_2 production in the clear chamber + the rate of O_2 consumption in the dark chamber over the course of the 3 – 5 day deployment. This method assumes that respiration that occurs in the daylight is equal to night-time respiration (Wetzel and Likens 1991; Strickland and Parsons 1972). Recent work has shown that daytime respiration is likely higher than dark respiration as a result of mitrochondrial respiration, photorespiration, and the Mehler Reaction (Roberts 2004). Therefore, this method likely underestimates GPP, although it is commonly used (Howarth and Michaels 2000).

Chlorophyll a and phaeophytin analysis. Three cores 2.7 cm in diameter were collected for chlorophyll a and phaeophytin analysis each time cores were collected for N_2 fixation (three times per season), and additional chlorophyll cores were collected occasionally when the *in situ* primary production chambers were deployed if SCUBA was used and if there was access to the sediment (maximum of three additional times per season). According to Arctic LTER protocol, the top 2 cm of each core were sectioned and the mud was collected in a whirl-pack bag and homogenized. Of this sample, 5 ml were subsampled for chlorophyll a analysis. Samples were frozen at -80^{o}C and transported back to The Ecosystems Center, Woods Hole, for analysis the following fall. Samples were analyzed using acetone extraction and a Shimadzu UV-1601 spectrophotometer with a syringe (piston) sipper and a 1 cm Hellma cell according to the Arctic LTER protocols, which were adapted from Lorenzen (1967). Because chlorophyll degrades rapidly in the sediments (Bianchi et

al. 1991), chlorophyll a was expressed as a proportion of total chlorophyll, which was estimated as the sum of chlorophyll a and phaeophytin.

Data analysis for whole-lake fertilization experiment. Benthic N_2 fixation, primary production, and total chlorophyll were analyzed using a repeated measures ANOVA in Proc Mixed in SAS version 9.1 (2002). Proc Mixed accounts for unbalanced sampling design and allows for random effects as well as repeated measures. Lake was treated as a random effect and treatment, lake depth (shallow, deep), year, and interactions among these variables were treated as fixed effects. We tested whether modeling covariance structure among repeated measures increased the overall model fit by performing the maximum likelihood test. In all cases, fit was substantially improved by using a compound symmetrical covariance structure that was specific for each lake. Modeling covariance among repeated measures is more conservative than assuming independence among measurements that occur closely in time. The most parsimonious model was developed by eliminating non-significant fixed effects one-by-one until the best model fit was determined by examining the AIC value. Data and model fit were checked for the assumption of normality, and because chlorophyll a/total chlorophyll is proportional, these data were arcsin-square-root transformed. No other variables required transformation.

In order to determine whether seasonal measurements of N_2 fixation were related to ambient lake light, a randomized coefficient analysis was also performed using Proc Mixed in SAS version 9.1 (2002). This analysis used the entire data set, including measurements of ambient N_2 fixation and the light level at which they were incubated, which was always based on measurements made on the day in which they were collected. Light was transformed by natural log because the response of N_2 fixation to light levels is not linear (see below). Lake was treated as a random effect,

and covariance structure among repeated measures was accounted for as described above.

N_2 fixation-light response curves. In addition to relating seasonal measurements of N_2 fixation to *in situ* light conditions as described above, we also performed a laboratory experiment to compute N_2-fixation–Irradiance (NI) curves in 2003. Three cores for N_2 fixation and one core each for ethylene production and consumption were collected from 3m and 6m depths in Fog 2 and E-5, and from 1 and 3m depths in E-6, and from 2.5 m in Fog 4. After determining that N_2-fixation rates were linear over long incubation times, cores were first incubated in the dark for 4 hours, and then at increasing light levels for a total of 5 levels up to 250 – 350 μE m^{-2} sec^{-1}, which is up to 5 – 6 times greater than ambient lake light levels. Incubations were conducted under Hydrofarm Radient System lamps and AgroSun Metal Halide and Sodium 1000W bulbs. Cores were incubated at ambient lake temperature conditions, which was 12 ^{o}C.

The model used to fit the light response curves was according to Stal and Walsby (2000), who used the Photosynthesis–Irradiance model based on Webb et al. (1974) to fit NI curves. The equation is:

$$N_{fix} = N_{max} * (1 - e^{-\alpha I / N_{max}}) + N_d$$

Where N_{max} is the maximum N_2-fixation rate achieved at saturation; α is the initial slope; N_d is the intercept, or N_2 fixation in the dark, and I is light (PAR) in μE m^{-2} sec^{-1}. This model had a better fit (R^2) and less-biased residuals than the rectangular hyperbola model recommended for fitting N_2-fixation–Irradiance curves by Staal et al. (2002). Proc NLIN in SAS version 9.1 (2002) was used to estimate the parameters N_{max}, α, and N_d to using N_2-fixation and irradiance data. Once the parameters were estimated, the half saturation constant (K_m) was calculated using the following equation:

$$K_m = Ln(2)*N_{max}/\alpha$$

Core nutrient addition experiment. In 2003, four cores were collected from 5 different locations in Fog 2 for a total of twenty cores. One core from each location was designated as Control, +N, +P or +N+P treatment. The target loading rates for N and P were in Redfield proportion (16:1). N was added as NH_4SO_4 at a rate of 1 μmole L^{-1} day^{-1} and P was added as KPO_4 at a rate of 0.0625 μmole L^{-1} day^{-1}. Because the cores were incubated for only 9 days to avoid possible core artifacts, the loading rate was higher than that of E-5 and E-6 by a factor of 14 times to increase the possibility of documenting a response. At the end of 9 days, two cores from each treatment were measured for production. Following the production measurement, the cores were used to measure N_2 fixation. The two cores on which production was measured were used for ethylene consumption and production blanks in the N_2-fixation assays, resulting in 3 replicates of N_2 fixation per treatment and two replicates for production. Following the experiment, one chlorophyll a sample per core was taken by sectioning the top two centimeters, homogenizing the sediment, and removing a 10 ml subsample. The sample was frozen at -80 ^oC and analyzed using an acetone extraction according to the procedure described above.

Gross Primary Production and Respiration in nutrient-addition cores. In a manner similar to the ARA chambers, measures of production were made using a chamber design that allowed the core tubes to fit a specially designed lid. The chambers were filled completely, with no gas headspace, and the lid had a water-filled port into which a data-logging O_2 probe (WTW Oxi 340i) was placed. This prevented the chamber from experiencing air exchange with the atmosphere while measurements were conducted. Changes in oxygen consumption and production were recorded every 15 minutes over periods of dark and ambient light levels, each lasting approximately 12 hours. Incubations were done at ambient lake temperature (12^oC).

Community Respiration (CR) was measured as the rate of O_2 consumption during the dark period of the incubation and multiplied over 24 hours. Gross Primary Production (GPP) was calculated as the rate of O_2 production during the light period of the incubation and multiplied by 24 hours (there are 24 hours of light in an arctic summer), and summed with CR. This method of measuring production is similar to the *in situ* method described above and is subject to all the same assumptions and caveats.

Data from the laboratory nutrient addition experiment were analyzed by one-way ANOVA and simple regression using Proc GLM in SAS version 9.1 (2002). Tukey's post-hoc tests were performed to determine significant interactions to correct for Type I error.

RESULTS.

Response of benthic processes to whole-lake fertilization. Benthic N_2 fixation showed no obvious seasonal patterns in either the fertilization lakes or reference lakes (Figure 2.2), so we compared annual means to compare treatment effects (Figure 2.3; Table 2.2). Benthic N_2 fixation was depressed in both the shallow and deep fertilization lakes relative to reference lakes (significant treatment effect $p=0.039$; Table 2.3; Figure 2.3). Benthic N_2 fixation declined from the pre-fertilization year (2000) and in each subsequent year in both the shallow and deep fertilization lakes (Table 2.2; Figure 2.3). The largest decrease in N_2 fixation occurred in the first year of fertilization in both lakes, and declined less in subsequent years of the experiment. This result is supported by the fact that 2000 was significantly different from years 2001 – 2003 ($p>0.0008$;Table 2.4). By the last year of measurement (2003), N_2 fixation had declined by about 75% compared with 2000.

These results are in contrast with the reference lakes Fog 2 and Fog 4, in which no clear pattern in N_2 fixation is shown between years, and 2000 is within the range of variability of measurements made in the following years (Figure 2.2). N_2 fixation increased from year to year in the deep reference lake, Fog 2, and therefore showed the opposite trend to the deep fertilized lake, E-5. Patterns in N_2 fixation also differed between the shallow lakes; in Fog 4, N_2 fixation declined 2000 – 2001 and increased in 2002 – 2003 in contrast with the consistent decline in N_2-fixation rates observed in E-6 throughout each year of the experiment (Figure 2.3).

Benthic gross primary production, respiration, and chlorophyll a also showed no seasonal pattern in the fertilized and reference lakes (Giblin et al. unpublished data), so data were averaged to provide an estimate of each benthic process for each year of the experiment (Figure 2.4). In contrast to N_2 fixation, fertilization did not affect benthic gross primary production (GPP) and respiration (CR) (non-significant treatment effect, $p>0.05$; Table 2.3). Furthermore, GPP and CR were not different between the pre-fertilization and post-fertilization years (non-significant year effect, $p>0.05$; Table 2.3; Figure 2.4). In year 2003, GPP increased in lake E-6 by about 50%, likely because CR also increased (Figure 2.4; Table 2.2); however, this effect is not statistically significant. GPP in E-5 and Fog 2 was significantly lower than in E-6 and Fog 4 by about 60% regardless of treatment or year (significant lake depth effect; $p<0.0001$; Table 2.3). Shallow lakes exhibited higher (i.e., more negative) respiration rate than deep lakes by about 40% regardless of treatment or year ($p=0.001$; Table 2.3).

There was also a significant treatment effect for the proportion of benthic chlorophyll a ($p<0.0001$; Table 2.3). In the treatment lakes, chlorophyll a was more than twice as high in 2003 as it was in 2000 (Figure 2.5). Benthic chlorophyll a increased each year following fertilization in E-6, but did not increase in E-5 until year

2003, leading to a significant year effect ($p<0.0001$; Table 2.3; Figure 2.5). In contrast, benthic chlorophyll a was variable from year to year in Fog 2 and showed no consistent yearly trend (Figure 2.5; Table 2.2). Chlorophyll a levels in the shallow reference lake, Fog 4, started with higher levels of chlorophyll a than E-6, and also increased throughout the experiment, ending with twice the amount of chlorophyll a that it had in year 2000 (Figure 2.5; Table 2.2). This is likely as a result of increasing thermokarst (melting permafrost and top soil slumping) activity along the shore of Lake Fog 4, which appeared to increase the sediment — and likely P — load to the lake. Other lake-wide changes (increased water-column chlorophyll and reduced light) were also documented in water-column data for Fog 4 (Arctic LTER database; and as shown below). Although patterns in N_2 fixation are confounded by the thermokarst activity in Fog 4, the significant treatment effect is maintained if the statistical analysis is repeated without data from Fog 4.

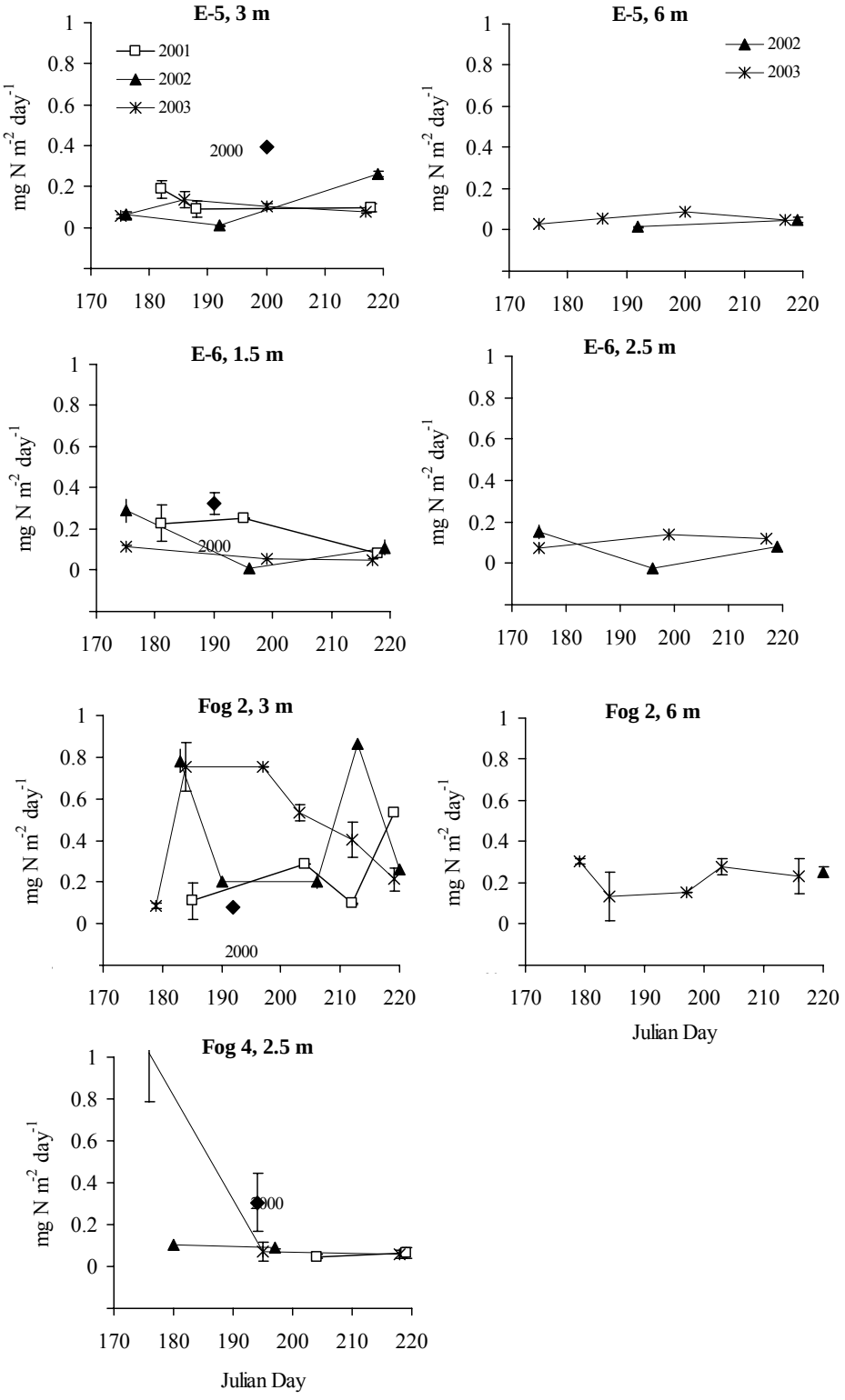

Figure 2.2 Seasonal benthic N_2 fixation in the fertilized (E-5 and E-6) and reference (Fog 2 and Fog 4) lakes.

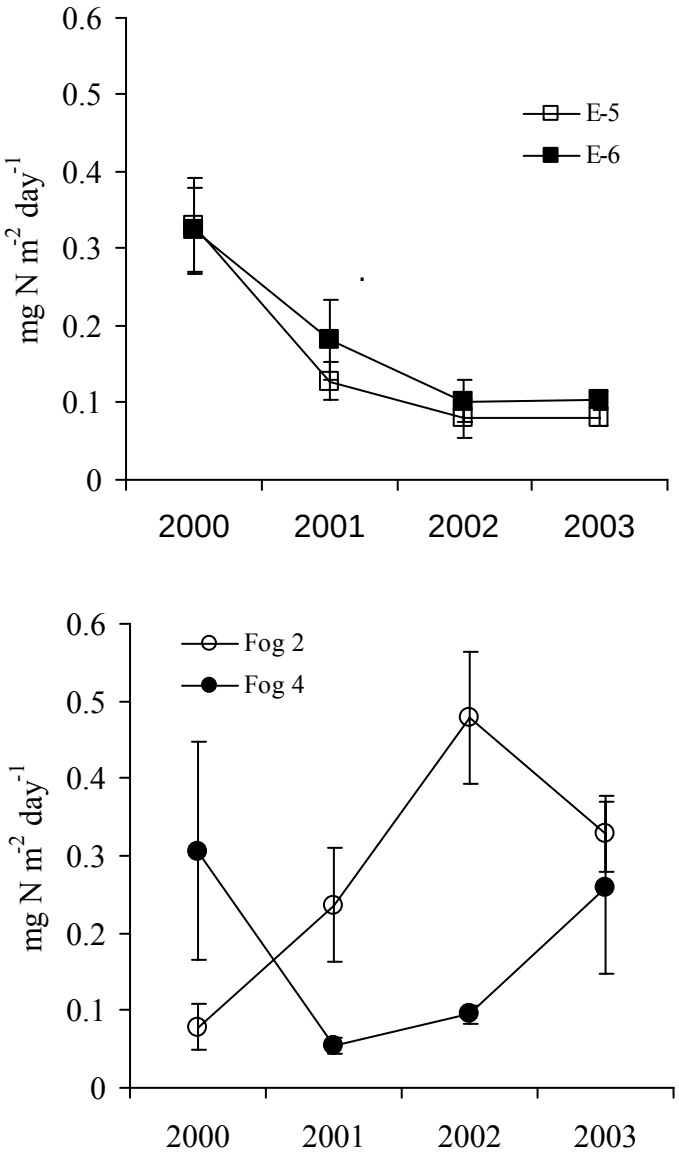

Figure 2.3 Graph showing benthic N_2 fixation in the fertilization lakes (top panel) and the reference lakes (bottom panel).

Table 2.2. Yearly averages for benthic N_2 fixation, benthic chlorophyll a, GPP, and CR in the fertilization and reference lakes.

	Benthic N_2 fixation (mg N m^{-2} day^{-1})						
	E-5, 3m	E-5, 6m	Fog 2, 3m	Fog 2, 6m	E-6, 1.5m	E-6, 3m	Fog 4, 2.5m
2000	0.39 ± 0.01	-	0.08 ± 0.01	-	0.33 ± 0.01	-	0.31 ± 0.01
2001	0.12 ± 0.03	-	0.25 ± 0.10	-	0.38 ± 0.23	-	0.05 ± 0.01
2002	0.11 ± 0.08	0.31±0.12	0.46 ± 0.15	0.25±0.01	0.14 ± 0.08	0.07± 0.05	0.09 ± 0.01
2003	0.09 ± 0.02	0.06±0.13	0.46 ± 0.11	0.22±0.03	0.07 ± 0.02	0.11± 0.02	0.38 ± 0.32

	Benthic Chlorophyll a (mg m^{-2})			
	E-5, 3m	Fog 2, 3m	E-6, 1.5m	Fog 4, 2.5m
2000	132 ± 4	100 ±3	118 ± 11	303 ± 6
2001	144 ± 39	438 ± 62	217 ± 37	382 ± 81
2002	142 ± 8	125 ± 12	303 ± 32	545 ± 28
2003	281 ± 22	254 ± 49	320 ± 29	694 ± 34

	Benthic GPP (mg C m^{-2} day^{-1})			
	E-5, 3m	Fog 2, 3m	E-6, 1.5m	Fog 4, 2.5m
2000	60 ± NA	110 ± NA	115 ± NA	132± NA
2001	65 ± 52	NA	158 ± 23	NA
2002	53 ± 15	149 ± NA	104 ± 15	66 ± NA
2003	30 ± 7	92± NA	203 ± 34	101± NA

	Benthic Community Respiration (mg C $m^{-2}$$day^{-1}$)			
	E-5, 3m	Fog 2, 3m	E-6, 1.5m	Fog 4, 2.5m
2000	-106 ± NA	-142 ± NA	-152 ± NA	-168 ± NA
2001	-106 ± 59	NA	-202 ± 14	NA
2002	-80 ± 11	-121± NA	-110 ± NA	-201± NA
2003	-83 ± 8	-133± NA	-197 ± 26	-144± NA

Table 2.3. Summary of repeated measures ANOVA models for benthic processes in the fertilized and reference lakes. The most parsimonious model was determined by using the maximum likelihood test and resulted in models that included only significant predictors of the Y variable.

	Numerator DF	Denominator DF	F-Value	p-value
N_2 Fixation (mg N_2 fixed m^2 day^{-1})				
Treatment	1	41	3.05	**0.039**
Year	3	41	16.91	**0.0002**
Gross Primary Production (mg C m^{-2} day^{-1})				
Lake Depth	1	23	39.19	**<0.0001**
Community Respiration (mg C m^{-2} $year^{-1}$)				
Lake Depth	1	23	11.51	**0.003**
Proportion Chlorophyll a				
Treatment	1	33	42.25	**<0.0001**
Year	3	33	22.81	**<0.0001**
Lake Depth	1	33	8.92	**0.005**

Table 2.4. Magnitude of significant effects for repeated measures ANOVAs for benthic processes in fertilized and reference lakes. Dummy variables are denoted by a period and are used to determine which variables are significantly different from one another by the corresponding p-value.

	Estimate	Standard Error	DF	T-Value	p-value
N_2 Fixation (mg N_2 fixed m^2 day^{-1})					
Treatment					
Fertilized	0.083	0.020	41	4.11	**.**
Reference	0.317	0.057	41	4.11	**0.0002**
Year					
2000	0.222	0.079	41	2.81	**0.008**
2001	0.048	0.036	41	1.36	0.182
2002	0.007	0.032	41	0.22	0.830
2003	0.083	0.020	41	4.11	**.**
GPP (mg C m^{-2} day^{-1})					
Lake Depth					
Deep	49.040	11.190	23	-6.26	**<0.0001**
Shallow	119.090	8.330	23	14.30	**.**
CR (mg C m^{-2} $year^{-1}$)					
Lake Depth					
Deep	-92.700	15.877	23	3.39	**0.001**
Shallow	-146.560	9.433	23	3.39	**.**
Proportion Chl a					
Treatment					
Fertilized	0.683	0.026	33	26.16	**.**
Reference	0.857	0.027	33	6.50	**<0.0001**
Year					
2000	0.373	0.044	33	-7.02	**<0.0001**
2001	0.515	0.028	33	-6.01	**<0.0001**
2002	0.567	0.031	33	-3.72	**0.0007**
2003	0.683	0.026	33	26.16	**.**
Lake Depth					
Deep	0.613	0.023	33	-2.99	**0.005**
Shallow	0.683	0.026	33	26.16	**.**

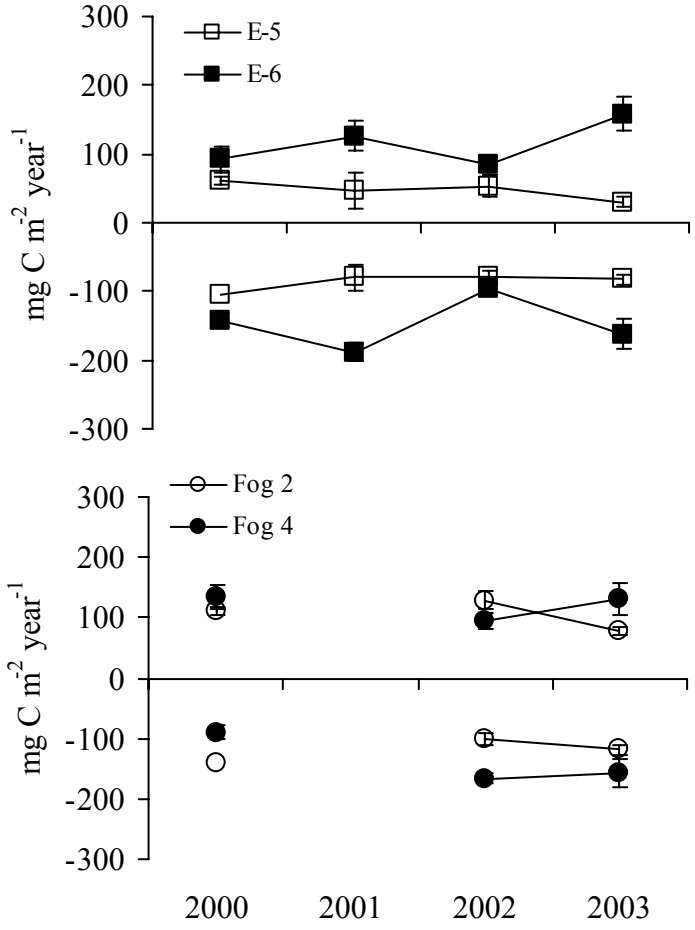

Figure 2.4. GPP and CR in the fertilized (top panel) and reference (bottom panel) lakes. No data are available from year 2001 from the reference lakes.

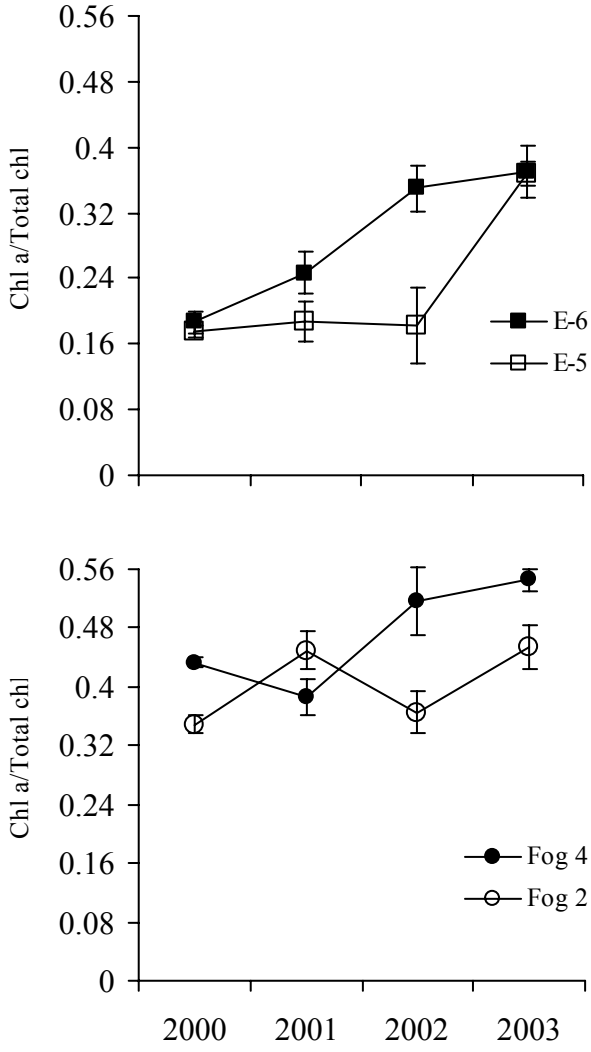

Figure 2.5. Annual averages of proportion of benthic chlorophyll a at the shallow stations in the fertilization lakes (E-5 and E-6; top panel) and the two reference lakes (Fog 2 and Fog 4; bottom panel).

Response of N_2 fixation to ambient light in fertilization and reference lakes.

The light extinction coefficients (k) generally increased in the fertilization lakes from the pre-fertilization year (2000) to year 2003 (Table 2.5; Figure 2.6). The light extinction coefficient increased by 0.5 in Lake E-6 at 1.5 m and in Lake E-5 at 3 m by 0.12. The light extinction coefficient also increased dramatically in Fog 4 by 0.71, while it was more variable from year to year in Fog 2 (Table 2.5; Figure 2.6). The

increase in k in Fog 4 was likely due to the thermokarst activity as described above. As light extinction coefficients increased in the fertilization lakes, ambient PAR at depth also decreased (Figure 2.6); however, in 2003, ambient PAR at depth was similar to the pre-fertilization year because 2003 was a sunnier year than the previous years of the experiment (Table 2.5).

Table 2.5. Light parameters for reference and treatment lakes 2000 – 2003. **A.** Ambient photosynthetically active radiation (PAR) for growing season (21 June – 10 Aug). **B**. Vertical extinction coefficient at depth for each lake for each year. The vertical extinction coefficient is the average of the extinction coefficients at each sampled depth in the reference and fertilization lakes. **C.** Average PAR at depth is given for each year in each lake.

A.

	Average growing season PAR ($\mu E\ m^2\ sec^{-1}$) ± SE
2000	371.92 ± 9.71
2001	372.98 ± 9.34
2002	323.16 ± 8.68
2003	495.04 ± 9.01

B.

	Average seasonal vertical extinction coefficient at depth			
	E-5, 3m	**Fog 2, 3m**	**E-6, 1m**	**Fog 4, 2.5m**
2000	1.08	0.64	1.82	1.02
2001	0.97	0.43	1.61	1.18
2002	1.33	0.45	2.22	1.27
2003	1.20	0.80	2.32	1.73

C.

	Average summer PAR at depth ($\mu E\ m^{-2}\ sec^{-1}$)						
	Lake E-5		**Lake Fog 2**		**Lake E-6**		**Fog 4**
	3m	**6m**	**3m**	**6m**	**1m**	**3m**	**2.5m**
2000	16.10	.	56.33	.	68.24	.	66.82
2001	21.44	.	102.29	.	80.73	.	39.22
2002	6.56	4.32	86.95	17.29	42.75	5.42	26.80
2003	16.39	1.67	46.53	24.65	50.17	7.67	17.90

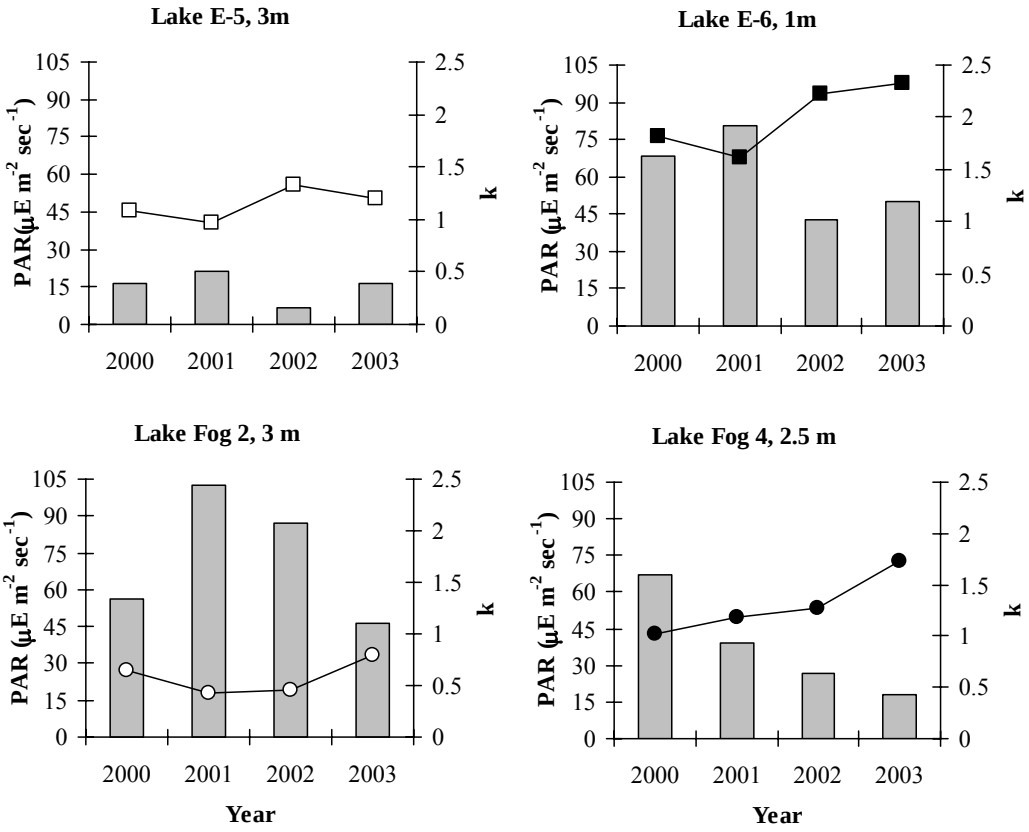

Figure 2.6. Light extinction coefficient, k (lines), and average ambient PAR (bars) for each year at shallow stations in fertilization and shallow reference lakes.

A random coefficient analysis using ambient light and measured N_2-fixation rates in the fertilization and reference lakes in all years showed that N_2 fixation was not related to ambient light in E-5, E-6, or Fog 4 ($p<0.05$; Table 2.6). (NI curves showed that N_2 fixation was related to light in some lakes at some depths in the laboratory; these results are discussed below.) N_2 fixation was related to ambient lake light only in Fog 2 ($p=0.006$; Table 2.6), which was responsible for the overall effect of light in the randomized coefficient analysis ($p=0.006$; Table 2.6).

Table 2.6. Results from randomized coefficent analysis relating N_2-fixation rates to ambient light in the fertilization and reference lakes for all years.

N_2 Fixation (mg N_2 fixed m^2 day^{-1})	Estimate	Standard Error	DF	T-Value	p-value
Intercept	-0.073	0.07	156	-1.04	0.300
Log Light ($\mu E\ m^{-2}\ sec^{-1}$)	0.057	0.021	156	2.74	**0.007**
	Slope	Standard Error	DF	T-Value	p-value
E5	0.0004	0.0005	156	0.81	0.420
E6	-0.0003	0.0003	156	-0.90	0.370
Fog 2	0.0010	0.0004	156	2.79	**0.006**
Fog 4	0.0001	0.0006	156	0.16	0.870

In addition to the lack of a response of N_2 fixation to light in the fertilization lakes as demonstrated by the random coefficient analysis, several other patterns also indicate that N_2 fixation was not strongly related to ambient light throughout the course of this experiment. For example, in year 2000, N_2 fixation was about 4 times higher in E-5 than in Fog 2 despite the fact that ambient lake light was lower in E-5 than Fog 2 (16 vs. 56 $\mu E\ m^{-2}\ sec^{-1}$ respectively; Table 2.6). Similarly, benthic N_2 fixation was higher in E-6 than in Fog 4, even though their light levels were similar (~66 – 68 $\mu E\ m^{-2}\ sec^{-1}$; Table 2.5). Finally, shallow stations at Lakes E-5 and E-6 behaved similarly in response to fertilization even though the shallow station in E-6 was at 1 m and had higher light availability than the shallow station at 3 m in E-5. Therefore, differences in light do not appear to explain inter-annual variation in N_2-fixation rates or the differences between lakes.

While light does not appear to explain between-lake differences in N_2-fixation rates, it may be important in explaining within-lake differences. Deep stations, which had lower light levels than shallow stations (Table 2.6), also had lower N_2-fixation rates (Table 2.2), but this was not always a statistically robust difference. In lake E-5,

the deep-station average for N_2 fixation was 0.05 ± 0.01, while the shallow-station average was 0.13 ± 0.03 (t-value = -1.85; p=0.08). N_2-fixation rates at deep and shallow stations were not significantly different from one another in lakes E-6 and Fog 2, although the means were higher at the shallow station in each lake (0.21 vs. 0.09 mg N m^{-2} day^{-1} for lake E-6 and 0.39 vs. 0.23 mg N m^{-2} day^{-1} for Fog 2). The difference in benthic N_2 fixation between shallow and deep stations was greatest in the fertilization lakes, which also had the greatest difference in light availability between stations (Table 2.2).

N_2 fixation–Irradiance curves. In the controlled laboratory experiment in which N_2 fixation was measured in response to increasing light levels, all of the shallow stations except in E-6 showed a positive, saturating response (Figure 2.7; Table 2.8). None of the deep stations except Lake Fog 2 showed a significant relationship with light, but the N_2-fixation rates were also very low. While both the shallow stations in E-5 and Fog 2 showed a similar response to light at low light levels (alpha = 0.007), Fog 2 had a higher N_{max} than E-5 (0.77 vs 0.51 mg N m^{-2} day^{-1} respectively). In addition, the half saturation constant for Fog 2 was nearly twice that for E-5 (76 μE m^{-2} sec^{-1} vs. 44 μE m^{-2} sec^{-1} respectively.) The deep station in Lake Fog 2 was similar to the response at the shallow station in Lake Fog 2, although it had a slightly smaller alpha (0.005) and a higher N_{max} resulting in a higher half saturation constant at 120 μE m^{-2} sec^{-1} (Table 2.8). Fog 4 also showed a positive relationship of benthic N_2 fixation with light, achieving N_{max} at 0.36 mg N m^{-2} day^{-1} and a half saturation constant of 120 μE m^{-2} sec^{-1}, while its fertilized counterpart, E-6, showed no relationship light.

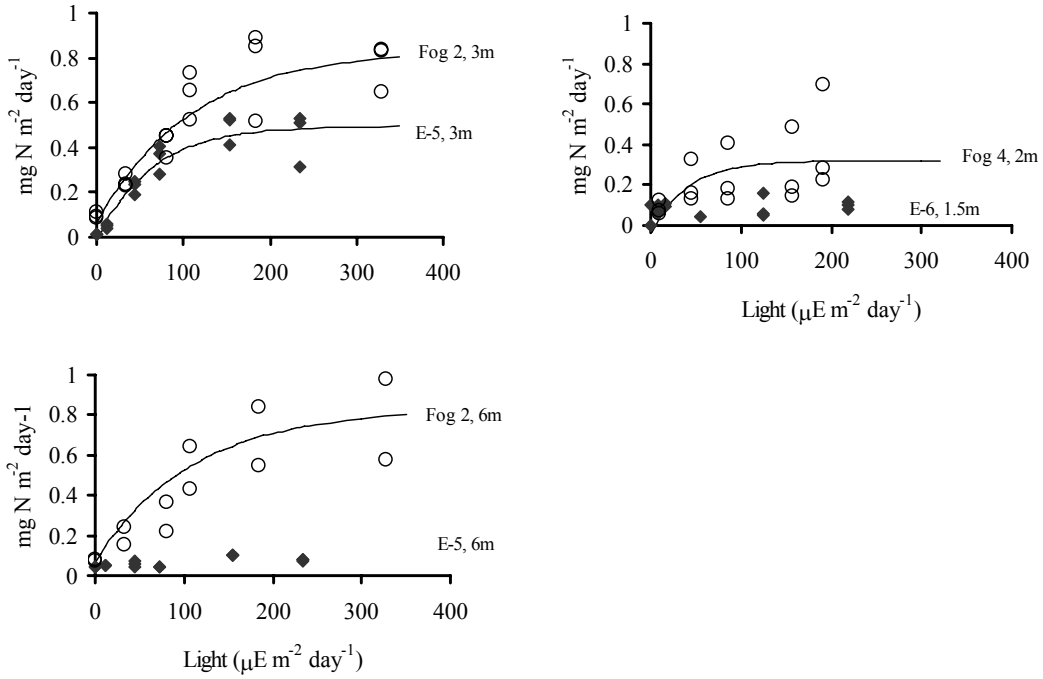

Figure 2.7. Light response curves for benthic N_2 fixation in the deep fertilization lake (E-5), the deep reference lake (Fog 2), the shallow fertilization lake (E-6), and the shallow reference lake (Fog 4). Benthic N_2 fixation in E-6 at 1.5 m and in E-5 at 6 m was not related to light level.

N_2-fixation response to nutrient addition to cores, Lake Fog 2. In the nutrient-addition laboratory experiment, benthic N_2-fixation rate significantly increased due to the addition of P by 0.13 mg N m^{-2} day^{-1}, or 38% relative to the control (p=0.03; Figure 2.8; Table 2.9). The +P treatment was also higher than the +N treatment by 0.15 mg N m^{-2} day^{-1}, or 46% (p=0.08; Figure 2.8; Table 2.8). There was no significant response of GPP or chlorophyll a to added nutrients, but there is a slight indication that GPP responded most to the +N treatments and chlorophyll responded most to the +N+P treatment (Figure 2.8). The molar N:P ratio in the ammonium and phosphorus concentrations measured at the end of the experiment was significantly higher than controls in the +N treatments (Figure 2.9; Table 2.8), and a simple linear regression analysis indicates that N_2 fixation was negatively related to N:P ratio (p=0.01 R^2 = 0.56).

Table 2.7. Light curve parameters for benthic N_2-fixation curves from shallow and deep stations in the fertilization and reference lakes. "NA" indicates that there was no significant relationship between light and benthic N_2 fixation.

Lake	Depth (m)	Treatment	N_{max}	alpha	N_d	$I_{1/2}$	R^2	p-value
E-5	3	Fertilized	0.51 ± *0.04*	0.008 ± *0.002*	-0.012 ± *0.08*	44.01	0.91	**<0.0001**
Fog 2	3	Reference	0.77 ± *0.09*	0.007 ± *0.002*	0.067 ± *0.06*	76.08	0.86	**<0.0001**
E-6	2	Fertilized	NA	NA	NA	NA	NA	NA
Fog 4	2.5	Reference	0.36 ± *0.081*	0.009 ± *0.006*	-0.04 ± *0.07*	27.76	0.53	**0.001**
E-5	6	Fertilized	NA	NA	NA	NA	NA	NA
Fog 2	6	Reference	0.87 ± *0.22*	0.005 ± *0.002*	0.05 ± *0.09*	120.08	0.79	**0.0008**

Table 2.8. ANOVA statistics for the nutrient core incubation experiment. There was a significant treatment effect for benthic N_2 fixation and for the ending N:P ratio molar concentrations. Treatment was not significant for chlorophyll a, gross primary production, or respiration.

	DF	Mean Square Error	F-Value	p-value
Benthic N_2-fixation				
Treatment	3	0.015	4.29	**0.04**
Error	8	0.003		
Total	11			
Chlorophyll a				
Treatment	3	1273	0.61	0.62
Error	16	2079		
Total	19			
Gross Primary Production				
Treatment	3	7313	0.44	0.74
Error	4	16754		
Total	7			
Respiration				
Treatment	3	1804	1.159	0.43
Error	4	1556		
Total	7			
N:P Ratio				
Treatment	3	537	3.58	**0.04**
Error	16	150		
Total	19			

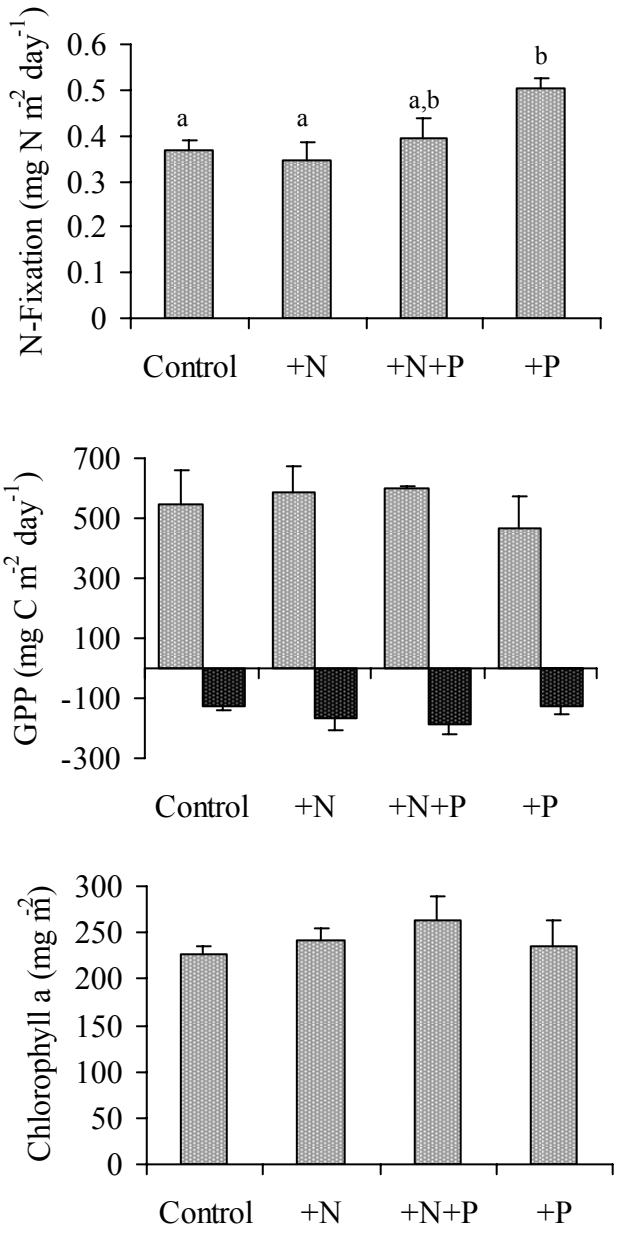

Figure 2.8. Results from a nutrient-addition experiment to intact sediment cores. N_2 fixation in the +P treatment was significantly higher than the control and the +N treatment (Tukey; $\alpha < 0.08$). Chlorophyll, GPP, and Respiration were not significantly different among treatments.

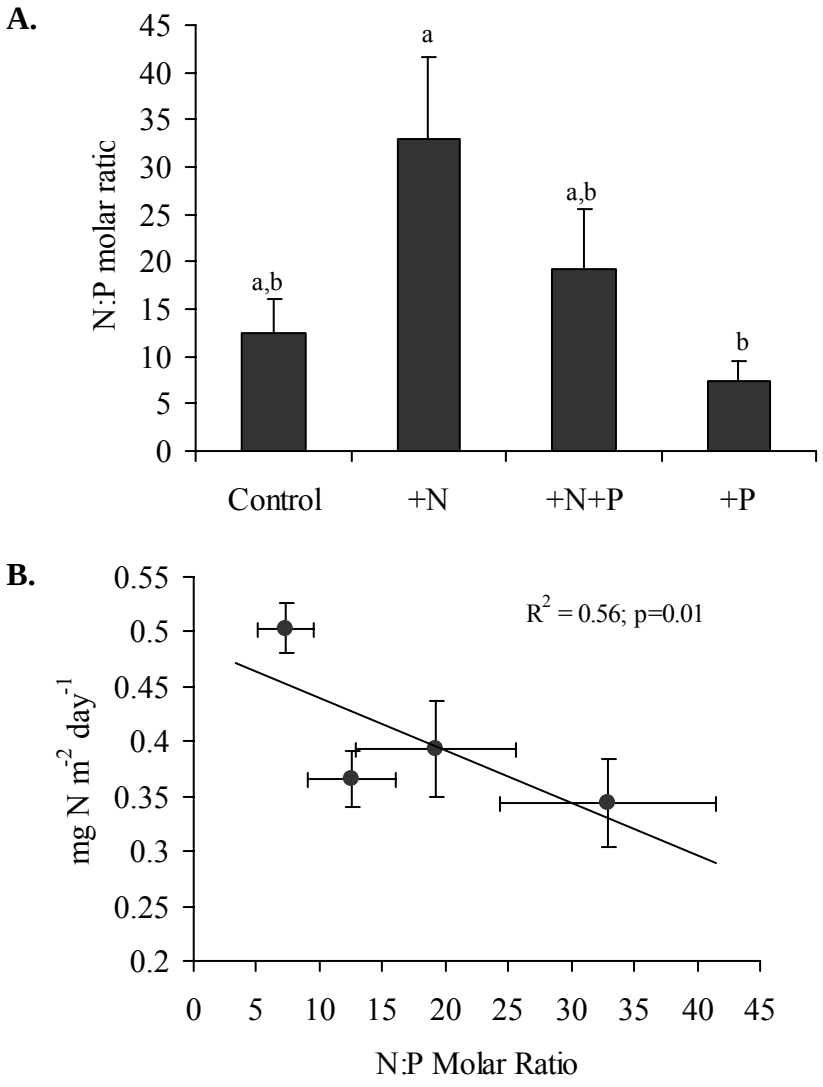

Figure 2.9. Relationship between N_2 fixation and N:P ratio in nutrient addition cores. **A**. The N:P molar ratio as measured by ammonium and phosphorus concentration was significantly higher in the +N treatment, and was approximately Redfield in the +N+P treatment. **B**. N_2-fixation rate was significantly related N:P ratio such that the lowest N:P ratio yielded the highest N_2-fixation rate.

DISCUSSION.

The study shows that benthic N_2 fixation declined by about 4-fold in both the shallow and deep lake in response to fertilization. There are few other studies that examine the effect of whole-lake fertilization on benthic N_2 fixation, but these results

are consistent with one; Bergman and Welsh (1990) found that in an N and P fertilized lake (N:P=14.6:1), epilithic N_2 fixation was suppressed by about 50% relative to their non-fertilized reference lake. Benthic N_2 fixation may have declined in fertilization lakes for one of several reasons: 1). Benthic primary production and/or biomass declined, causing a decline in the demand for N; 2). Light reaching the benthos declined, and autotrophic N_2 fixation became light-limited; or 3). Increased N availability by fertilization reduced the favorability and competitive advantage of N_2 fixation. The *in situ* patterns in GPP, CR, and chlorophyll a in conjunction with the laboratory experiments provide insight into which mechanism may be causing this observed pattern.

Chlorophyll a proportion increased by year 2003 in both fertilization lakes. These results are in contrast with those of Nydick et al. (2004b), who showed that epipelic chlorophyll a biomass did not respond to added nutrients over the course of a summer of fertilization to encloses in two oligotrophic lakes in Wyoming. However, response times for benthic chlorophyll may be long, especially in the Arctic, where turnover time is nearly an entire summer (Chapter 3), and it may take several years to be able observe a treatment effect (e.g., O'Brien et al. 2005). This lag time is shown in the deep fertilization lake, E-5, which did not respond to treatment until 2003. Benthic algae may also adapt to low light levels by increasing chlorophyll a content (Wetzel 2001), and this response in our data may account for the fact that the increase in chlorophyll a proportion is not accompanied by increases in GPP or CR.

Benthic GPP remained relatively constant in the fertilization lakes, indicating that autotrophic N demand probably did not change. The results of this whole-lake fertilization experiment contrast those of an experiment in Peter Lake, Michigan, which showed that epipelic production declined in response to fertilization as a result of reduced light availability (Vadeboncoeur et al. 2001). However, the fertilization

rate in that experiment was an order of magnitude greater per *day* than that of this study per *year* (i.e., 31 μM P m^{-3} day^{-1} in their study vs. 0.25 μM P m^{-3} $year^{-1}$ in this study). As a result, light to the sediment bottom was not drastically reduced in response to fertilization (i.e., light was penetration reduced by about 75% in the Vadaboncouer et al. study whereas this study showed a decline of 15 – 20%; Table 2.5). The fact that GPP and CR have not increased indicate that N demand by primary producers has not declined as a result of fertilization, but has likely stayed the same. Thus, benthic N_2 fixation probably has not declined as a result of decreased N demand by primary producers in the sediments.

Ambient lake light at depth declined throughout the treatment period because the light extinction coefficients increased (Figure 2.6); however, the summer season in 2003 had about 140 μE m^{-2} sec^{-1} more light than the average of the previous 3 years (Table 2.5), and the decline in light availability was not very dramatic (15 – 20%; Table 2.5). Surprisingly, N_2 fixation was related to ambient light availability over the years of the experiment only in the deep reference lake, Fog 2, and not in the fertilization lakes. Although light declined most dramatically (nearly 4-fold) in the shallow reference lake due to termokarst activity, N_2 fixation was not related to light in that lake, either. These results suggest that differences in ambient light did not explain inter-annual variation in N_2 fixation, nor could they explain the differences in N_2 fixation between reference and fertilization lakes over the course of the experiment.

These results seem to contrast with results from the NI curves, which showed that N_2 fixation was significantly related to light availability in the laboratory for lakes Fog 2, E-5, and Fog 4. This discrepancy could be due to the fact that the range over which we conducted the light-response incubations (0-250 μE m^{-2} sec^{-1}) far exceeded the change in light availability in the experimental lakes as a result of fertilization (6 – 80 μE m^{-2} sec^{-1}; Table 2.5). N_2-fixation rates predicted by the NI curves are similar to

actual N_2 fixation at the seasonal light levels shown in Table 2.5; however, the N_2-fixation rates may be within the variability predicted by the NI model, which would prevent us from determining a significant relationship between ambient light and benthic N_2 fixation. The short duration of the laboratory experiment also means that N_2-fixing community may not have had time to adapt to low light levels, as they would over the course of several years.

Although light cannot explain the decline of N_2 fixation in the fertilization lakes, light availability may explain within-lake variation in N_2 fixation as a function of depth. For example, N_2 fixation rates at the deep station are generally lower than they are at the shallow stations (Table 2.2). The deep station in Fog 2 showed a higher ½ saturation constant than the shallow station, indicating more light limitation at depth. In E-5, the deep cores had no significant relationship with light, which may be due to a low N_2-fixing biomass at the depth. Since we express the NI curves on an areal basis rather than on a biomass basis, low biomass may reduce our ability to detect a light response. Finally, ½ saturation constants for each lake were higher than average seasonal ambient PAR (Table 2.7; Table 2.5), suggesting that the other lakes may also be light limited. As such, these results indicate that light is an important factor controlling N_2 fixation, and these curves could be used to model N_2 fixation over depths over a season and applied to estimate seasonal rates of N_2 fixation (e.g. Higgins et al. 2001; Bergmann and Welch 1990, Grimm and Petrone 1997); however, they cannot be used to predict differences between lakes or responses due to fertilization. Another mechanism besides light acts to control N_2 fixation in the fertilization lakes.

The third hypothesis to explain the reduction of N_2 fixation to fertilization is that increased N availability from fertilization reduced the favorability of N_2 fixation. Results from the core incubation indicate that nutrient addition may be responsible,

but the exact mechanism is unclear. N_2 fixation was significantly negatively related to the N:P ratio in the treatments such that N_2 fixation was lowest in the +N treatment and highest in the +P treatment (R^2=0.56; p=0.01; Figure 2.9). This result is consistent with water-column studies that indicate that N_2 fixers gain a competitive advantage when N supply is low relative to P and can compensate for N limitation (Schindler 1977, Findlay et al. 1994; Hendzel et al. 1994; Flett et al. 1980), and suggests that benthic N_2 fixation has similar responses to nutrient controls as water-column N_2 fixation. When N_2 fixation is compared among treatments, however, there was no effect of added N either alone or together with P (Tukey $p<0.05$; Figure 2.8; Table 2.8). These results are inconsistent with the results of the whole-lake fertilization experiment in which N_2 fixation declined with the addition of N and P, and also those of Bergman and Welsh (1990) in which N_2 fixation declined in an N- and P- fertilized lake in the NWT, Canada.

Other researchers have shown that high concentrations of inorganic N suppress the synthesis of nitrogenase, but not necessarily the activity of nitrogenase that has already been produced (Pearl et al. 1981; Horne et al. 1979). This response may not be detected if N_2-fixer turnover is long relative to the length of the experiment. N_2 fixers are known to be slower-growing than many algal species (Chan et al. 2004), and benthic algal turnover in these systems is about two months (Chapter 3). It possible that high ammonium concentrations in the core experiment suppressed the production of new nitrogenase, but the experiment was not long enough to detect that kind of change.

It is unlikely that increased inorganic N concentration in the whole-lake experiments caused the observed decline in N_2 fixation, because added nutrients appeared to be taken up quickly, and water-column N concentrations remained very low throughout the course of the experiment (Arctic LTER Database). There is little

evidence for increased N concentration in the sediments as a result of fertilization, although ammonium flux in the dark chambers (used to measure benthic respiration) was detected in Lake E-6 in 2004, indicating that mineralization of organic matter may be starting to exceed heterotrophic N demand (Giblin unpublished data). If this N is available for uptake by the autotrophic community, the advantage of fixing N_2 may be reduced.

Benthic communities have a significant capacity for N uptake, and it has been shown to exceed that of phytoplankton communities in other oligotrophic systems. For example, Axler and Reuter (1996) showed that more than 50% of an added ^{15}N-NO_3 tracer was taken up by sediments and periphyton in Castle Lake, while less than 9% of it was recovered in the water column. In a series of mesocosm experiments in oligotrophic mountain lakes in which ^{15}N-NO_3 tracer was added in conjunction with fertilizer, Nydick et al. (2004a) also showed that a significant portion was taken up by sediments and periphyton. Increased N availability in the sediments — either by mineralization of organic matter or by direct uptake of N from added fertilizer — could alleviate N limitation or reduce the competitive advantage of N_2 fixers. It is likely that N_2 fixation was suppressed in the whole-lake fertilization experiment because increased N input from fertilization satisfied some of the N demand by autotrophs in the benthos.

The results from the whole-lake fertilization experiment and the core incubation experiment show that N_2 fixation was more sensitive to added nutrients than chlorophyll or production, indicating that changes in nutrient status can significantly alter the pathway of new N input through N_2 fixation to lakes before changes in other benthic processes are evident. Thus, low-level nutrient additions of N and P may suppress N_2 fixation and maintain these systems at N-limitation status through the early stages of eutrophication.

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CHAPTER 3

THE EFFECTS OF GRAZING BY THE SNAIL *LYMNAEA ELODES* ON BENTHIC NITROGEN FIXATION AND PRIMARY PRODUCTION IN OLIGOTROPHIC, ARCTIC LAKES

ABSTRACT.

This study assessed whether grazing by the snail *Lymnaea elodes* limits the rate of benthic N_2 fixation and primary production in N-limited oligotrophic lakes near Toolik Field Station, Arctic Long Term Ecological Research (LTER). I also tested whether snail excretion increased N supply and the N:P supply ratio, which could indirectly affect the rate of N_2 fixation. We performed an *in situ* randomized block experiment in two lakes over 3 years in which snail density was manipulated and compared to open cage controls. Randomized coefficient regression analysis showed that snails significantly decreased areal rates of N_2 fixation in both lakes in all years. Snails did not, however, significantly affect measures of benthic production, including gross primary production, respiration, net ecosystem production, or chlorophyll biomass. The molar $N\text{-}NH_4^+$:$P\text{-}PO_4^+$ excretion ratio was 4.8, which is well below that of benthic algae, indicating that snails likely exacerbated N limitation rather than reduced it. Furthermore, the excretion rate of $N\text{-}NH_4^+$ was very low relative to other sources of N from decomposition and N_2 fixation. Thus, snail excretion was probably not an important factor in reducing N_2 fixation. While the mechanism by which *Lymnaea elodes* caused a decline in N_2 fixation is unknown, the treatment effect was small, accounting for a reduction of N inputs of only 0.12 mg N m^{-2} $summer^{-1}$ or by 0.85 – 1.8%. Because N fixation is a new N input able to support new production, however, this effect may be important over long time scales. Comparisons with the literature indicate that snails may be unique grazers, and more work is needed in order to

determine how snails affect N_2 fixation, N supply via excretion, and production in a variety of environments.

INTRODUCTION.

Biological nitrogen (N) fixation of atmospheric N_2 by free-living cyanobacteria in aquatic environments is common, and in many ecosystems, it can account for a significant portion of the biologically available N inputs. In lakes of moderate to high productivity, N input from N_2 fixation is generally sufficient to alleviate N limitation of net primary production, so that phosphorus is considered the ultimate limiting nutrient (Schindler 1977). In many oligotrophic, freshwater systems and estuaries, however, N_2 fixation by cyanobacteria does not compensate for N limitation, and N limitation is maintained over relatively long time scales (Howarth et al. 1988a; Vitousek et al. 2002).

Grazing of free-living cyanobacteria is increasingly recognized as a possible controlling factor of N_2 fixation in N-limited aquatic systems. Recent research has shown that grazing can limit the filament size, heterocyst production, and growth rate of filamentous cyanobacteria in some freshwater environments (Schaffner et al. 1984; Chan et al. 2004). In estuarine environments, grazing plays a significant role in maintaining N limitation (Howarth et al. 1999; Marino et al. 2002; Chan et al. 2004; Marino et al. 2006). Despite the fact that grazing is demonstrably important to understanding the dynamics of N_2 fixation, N limitation, and primary production of whole ecosystems, only a handful of published studies have examined the effect of grazing on N fixation in oligotrophic systems. Most were conducted in tropical marine ecosystems (Wilkinson and Sammarco 1983; Williams and Carpenter 1997), and none to our knowledge has been done in oligotrophic lake ecosystems.

N limitation is prevalent in many oligotrophic lakes - e.g., East African Rift Lakes (Bootsma and Hecky 2003); Lake Tahoe (Goldman 1988); Castle Lake (Axler and Reuter 1996); and many other high-latitude and high-altitude lakes (Morris and Lewis 1988; Levine and Whalen 2001). In these environments, benthic processes often drive whole-lake production and nutrient dynamics because water column production is low, light penetration is high, and littoral zones are large (Ramlal et al. 1994; Vadeboncoeur et al. 2003). Benthic algae comprise a significant portion of whole-lake productivity (Wetzel 1964; Vadeboncoeur et al. 2001), and they are an important component of lake food webs (Hecky and Hesslein 1995; Sierszen et al. 2003). N_2-fixing filamentous cyanobacteria are common on the sediment in oligotrophic lakes (Moeller and Roskoski 1978; Loeb and Reuter 1981), and because other inputs are low, inputs of new N through autotrophic N_2 fixation may be important to their N budgets (Moeller and Roskoski 1978; Reuter et al. 1986; Bergmann and Welch 1990; Higgins et al. 2001). Understanding the role of grazers in controlling benthic N_2 fixation and production in oligotrophic lakes is essential to understanding how these lakes store carbon, cycle nutrients, and fuel their food webs.

In the benthic environment, grazing may be one of the most important factors controlling cyanobacterial growth, biomass, and fixation rate because underlying reducing conditions could help alleviate phosphorus and trace metal limitation (Howarth et al. 1988a). However, the effects of grazing on benthic cyanobacteria and N_2 fixation are unclear, and they have been shown to exhibit both positive and negative effects. For example, direct consumption by snail grazers has caused the reduction of cyanobacterial biomass in some freshwater benthic environments (McCollum et al. 1988; Tuchman and Stevenson 1991). On the other hand, selective grazing of non-cyanobacterial algal species could supply cyanobacteria with a competitive advantage such that they are able to increase in biomass (Cattaneo and

Kalff 1986; Rosemond et al. 1993). Grazing can also stimulate N_2 fixation by maintaining algal community in early successional stages, which is often dominated by N_2 fixers (Wilkinson and Sammarco 1983); or, grazers may increase light availability to N_2 fixers by canopy or epiphyte clipping and/or sediment removal (Dodds and Castenholz 1987; Power et al. 1988; Flecker 1996; Williams and Carpenter 1997).

Grazing may also affect N_2-fixation rates indirectly by exacerbating or alleviating N limitation through excretion of inorganic nutrients. Grazers can supply a significant portion of inorganic nutrient (N and P) demand by primary producers (Grimm 1988; Hall et al. 2003), and the excretion rate as well as the N:P excretion ratio can affect the degree of nutrient limitation and the identity of the limiting nutrient (Elser et al. 2000; Vanni 2002). If excretion drives the primary producer community toward N limitation, N_2-fixing cyanobacteria may have a greater competitive advantage, and N_2-fixation rates may increase; conversely, if excretion increases N supply, N_2 fixation may be suppressed. In a laboratory experiment, Hillebrand et al. 2004) showed that the presence of snail grazers in an N-limited mesocosm increased N availability and consequently the N content of the periphyton community, suggesting that snail excretion was an important supply of the limiting nutrient. A conceptual model by Elser 1999) proposes that cyanobacteria blooms may be suppressed when *Daphnia sp.* are present because their high N:P excretion ratio alleviates N limitation. To our knowledge, no one has tested the effects of grazer excretion on N_2 fixation itself, and few experiments have shown that the excretion ratio of dominant grazers affects N limitation *in situ*.

Here I report the results of *in situ* grazing experiments in N-limited, oligotrophic lakes on the North Slope of Alaska in the vicinity of Toolik Field Station (Arctic Long Term Ecological Research Site). I examine the effects of benthic

grazing by the snail *Lymnaea elodes* on benthic primary production and N_2 fixation. I also measured the snail excretion rate of nitrogen and phosphorus to assess how much N snails could supply to primary producers relative to N_2-fixation rates. *Lymnaea elodes* is the dominant benthic grazer in oligotrophic arctic lakes in the vicinity of Toolik Lake (Cuker 1983; Hershey 1990) and is a principle prey item of the top fish predators, Lake Trout and Arctic Char (Merrick et al. 1992; Hershey et al. 1999), but little is known about how they affect community structure of benthic algae, benthic production, or nutrient dynamics on soft sediment in these arctic lakes.

METHODS.

Site Description. Toolik Field Station is located off the Trans-Alaskan Pipeline haul road (Dalton Highway) in the northern foothills of the Brooks Mountain Range in arctic Alaska (68°37′N, 149°35′W), about midway (150 miles) between the Arctic Circle and Prudhoe Bay (Figure 3.1). The area is underlain by continuous permafrost, and the landscape is rolling tundra terrain. The lakes in this region are generally shallow (3 – 15 m) glacial kettles. Ice-free season is mid-June to mid-September, and maximum epilimnetic temperatures range from 13 – 18 °C. Lakes in this region are dimictic and ultra oligotrophic (Miller et al. 1986), and water column concentrations of ammonium, nitrate, and phosphate are near detection limit, with concentrations below 0.1 μmol L^{-1} for these major nutrients. Water column ^{14}C-primary production ranges from 12 – 16 g C m^{-2} $year^{-1}$ (Miller et al. 1986). Light penetration is high, and secchi depth ranges 6 – 10 m in the summer. Summer thermocline depth is about 5 m, and bottom O_2 concentrations in most lakes remain high throughout the summer (7 – 8 mg L^{-1}). Because water column production is low and bottom oxygen concentrations are near saturation, many lakes contain oxidized layers of Mn and Fe at the sediment–water interface (Cornwell and Kipphut 1992). In

general, lake bottoms comprise extremely fine-grained, unconsolidated sediment. The N-fixing cyanobacteria *Nostoc sp.* form balls as large as 2 cm in diameter and are visibly prevalent on sediment surfaces in many lakes, including the lakes in this study (personal observation). Sediments also contain *Anabaena sp.* and other genera of filamentous cyanobacteria (personal observation).

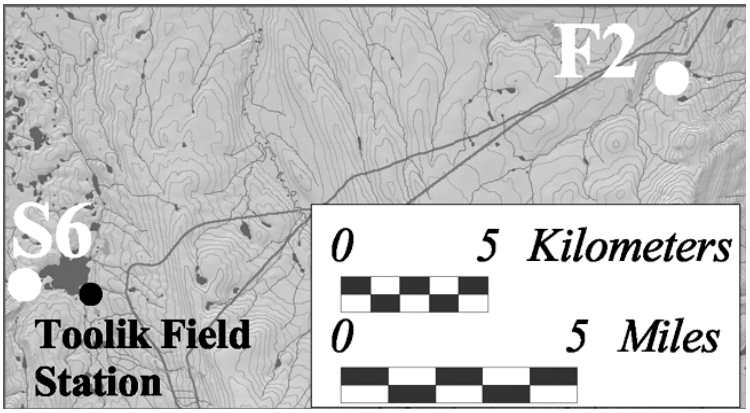

Figure 3.1. Map showing location of lakes Fog 2 (labeled F2) and S6 relative to Toolik Field Station on the North Slope of Alaska, 68°37′N, 149°35′W. Refer to Figure 1.1 in Chapter 1 for map of Alaska showing the location of Toolik Field Station.

Experiments were carried out in two lakes. Fog 2 is a headwater lake on young glaciated surface originating from the young advance of the Itkillik Phase II glaciation 12,000 – 25,000 ybp (Hamilton 2003). Lake Fog 2 has a surface area of 5.6 ha, maximum depth of 20 m, and an average depth of 7.8 m. Incubation experiments of water column production show strongest responses to N addition followed by N&P addition, indicating primary N limitation and secondary P limitation (Gross unpublished data). Fog 2 has relatively high rates of benthic production (up to 300 mg C m^{-2} day^{-1}; Chapter 2). Lake Fog 2 contains Arctic Char, which prey upon *Lymnaea elodes*, and likely control its size, distribution, and density by top-down control (Hershey et al. 1999).

Lake S-6 is also on glaciated surface originating from the Itkillik Phase II glaciation, but is smaller than Lake Fog 2. It is 1.1 ha and has a maximum depth of 8.5. The average depth is not known, but it is estimated to be about 4 – 5m based on a depth profile performed along transects using sonar from a rubber raft. Lake S-6 has higher benthic production than Fog 2 (up to 750 mg C m^{-2} day^{-1}, Chapter 2) and a higher conductivity (215 μS cm^{-1}). Lake S-6 does not contain fish species that prey upon *Lymnaea elodes.*

Experimental Design. I used a randomized block design in which snail density was manipulated in each treatment. The experiment was performed *in situ*, and cages were deployed using SCUBA at 3 – 4.5 m depth. Because of the heterogeneity in benthic environments, the experiment was conducted in lake Fog 2 in years 2001, 2002, 2003 and in S-6 in year 2002 (Table 3.1). There were 2 – 3 blocks depending on the year, and each block had three treatments: Snail exclusion (zero snails), low density, and high density. Low- and high-density treatments varied from year to year (Table 3.1). In year 2001, the low-density treatment had 12 snails m^{-2} and the high-density treatment had 48 snails m^{-2}. In year 2002, the low-density treatment had 24 snails m^{-2}, and the high-density treatment had 60 snails m^{-2}. In year 2003, the low-density treatment had 36 snails m^{-2}, and the high-density treatment had 72 snails m^{-2}. In year 2003, each block had a two replicates for each treatment. In years 2001 and 2002, there was one cage per treatment. To minimize variation in biomass, snails between 19.5 and 22 mm were used. These snails weighed 0.8 – 1.07 grams by length–weight regression (ln (g) = ln (mm) x 2.79 – 8.53; R^2=0.76; p<0.001; n=30).

Snail cages were constructed by cutting the bottoms off 5-gallon buckets 0.25 m^2 in area and covering the tops with plastic mesh screen with holes 4 mm in diameter to enclose or exclude snails. In years 2001 and 2003, windows were cut in the sides of the buckets to allow water exchange through the cage and to prevent shading. Each

window was covered with the plastic mesh, which reduced light by about 25%. To control for cage effects which included reduced light, an open control was used whose open sides allowed free access to the sediment by snails. In year 2002, I used a similar bucket design but without the windows to reduce the buoyancy of the cages. However, I was concerned that this bucket design exhibited greater cage effects (see results below), so windows were used again in 2003.

Table 3.1. Experimental information for *in situ* randomized block design grazing experiment in lakes Fog 2 and S-6 in years 2001 – 2003 to examine the effect of snail density on benthic N_2 fixation and primary production.

	Experimental Design			
Year	2001	2002		2003
Lake	Fog 2	Fog 2	S-6	Fog 2
Number of Blocks	3	3	2	2
Replicates/Treatment	1	1	1	2

	Snail Density Treatments (# m^{-2})			
Year	2001	2002		2003
Lake	Fog 2	Fog 2	S-6	Fog 2
Zero Snails	0	0	0	0
Low Density	12	24	24	36
High Density	36	60	60	72

	Ambient Conditions			
Year	2001	2002		2003
Lake	Fog 2	Fog 2	S-6	Fog 2
Temperature (^oC)	10	13	13	12
Light (μmol m^{-2} sec^{-1})	268	180	180	201

The experiments were conducted in the mid summer season (July) and completed by early August. Once the experiment was deployed, snails were allowed to graze for approximately three weeks. At the end of the experiment, SCUBA was used to collect intact sediment cores from within each bucket with an undisturbed sediment–water interface. Any snails present in the intact cores were removed, and these cores were then used to make measurements of primary production, N_2 fixation, chlorophyll biomass, and algal composition. One core 2.7 cm in diameter was

collected from each cage for chlorophyll and algal composition analysis. Two cores 9.5 cm in diameter and 30 cm tall were collected for N_2 fixation and primary production assays. The larger core used for N_2 fixation and primary production assays was inserted into the sediment up to about 10 cm, which left about 20 cm of water headspace. The mud cores were transported from the field to Toolik Field Station via helicopter in a water-filled, dark cooler. Incubations were performed in an incubation facility at ambient temperature and light conditions for production and N_2-fixation measurements.

Ambient snail densities were determined by laying a 10 m transect line between 3 – 5 m depth and swimming (with SCUBA) along the transect line and counting the number of snails 1 m to one side of the line. Three transects were conducted at the start, midpoint, and end of the experiment and averaged for the purposes of data analysis.

Chlorophyll a and phaeophytin analysis. Chlorophyll a and phaeophytin samples were sectioned from the top 2 cm of each core. Of the top two centimeters of sediment, five mls were subsampled each for chlorophyll and algal composition. Chlorophyll samples were frozen at –80^{o}C and transported back to The Ecosystems Center, Woods Hole, for analysis the following fall. Samples were analyzed using acetone extraction and a Shimadzu UV-1601 spectrophotometer with a syringe (piston) sipper and a 1 cm Hellma cell according to the Arctic LTER protocols, which were adapted from Lorenzen 1967). Chlorophyll a and phaeophytin measurements were summed to estimate total chlorophyll biomass, and total chlorophyll was used in the statistical analysis. Total chlorophyll is often considered a better proxy for algal biomass because the degradation of chlorophyll a to phaeophytin pigments can be rapid, especially in sediments (Bianchi et al. 1991).

Rates of N_2 fixation*:* N_2 fixation in mud cores was measured by the acetylene reduction assay (ARA), which quantifies the reduction of acetylene (C_2H_2) to ethylene (C_2H_4) by the nitrogenase enzyme (Hardy et al. 1968). Core tubes were 9.5 cm in diameter (70.9 cm^2 in area) and 30 cm tall. They became ARA incubation chambers when they were placed between two 1 cm thick clear polycarbonate rectangular plates, which were held together by nylon-threaded rods. The rectangular plates had a groove and O-ring fitting, which prevented leaking. Mud cores had about 1 L overlying lake water and 100 ml gas headspace. The top plate had a bulkhead-style septum port to allow sampling of the headspace, and the water–gas interface had an externally operated magnetic stirring apparatus to maintain the water and gas phases in equilibrium. The magnet was ~15 cm above the mud surface so that the sediments were not disturbed by the gentle stirring. Measurements of N_2 fixation in the overlying water proved negligible.

The volume of the water phase was determined by measuring the height of the water from the mud surface to the surface of the water to the nearest mm. Because the area of the core tube is known, volume can be calculated. Volume of the gas headspace was determined when the incubation was completed by weighing the core on a 2 kg scale, then filling the tube to the top with water and re-weighing it. The volume of the headspace was determined by subtracting the initial core weight from the final weight. The total amount of ethylene produced present in the gas and water phase was determined using Henry's Law. The difference in ethylene solubility due to temperature was accounted for according a temperature relationship presented in Sander 1999).

In addition to the ARA, ethylene consumption and production control blanks were included for each treatment to apply corrections if necessary. Soils and sediments can contain heterotrophic bacteria known to consume ethylene (Elsgaard

and Andersen 1998; Jackel et al. 2004) whereas diatoms and higher plants can produce ethylene (Abeles et al. 1992; Lee and Baker 1992). I did not sample areas that contained macrophytes, but these sediments contain a significant diatom community. For ethylene consumption controls, a known amount of ethylene was added in the range of expected ethylene production from N_2 fixation (usually ~1.5 μL ethylene), and no acetylene was added. For the production control cores, no ethylene or acetylene was added. Ethylene was rarely produced without the presence of acetylene, but ethylene consumption ranged from 5 – 15 % over the course of the 4-hour incubations. Independent ethylene consumption experiments confirmed that the ethylene loss rate was also linear for the duration of the incubation, and I applied the correction for each treatment assuming a linear consumption rate. Corrections were minor and did not change qualitative results.

Acetylene was introduced as saturated water, which reduces contamination by ethylene common in many acetylene tanks (Marino et al. 2003). Saturation was achieved by bubbling acetylene through water for about 25 minutes while on a stir plate to ensure that the water is saturated and in equilibrium with the atmosphere (see Marino et al. 2003 for details). Because some acetylene tanks can contain substantial amounts of ammonia which may affect N_2-fixation rates, acetylene was first bubbled through a 10% sulfuric acid solution to trap any ammonium present before bubbling it through the lake water. This procedure resulted in ammonium concentrations in the acetylene water that were similar to lake water concentrations near detection. Incubations lasted 4 – 6 hours over which ethylene production was linear. Ethylene samples were analyzed on a Shimadzu GC 8-A Flame Ion Detector (FID) using a Porapak N column, mesh size 80/100.

It is desirable to calibrate the ARA by measuring the incorporation of $^{15}N_2$ in N-fixing organisms because the relationship between moles of ethylene produced and

moles of N fixed is not always fixed at the assumed 3:1 theoretical value and may in fact be closer to 4:1 (Graham et al. 1980; Howarth et al. 1988b). For anaerobic sediments from the Narragansett Bay where N_2 fixation is most likely heterotrophic, Seitzinger and Garber 1987), determined that conversion factors could vary from 10:1 – 100:1 over an incubation period lasting 26 days. Because these sediments are over-lain by oxygenated water, and most of the N_2 fixation is likely autotrophic, this study system is not comparable. In addition, N_2-fixation rates are low in the Arctic (0.2 – 3 $\mu g\ m^{-2}\ day^{-1}$), and a pilot study in 2001 indicated that an isotope calibration method using $^{15}N_2$ gas is suspect because it requires long incubations (+1 week) in order to measure incorporated ^{15}N levels against sediment background N. Overly long incubations introduce a strong potential for core artifacts and make it difficult to calibrate to ARA incubations, which are much shorter (4 – 6 hours). Other measured conversion factors for cyanobacteria from benthic environments are reasonably constrained, ranging from 1.9 to 5.4 (Howarth et al. 1988b). Thus, a theoretical 3:1 conversion factor is a reasonable assumption, and it allows us to compare with the vast majority of other studies that also use this ratio to report and compare measurements (e.g., Howarth et al. 1988b; Alexander et al. 1989; Grimm and Petrone 1997; Higgins et al. 2001).

Gross Primary Production, Net Ecosystem Production, and Respiration. In a manner similar to the ARA chambers, measures of production were made using a chamber design that allowed the core tubes to fit a specially designed lid. The chambers were filled completely with no gas headspace, and the lid had a water-filled port into which a data-logging WTW O_2 probe was placed. This prevented the chamber from experiencing air exchange with the atmosphere while measurements were taking place. Changes in oxygen consumption and production were recorded every 15 minutes over periods of dark and ambient light levels, each lasting

approximately 12 hours in length at ambient temperature (Table 3.1). Net Ecosystem Production (NEP) was calculated as the rate of O_2 production during the light period of the incubation and multiplied by 24 hours because there are 24 hours of light in an arctic summer. Community Respiration (CR) was measured as the rate of O_2 consumption during the dark period of the incubation and multiplied over 24 hours. Gross Primary Production (GPP) was calculated as the sum of NEP and Respiration over a 24 hour period because the Arctic has 24 hours of daylight during the summer months.

Snail excretion. I measured ammonium excretion by snails by collecting snails from the sediments of Fog 2 and immediately enclosing them in acid-washed, individual bottles containing 0.2 μ filtered lake water. The lake water was filtered to remove phytoplankton and bacteria to minimize the possibility of N uptake and microbial N transformations during the course of incubations. Bottles with snails were stored in the incubation facility at ambient light and temperature conditions (180 μmol $m^{-2}sec^{-1}$ and 12°C). Excretion rate was calculated by measuring ammonium and phosphorus concentration before and after the snail incubation and expressed as μmoles $time^{-1}$ snail $biomass^{-1}$. In order to ensure that I could document changes in ammonium concentration, the incubation time was 10 hours long. However, the lack of food resources available to the snail during the long incubation period may cause underestimation of the excretion rate. To account for this possible source of error, I calculated a range for the excretion rate. For the upper estimate, I assumed that all of the N and P was excreted in the first hour, and for the lower estimate I assumed that the N and P was excreted over 10 hours.

Data Analysis. At the end of the *in situ* experiment, an effort was made to recover the snails that were added to the buckets at the beginning of the experiment, but because diver visibility is reduced while working in soft-sediment, recovery rates

varied from year to year. In year 2001, 67% of the snails were recovered at the end of the experiment; in year 2002 71% were recovered; and in year 2003, 47% were recovered. In total, only 3 dead snails were found over 3 years and two lakes. Thus, the snail density in the analysis is based on the number of added snails rather than the number recovered.

Because snail density in the low and high treatments varied in each year, data were analyzed using a randomized coefficient linear regression model with snail density as a continuous variable rather than using a Randomized Block ANOVA, which requires each treatment to be categorical. I controlled for block variability using Proc Mixed in SAS 1999) in which Block and Block x Year x Lake were treated as random effects, and snail density, lake, and year were treated as fixed effects. The model generated regression equations with estimates of intercept and slope (as related to snail density) that also generated estimates of standard error. When terms were not significant, they were eliminated so as to increase the power of the analysis for the remaining significant factors. Two of the N_2-fixation variables (biomass-specific N fixation and N-fixed as a proportion of N demand) were natural log transformed to improve their distribution under the assumption of normality and to stabilize variance.

Some data were eliminated for logistical and weather related reasons. In year 2002, Block 2 in S-6 required that the cores be extruded into different core-tubes because the cores sank too deep in the soft sediment and there was not an adequate headspace; further, some of the cores in Block 2 were sloshed in transport, which caused significant sediment disturbance. Thus, Block 2 in S-6 was not included in the analysis. In year 2003, Block 3 in Fog 2 was left in the lake 9 days longer than Blocks 1 and 2 because weather prevented us from accessing the lake by helicopter. By then, there were several hours of darkness each night, and the water temperature was 2 – 3 ^{o}C colder according to our dive computers. No snails were recovered, possibly

because they bury themselves in the sediment over the winter (Eisenberg 1966), and significant cage effects were shown. I therefore eliminated Block 3 in Fog 2 in year 2003 from the analysis. Even though logistical difficulties meant that retrieving the experiment was problematic, similar patterns are shown in different lakes in different years. Thus, our results are robust. In addition, the statistical analysis in Proc Mixed accounts for missing data and does not assume equal variance in its analysis of randomized block designs (SAS 1999).

RESULTS.

Cage effects. T-tests between ambient and control cages did not show significant differences for N_2 fixation, production, or biomass variables in years 2001 and 2003 ($p>0.05$ for all variables; Table 3.2). However, in year 2002, controls in Fog 2 showed significantly lower values than ambient cores for areal rates of N_2 fixation and respiration ($p<0.05$; Table 3.2). Also in year 2002, NEP was lower in control cages than ambient cages in S-6, but this difference was marginally significant ($p=0.07$; Table 3.2). As noted above, differences between ambient and control cages in 2002 were probably due to the design of the cage. However, the inclusion of year 2002 data in the randomized coefficient analysis did not change the statistical outcomes or the relationship between snail density and response variables, so 2002 data were included in the analysis.

The effect of grazing. Snail density was not a significant factor controlling benthic chlorophyll biomass in Fog 2 or S-6 in any year ($p>0.05$; Table 3.3). This is consistent with the fact that snail density did not affect measures of benthic metabolism including GPP, NEP, or CR ($p>0.05$; Table 3.3). In contrast, areal-specific and biomass-specific N_2 fixation as well as the proportion of N demand met

by N_2 fixation declined significantly with increasing snail density in both Fog 2 and S-6 and across years ($p<0.05$; Table 3.3; Figure 3.2).

When N_2 fixation was expressed as an areal rate, there was no significant year-to-year variation in Fog 2 (Table 3.4; Figure 3.2A). When the yearly data were pooled for Lake Fog 2 and compared to S-6, snail density reduced areal rates of N_2 fixation in a statistically similar manner, and the relationship between snail density and areal-specific N_2 fixation resulted in parallel slopes between the two lakes (Table 3.4; Figure 3.2A). Although the slopes relating snail density to areal rates of N_2 fixation were similar between Fog 2 and S-6, the intercept was significantly higher in S-6 than in Fog 2 (Table 3.4; Figure 3.2A). This indicates that S-6 has naturally higher rates of N_2 fixation even though it has a two-fold higher ambient snail density (Figure 3.3). This is likely due to the fact that lake S-6 is more productive than Lake Fog 2, and may have stronger positive bottom-up (nutrient) influences on N_2 fixation.

Biomass-specific N_2 fixation was also negatively related to snail density. However, neither lake nor year were significant factors (Table 3.3; Figure 3.2B). As a result, one regression line was generated from data pooled for S-6 and Fog 2 and across years 2001-2003 (Table 3.4, Figure 3.2B). This relationship is due to the fact that chlorophyll a biomass did not vary significantly between lakes or years and that chlorophyll was not drastically different between lakes Fog 2 and S-6 (Table 3.3, Figure 3.4).

Table 3.2. Mean values for ambient (A) measurements and cage controls (C) for years 2001 – 2003 in lakes Fog 2 and S-6. Standard error is in parentheses. The only significant differences by T-Test between ambient and cage controls occurred in year 2002 when cages had a different design (see text).

	N_2 Fixation (mg N m^{-2} day^{-1})					GPP (mg C m^{-2} day^{-1})					NEP (mg C m^{-2} day^{-1})				
	A	C	*df*	*t*	*p*	A	C	*df*	*t*	*p*	A	C	*df*	*t*	*p*
2001															
Fog 2	0.097 (0.01)	0.24 (0.07)	5	-2	0.1	142 (14)	126 (10)	5	0.86	0.4	29 (3)	33 (1)	5	-1.14	0.3
2002															
Fog 2	0.88 (0.04)	0.39 (0.12)	5	3.88	*0.02*	375 (30)	367 (50)	5	0.16	0.9	105 (34)	163 (37)	5	-1.15	0.3
S-6	0.36 (0.1)	0.35 (0.1)	5	0.99	0.93	713 (20)	647 (47)	5	1.46	0.2	409 (20)	263 (72)	5	2.26	*0.07*
2003															
Fog 2	0.27 (0.06)	0.24 (0.03)	14	0.38	0.71	405 (31)	412 (10)	14	-0.2	0.9	243 (17)	265 (20)	14	-7.59	0.46

	Resp (mg C m^{-2} day^{-1})					Total Chlorophyll (μg m^{-2} day^{-1})				
	A	C	*df*	*t*	*p*	A	C	*df*	*t*	*p*
2001										
Fog 2	-113 (17)	-93 (11)	5	-0.9	0.4	NA	NA	NA	NA	NA
2002										
Fog 2	-270 (6)	-204 (17)	5	-4.1	*0.01*	310 (54)	350 (47)	5	3.23	0.9
S-6	-304 (12)	-384 (31)	5	2.75	0.4	708 (83)	671 (109)	5	0.27	0.8
2003										
Fog 2	-161 (31)	-147 (12)	14	-0.3	0.75	426 (25)	459 (39)	12	-0.8	0.5

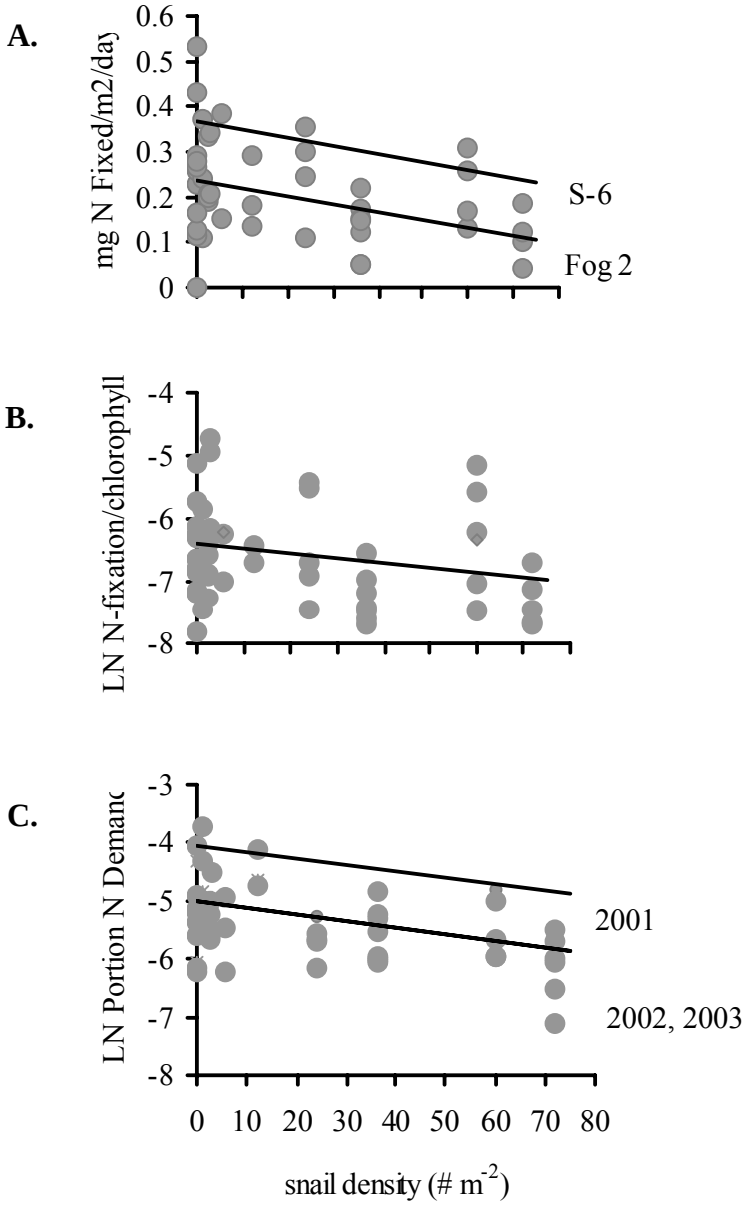

Figure 3.2. Results from randomized-coefficient analysis relating snail density to measured of N_2 fixation. The statistical analysis takes into account block, lake, and year variability, and the points depicted are data points from all blocks and all years. **A.** N_2 fixation on an areal basis declines with increasing snail density. Lake S-6 has a similar relationship as Fog 2, but has a significantly higher intercept. **B.** N_2 fixation per chlorophyll a biomass declines with increasing snail density. There was no significant difference between years or between lakes. **C.** The proportion N demand met by N_2 fixation declines with increasing snail density. This relationship was not different between lakes, but was significantly different in 2001 compared to 2002 and 2003.

Table 3.3. Significant factors from randomized coefficient regression for each of the measured variables in the experiment.

Model Info		Numerator DF	Denominator DF	F-Value	p-value
N_2 Fixation (mg N m^{-2} day^{-1})	Grazer Density	1	9	11.47	**0.008**
	Lake	1	8	17.15	**0.003**
GPP (mg C m^{-2} day^{-1})	Lake	1	6	29.37	**0.002**
	Year	2	6	18.34	**0.003**
NEP (mg C m^{-2} day^{-1})	Lake	1	6	11.11	**0.016**
	Year	2	6	22.26	**0.002**
Community Respiration (mg C m^{-2} day^{-1})	Lake	1	6	51.59	**<0.001**
	Year	2	6	12.68	**0.007**
Portion N Demand met by N_2 fixation	Grazer Density	1	6	15.41	**0.004**
Total Chl (mg m^{-2})	Lake	1	5	5.31	**0.069**
N_2 fixation per Chlorophyll Biomass	Grazer Density	1	8	5.74	**0.044**

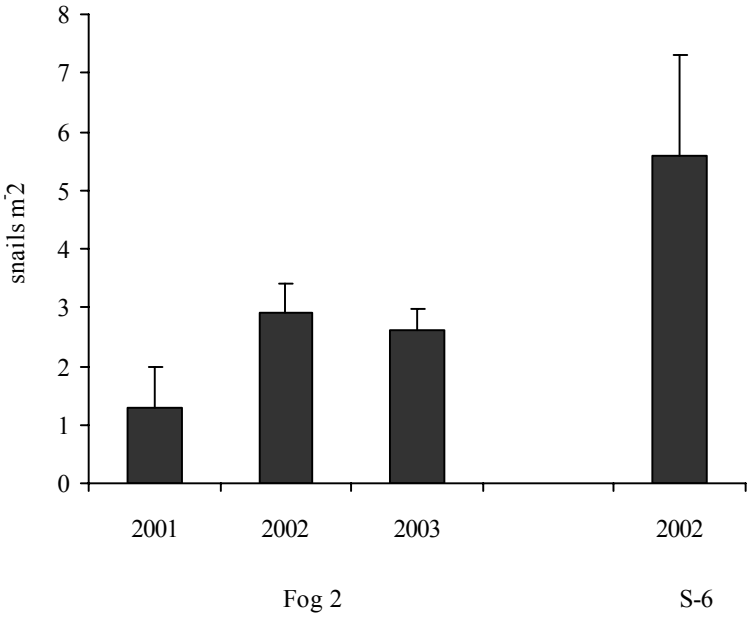

Figure 3.3. Snail density in lake Fog 2 and S-6 for each year of the experiment. Snail density was determined by counting snails 1 m from a 10 m transect line (see text). Snail density was significantly higher in Lake S-6 than in Lake Fog 2. Snail density was lower in 2001 in lake Fog 2 than in subsequent years.

I also expressed N_2-fixation rate as a proportion of N demand by primary production. Since we are not able to measure net primary production (NPP) using the oxygen production and consumption methods described above, I assumed that NPP was 75% of measured GPP. This assumption is simply a linear scaling factor and therefore does not affect our ability to compare among treatments. Other benthic ecologists have made similar assumptions (e.g., Anderson et al. 2003; Sundback et al. 2004). Based on a C:N molar ratio for benthic algae of 17:1 (Hillebrand and Sommer 1999), I calculated that N_2 fixation provided between 0.2 – 2.5 % of N demand by primary production across snail density treatments and that the portion of N demand that is met by N_2 fixation significantly declined with increasing snail density (Table 4, Figure 2C). This relationship was similar between Fog 2 and S-6. However, GPP (and hence NPP) was lower than in 2001 in Fog 2 than in subsequent years. This resulted in a higher proportion of N demand that was met by N_2 fixation, and a higher intercept in Figure 2C.

Table 3.4. Linear regression parameters and standard errors relating snail density to N_2 fixation. Significant factors (lake or year) are shown depending on which were significant in the regression model. For areal rates of N_2 fixation, S-6 served as a dummy variable and for the variable describing the portion N demand met by N_2 fixation, year 2003 served as a dummy variable. For per-biomass N_2 fixation, there was no significant lake or year effect. As a result, lake and year were pooled to generate one regression model relating snail density to per biomass N_2-fixation rates.

		Estimate	Standard Error	DF	T-Value	p-value	Meaning of the p-Value
N_2 fixation (mg N fixed m^{-2} day^{-1})							
Lake S-6	Intercept	0.235	0.033	8	11.22	<.0001	a
	Snail Density (slope)	-0.002	0.001	9	-3.39	0.008	b
Lake Fog 2	Intercept	0.106	0.031	8	-4.14	0.003	c
	Snail Density (slope)	-0.002	0.001	9	-3.39	0.008	b
Portion N Demand met by N_2 fixation							
2001	Intercept	-4.036	0.241	7	4.08	0.005	d
	Snail Density (slope)	-0.011	0.003	9	-3.92	0.004	b
2002	Intercept	-5.001	0.216	7	0.09	0.933	e
	Snail Density (slope)	-0.011	0.003	9	-3.92	0.004	b
2003	Intercept	-5.020	0.156	7	-32.18	<0.001	a
	Snail Density (slope)	-0.011	0.003	9	-3.92	0.004	b
N_2 fixation per Chlorophyll Biomass (mg N_2 Fixed/mg Chl m^{-2} day^{-1})							
Both Lakes, All Years	Intercept	-6.385	0.203	8	-31.41	<0.001	a
	Snail Density (slope)	-0.008	0.003	8	-2.40	0.044	b

[a]Intercept is significantly different than zero; [b]Slope is significantly different than zero; [c]Intercept is significantly different than Lake S-6; [d]Intercept is significantly different than year 2003; [e]Intercept is similar to year 2003

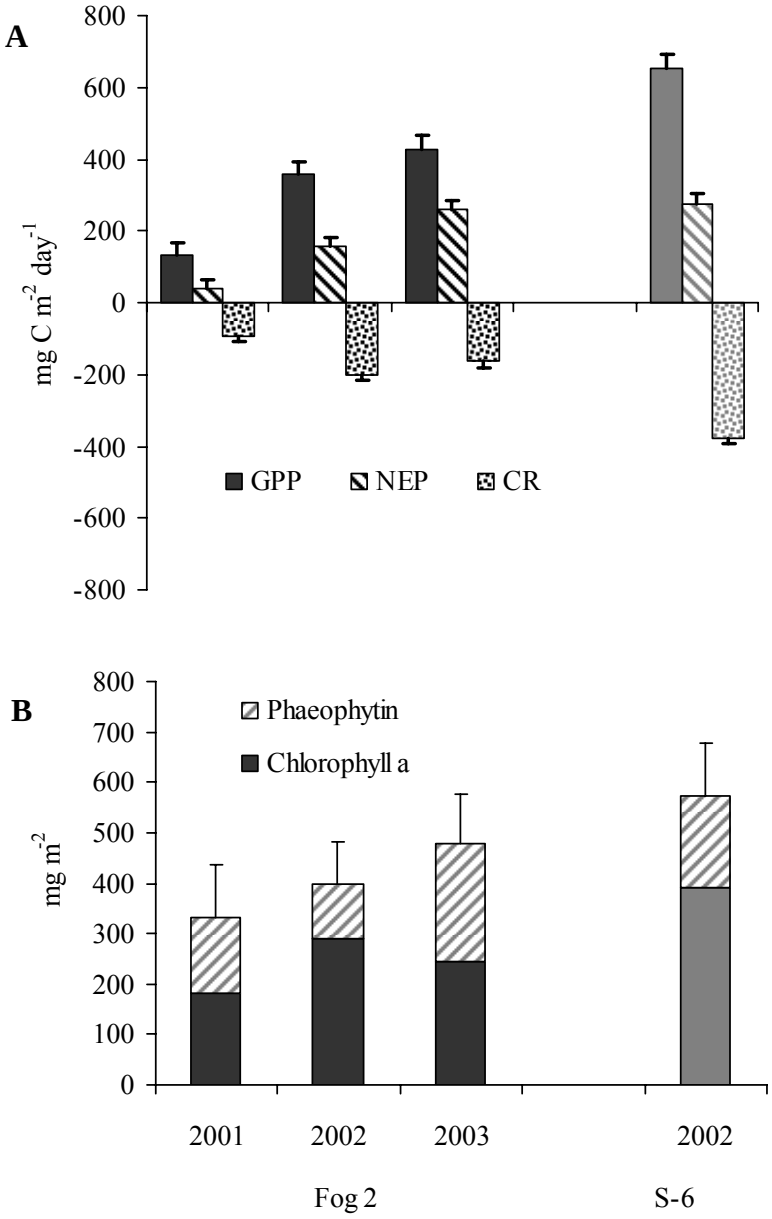

Figure 3.4. Variation in measures of metabolism and benthic chlorophyll biomass in Fog 2 and S-6 for years 2000 – 2003. **A**. Gross Primary Production, Net Ecosystem Production, and Community Respiration. **B.** Total chlorophyll biomass..

Lake and year comparisons. Because snail density did not affect chlorophyll biomass or measures of ecosystem metabolism, data were pooled across snail density treatments to compare these parameters among lakes and years (Table 3.5, Figure 3.4).

Lake S-6 had a significantly higher benthic gross production and respiration as well as slightly higher total benthic chlorophyll levels than Lake Fog 2 (Table 3.5, Figure 3.4). In year 2001, GPP, NEP, and CR were all significantly lower in lake Fog 2 than in years 2002 and 2003 (Table 3.5, Figure 3.4). Snail density was lower in Lake Fog 2 in year 2001 than in 2002.

Snail excretion. For an average snail of 20 mm, our range of estimated excretion rates for phosphorus ranged from $2.76x10^{-4}$ to $3.1x10^{-3}$ mg $P\text{-}PO_4$ day^{-1} (n=16). Our estimate for ammonium excretion was $4.4x10^{-4}$ to $5.2x10^{-3}$ mg $N\text{-}NH_4$ day^{-1} (n=16). Assuming that net primary production is about 75% of ambient gross primary production (or about 300 mg C m^{-2} day^{-1}) and that benthic algae have a C:N ratio of 6 by weight (Hillebrand and Sommer 1999), one snail supplies 0.001 – 0.017% of the N demand per day, supporting 0.003 – 0.03 mg C m^{-2} day^{-1} of net primary production. Even in the highest density treatment (72 snails m^{-2}), this supply rate is small, 0.03 – 0.37 mg N m^{-2} day^{-1} or 0.1 - 1.23% of the N demand by primary producers. Over the duration of the experiment (approximately three weeks), snails in the high-density treatment could support 4.8 – 55.7 mg C m^{-2}. The molar $N\text{-}NH_4$:$P\text{-}PO_4$ ratio excretion ratio was very low relative to algal N:P ratio, averaging 4.8 ± 1.42 (SE) (Figure 3.5).

Table 3.5. LS Means estimate of Net Primary Production, Gross Primary Production, Community Respiration, and Total Chlorophyll for Lake Fog 2 and S-6 for years 2001 – 2003.

	Estimate	Standard Error
GPP (mg C fixed m^{-2} day^{-1})		
Lake Fog 2		
2001	131	35
2002	360	34
2003	426	40
Lake S-6		
2002	652	42
NEP (mg C fixed m^{-2} day^{-1})		
Lake Fog 2		
2001	38	23
2002	157	23
2003	262	24
Lake S-6		
2002	277	28
Community Respiration (mg C fixed m^{-2} day^{-1})		
Lake Fog 2		
2001	-92	15
2002	-203	15
2003	-163	18
Lake S-6		
2002	-376	19
Total Chlorophyll (mg chlorophyll a + Phaeophytin m^{-2})		
Lake Fog 2		
2001	335	103
2002	398	83
2003	478	99
Lake S-6		
2002	575	102

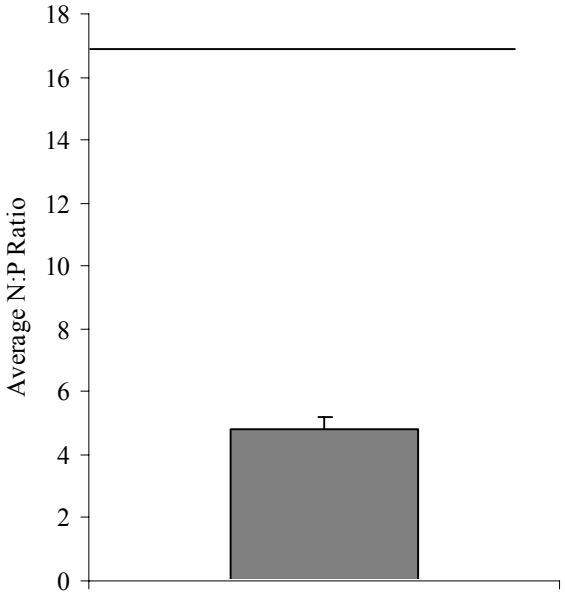

Figure 3.5. Molar N:P excretion ratio of Lymnaea elodes as N-NH4 and P-PO4. The line denotes the optimum N:P ratio for benthic algae as determined by Hillebrand and Sommer (1999).

DISCUSSION.

Grazing by the snail *Lymnaea elodes* caused a decline in areal and per-biomass rates of N_2 fixation. However, grazing did not cause declines in measures of ecosystem metabolism (net ecosystem production, gross primary production, or respiration); nor did snails cause a decline in total chlorophyll biomass. These results suggest that snails can reduce N inputs to N-limited arctic lakes without altering production in the benthic littoral environments. These results are robust over time (years) and in multiple lakes.

How can snails cause changes in N inputs through the reduction of N_2-fixation rates without causing changes in primary production or algal biomass? One possibility is that cyanobacteria are more sensitive to direct grazing pressure and recover more slowly from a grazing disturbance than other species of algae. Even though many

cyanobacteria are protected by cyanotoxins and can have morphological adaptations that reduce their susceptibility to certain species of grazers (Reynolds 1987, Dodds and Castenholz 1987), they have been shown to be sensitive to direct grazing pressure in pelagic environments (Schaffner et al. 1984; Chan et al. 2004; Chan et al. 2006). Fewer studies exist in benthic environments, but a number of other studies (Table 3.6) indicate that snails can have negative effects on cyanobacterial biomass. Of 6 grazing studies examining the effect of snails on periphyton community structure, five of them documented a decline in cyanobacterial biomass (Lowe and Hunter 1988; McCollum et al. 1988; Tuchman and Stevenson 1991; Armitage and Fong 2004; Evans-White and Lamberti 2005), and only two of them documented an increase (Catteneo and Kalff 1986; Rosemond et al. 1993). To examine whether snail grazers were reducing cyanobacterial abundance, I also enumerated algal community composition of the epipelon for all treatments in Fog 2 in year 2001 and for all treatments in both Fog 2 and S-6 in year 2003. The number of filamentous cyanobacteria colonies was not reduced in the high snail density treatments, either in absolute counts or proportionally (Appendix 3). Of the studies listed in Table 3.6, this study is the only one that documented no change in cyanobacterial abundance.

Another way in which grazing could reduce N_2 fixation is by reducing the size of cyanobacterial filaments (Schaffner et al. 1984; Chan et al. 2004; Chan et al. 2006). Because filamentous cyanobacteria often fix N in heterocysts, which are energetically expensive to maintain, the ratio of photosynthetic cells to heterocyst number may affect growth rates. Similar patterns have been shown in both a freshwater environment (Schaffner et al. 1984; Chan et al. 2004) and estuaries (Howarth et al. 1999; Chan et al. 2006; Marino et al. 2006,) such that grazed colonies were shorter in length and had slower growth rates than longer chains. In the samples that were enumerated for algal community composition, I did not see evidence from

measurements of cyanobacterial filaments that the length was reduced in the high density treatments relative to controls (Appendix 3.).

These results showing a reduction of N_2 fixation with increased grazer density are in contrast with a number other studies that have shown a positive response of benthic N_2 fixation and/or cyanobacterial biomass to grazing. These studies tend to include fish species as part of the grazing community (Table 3.6). Of these, authors hypothesize that grazing maintains algal community structure in early successional stages, which are dominated by cyanobacteria (Wilkinson and Sammarco 1983; Flecker 1996); or that grazers clear epiphytes from slower-growing cyanobacterial mats (Power et al. 1988). In one exception, Dodds and Castenholz (1987) found that a snail cleared epiphytes from *Nostoc puniforme* colonies, which increased their growth rates; however, they did not examine the effect of snail grazing on algal benthic community structure as a whole. Williams and Carpenter (1997) also documented that a sea urchin caused an increase in N_2 fixation, but did not find changes in community structure that supported that observation and acknowledged that more work was needed to determine whether an increase in N_2 fixation was due to community composition shifts.

Table 3.6. Comparison of results from benthic grazing studies that enumerated the response of N-fixing cyanobacteria or measured N_2 fixation.

Study	Benthic Grazer	Ecosystem type	Cyanobacteria response	N_2 fixation
This Study	Snail (*Lymnaea elodes*)	*In situ*,oligotrophic lakes near Toolik Lake, Alaska	No change in cyanobacterial colony number or length with increasing snail density	N_2 fixation reduced by snails
Armitage and Fong 1994	Snail (*Cerithidea californica*)	*In situ,* Tidal-flats, Mugo Lagoon, Southern California	Cyanobacterial pigment zeaxanthin declined when snails were present	N_2 fixation not measured
Cattaneo and Kalff 1986	Snail (*Annicola sp.*) and meiofauna assemblage including chironomids, oligochaetes, and cladocerans	*In situ*, Lake Memphremagog, Quebec	Filamentous cyanobacteria increased when snails were present, especially *Gloeotrichia pisum*	N_2 fixation not measured
Dodds and Castenholz 1987	Snail (*Vorticifex effusa*)	*In situ*, Mare's Egg Spring, Oregon	Epiphytes were removed from *Nostoc puniforme* colonies, increasing growth rate	N_2 fixation not measured
Evans-White and Lamberti 2005	Snail (*Lymnaea livescens*)	Recirculating stream, northern Midwest	Percent cyanobacterial biovolume was reduced by ~27% in the snail treatments relative to no-grazer controls	N_2 fixation not measured
Lowe and Hunter 1988	Snail (*Physa integra*)	*In situ*, Spring Lake, northern Michigan	Filamentous cyanobacteria *Schizothrix sp. a*nd other genera of cyanobacteria reduced in high density treatment	N_2 fixation not measured
McCollum et al. 1998	Snail (*Physella heterostropha*)	Aquaria (Water and snails collected from freshwater ponds in North Carolina)	Cyanobacterial cell biovolume and cell number were reduced when snails were present	N_2 fixation not measured
Tuchman and Stevenson 1991	Snail (*Elimia livescens*)	Flow-through mesocosms near Douglas Lake, MI and Kentucky Lake, Kentucky	Filamentous cyanobacteria *Schizothrix calcicola* and *Phormidium tenue* was reduced when snails were present	N_2 fixation not measured

Table 3.6. (Continued).

Study	Benthic Grazer	Ecosystem type	Cyanobacteria response	N_2 fixation
Rosemond et al. 1993	Snail (*Elimia clavaeformis)*	Flow-through channels and enclosures, woodland stream (Walker Branch, North Carolina)	Cyanobacteria with prostrate morphologies increased when snails were present	N_2 fixation not measured
Flecker et al. 1996	Detrivirous fish (*Prochilodus mariae*)	*In situ,*Tropical stream (Rio Las Marias, Venezuela)	Number of *Calothrix sp.*filaments was higher on rock substrates exposed to grazing.	N_2 fixation not measured
Gettel and Flecker unpub.	Armored catfish (*Ancistrus triradiatus)*	*In situ*,Tropical stream (Rio Las Marias, Venezuela)	Community composition not enumerated	N_2 fixation increased
Power et al. 1988	Native grazing assemblage including Catfish (*Campostomoa anomalum*), minnows, and snails	*In situ*, stream in Ozark Mountains	Filamentous cyanobacterial felts comprised of *Calothrix sp.* developed on grazed substrate and were overgrown by epiphytic diatoms on ungrazed substrate	N_2 fixation not measured
Williams and Carpenter 1997	Sea urchin (*Diadema antillarum*)	*In situ*, Coral reef, Tague Bay, St. Croix, Virgin Islands	Shift in algal community composition was probably not responsible for decline in N_2 fixation	N_2 fixation increased when grazers were present
Wilkinson and Sammarco 1983	Damselfish (*Hemiglyphidodon plagiometopon*) and native grazing fish	*In situ,* Britomart Reef, Great Barrier Reef	Percentage substrate containing cyanobacteria was higher on grazed substrate	N_2 fixation increased when grazers were present

It could be that large, scraping invertebrate grazers such as snails affect cyanobacterial communities differently than fish grazers. Snails may be less selective than fish species, and the susceptibility of algal and cyanobacterial species to snail grazing may be based on morphology rather than food quality (Cattaneo and Kalff 1986; Lowe and Hunter 1988). Of all the studies documented in Table 3.6, only two showed an increase in cyanobacteria in response to snail grazing (Cattaneo and Kalff 1986; Rosemond et al. 1993). In the Cattaneo and Kalff (1986) study, slower-growing filamentous cyanobacteria became dominant when snails were present, perhaps as a result of the lower grazer turnover rate associated with large snails compared to smaller sized chironomids, cladocerans, and oligochaetes present in other treatments. The increase in cyanobacteria in the Rosemond et al. (1993) study was in prostate forms of cyanobacteria, leading them to suggest that morphological resistance to grazing was an important component in structuring the periphyton community. Other studies also support this idea. McCollum et al. (1988) tested the hypothesis that colonial cyanobacteria would comprise a greater proportion of the periphyton community in the presence of snail grazing because cyanobacteria are considered unpalatable to snails (Cattaneo and Kalff 1986; Bronmark 1989; Porter 1977). Instead, they found that filamentous cyanobacteria were significantly reduced in the snail treatments, and suggested that snails dislodged or inhibited them from growing by the mechanical act of grazing. Hillebrand et al. 2000) also found that grazing resistance of periphyton communities was related to algal growth form in which large, erect species were more susceptible to grazing and smaller, more prostate forms were more resistant. While these patterns are well documented, the link between grazer species, subsequent effects on algal community structure, and N_2 fixation has not yet clearly been made.

Under some circumstances, grazers may alter nutrient regimes by increasing the supply rate of inorganic nutrients or the supply ratio of limiting nutrients such as N and P (Vanni 2002; Hillebrand et al. 2004; Evans-White and Lamberti 2005). In turn, excretion may affect nutrient limitation status and the relative competitive advantage of N-fixing cyanobacteria (Elser 1999; Elser and Urabe 1999). In this study, snails excrete a surprisingly low N:P molar ratio (~4.8; Figure 3.5), which is well below the 17: 1 molar ratio for benthic algae determined by Hillebrand and Sommer (1999). Little data exist for excretion for both N and P for other snails, but Evans-White and Lamberti (2005) showed that the snail *Elimia livescens* excretes a higher N:P molar ratio than *Lymnaea elodes,* ranging from 7 – 50. Their study indicated that the epilithon may have become less N-limited in the presence of snails, but our study indicates that the low N:P excretion ratio likely exacerbated N limitation rather than alleviated it.

If grazers increase the supply rate of inorganic N, N_2 fixation may be suppressed regardless of the N:P supply ratio (Howarth et al. 1988a). However, N-NH_4^+ excretion rate appears to be low relative to other sources of N. These sources include relatively high concentrations of ammonium in the underlying interstitial water (e.g., 0.18 – 0.38 mg N l^{-1} in Toolik Lake; Alexander et al. 1989) and decomposition, which based on estimates of heterotrophic respiration and the assumption that the sediment C:N molar ratio (including detritus) is ~12 (Giblin et al. unpublished data), supplies 7 – 17 mg N m^{-2} day^{-1} or 13 – 60% of autotrophic N demand. In our system, N excreted by *Lymnaea elodes* meets almost none of the N demand by primary production (<<1%). These results are consistent with a previous experiment in which Cuker (1983) showed that excretion by *Lymnaea elodes* from Toolik Lake did not increase algal production of periphyton communities on rocks, and Evans-White and Lamberti (2005) also showed that chlorophyll a biomass did not

decrease in snail grazing treatments relative to non-grazer controls. In our study, N-supply by snail excretion is low relative to the demand by primary producers, and it is also small relative to other sources of N.

Cuker (1983) also found that snails egest viable cells that photosynthesize at 80% the rate of ungrazed substrates on a per-biomass basis and hypothesized that these viable cells may take up inorganic N within the snail gut. This could account for the very small measured N-excretion rate as well as the lack of change in production and chlorophyll biomass that I detected in this experiment. This also suggests that the observed decline in N_2 fixation could be a result of lowered N demand from recycled N within the snail gut.

Although the mechanism by which snails reduce N_2 fixation in the benthos is unknown, both the supply of N by snail excretion and the reduction of N_2 fixation by snails at ambient densities is small. At ambient snail density of ~4 snails m^{-2}, N-supply by snail excretion is orders of magnitude lower than N_2 fixation (0.002 – 0.02 mg N m^{-2} day^{-1} vs. 0.1 – 0.4 mg N m^{-2} day^{-1}; Table 3.2). N_2 fixation was reduced by each snail by 0.002 mg N m^{-2} day^{-1}, or over the course of a 60-day growing season, 0.12 mg N m^{-2} $summer^{-1}$. This reduction is only 0.85 – 1.8% of the N_2-fixation rate in the zero snail treatments in S-6 and Fog 2. Although the reduction in N fixation is small, N fixation represents a new input of N which can support new production, whereas N excretion represents recycled N. Thus, the reduction of N_2 fixation by *Lymnaea elodes* may be important to lake productivity over long time scales. In addition, *Lymnaea elodes* densities are quite high in some lakes and reservoirs in the temperate latitudes and can reach 100's per square meter (Eisenberg 1966). These results indicate that *Lymnaea elodes* may be important factors affecting N_2 fixation, N supply and production where natural densities are higher.

Our results are unusual in that snails caused no change in production and chlorophyll biomass. Most grazing studies show a decrease in algal biomass with increasing grazer density (Steinman 1996; Liess and Hillebrand 2004). However, in a review of grazing effects in freshwater benthic environments, Steinman (1996) noted that whenever areal specific primary production either increased or did not change in response to grazers, snails were the herbivore involved. He hypothesized that some snails may exhibit relatively low grazing pressure because of their slower movements, lower consumption rates, and low mobility.

This observation and our results are in contrast with those of Cuker (1983), who showed that on rock substrates in Toolik Lake, *Lymnaea elodes* reduced chlorophyll a biomass, primary production. However, epilithic production is two orders of magnitude smaller (0.4 g m^{-2} $year^{-1}$; Yeakel 1978) than epipelic production (18 g C m^{-2} $year^{-1}$; our data) assuming a 60-day growing season. Thus, production on sediment may be less sensitive to grazing disturbance than on rock substrates. Calculations of algal turnover rate (our data) and estimates of grazing rate from Cuker (1983) further support that epipelic production may be sufficiently high to compensate for grazing by *Lymnaea elodes.*

Our data show that grazing by the snail *Lymnaea elodes* does not exert an important control on benthic primary production or algal composition in these arctic lakes. Although snail grazing can reduce N_2 fixation, this effect is relatively small at ambient densities. These results support other studies that indicate snails may be fundamentally different from other grazers in how they affect benthic production and N cycling. However, to date, only a few studies have directly measured the response of N_2 fixation to benthic grazing, and all the other studies were done in the tropics. More work will be needed to determine how different grazers impact nitrogen fixation in a variety of environments.

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APPENDIX 1. PATTERNS IN BENTHIC N FIXATION ACROSS THE ARCTIC LANDSCAPE

Introduction. In the vicinity of Toolik Field Station in Northern Alaska (Figure 1.1), Pleistocene valley glaciations have occurred for periods of time between 12,000 to 800,000 years before the present (Hamilton 2003). Three dominant advances and retreats occurred in nearby drainages, exposing landscapes at different times. The oldest landscape, the Sagavanirktok, was exposed 250,000 – 800,000 years ago; the intermediate glaciation, the Itkillik I, was exposed 25,000 – 50,000 years ago, and the youngest Itkillik II glaciation was exposed 10,000 – 12,000 years ago. Because of weathering, older surfaces have low phosphorus (P) and calcium (Ca) availability when compared with younger surfaces (Hobbie et al. 2002, Hobbie and Gough 2002). Lake chemistry follows these general patterns in Ca and P chemistry (Kling et al. 1992; Figure A1.1; data from Arctic LTER database), and lakes on younger surfaces generally have higher rates of benthic primary production (Figure A1.2; unpublished data from A. Giblin and G. Kipphut).

N fixation has been shown to increase with increased P inputs (e.g., Schindler 1977; Chapter 2) and to decline in response to snail grazing (Chapter 3). Snail grazers are more prevalent on young surfaces and absent in lakes on old surfaces, presumably because of a decline in Ca availability or other biogeographical constraints (Hershey, pers. com.) Therefore, landscape age may confound patterns in N fixation across the age gradient by directly influencing P availability or the N:P ratio (e.g., Chapter 2) or by indirectly influencing the presence of snail grazers (Chapter 3). In order to determine whether landscape age is related to N-fixation rates in benthic environments, I conducted two lake surveys across the chronosequence for benthic N fixation in year 2002 (described in Chapter 1) and in year 2000 (described below). I

also examined whether N fixation was related to other variables such as gross primary production (GPP), benthic chl a, and light (as photosynthetically active radiation, or PAR).

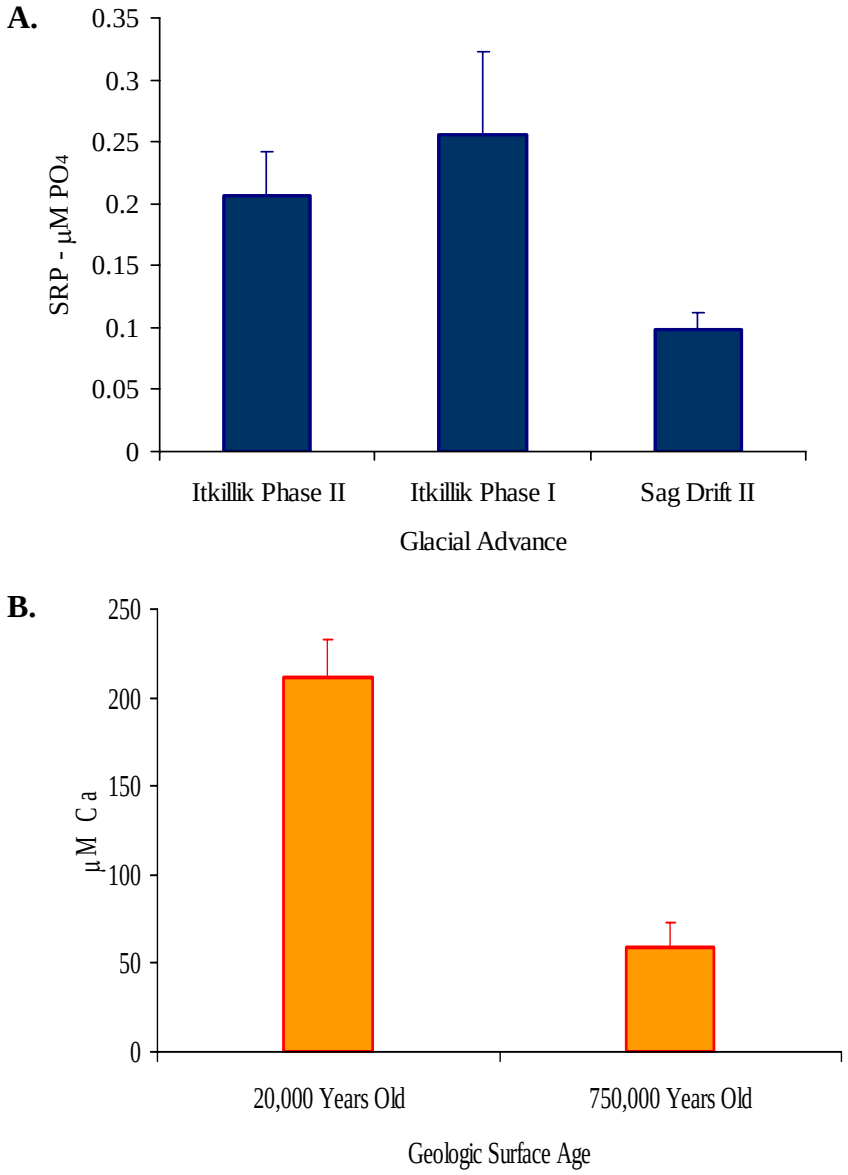

Figure A1.1. Relationship of geologic surface and Ca and PO_4 concentrations in lakes in the vicinity of Toolik Field Station. **A.** Average PO_4 concentrations for lakes on Iktillik I (12,000 years old), Itkillik II (20,000 years old), and Sagavanirtok (500,000-800,000 years old) surfaces. **B.** Average Ca for Itkillik Phase II and Sagavanirktok surfaces. No data were available for Itkillik Phase I from the Arctic LTER database for Ca.

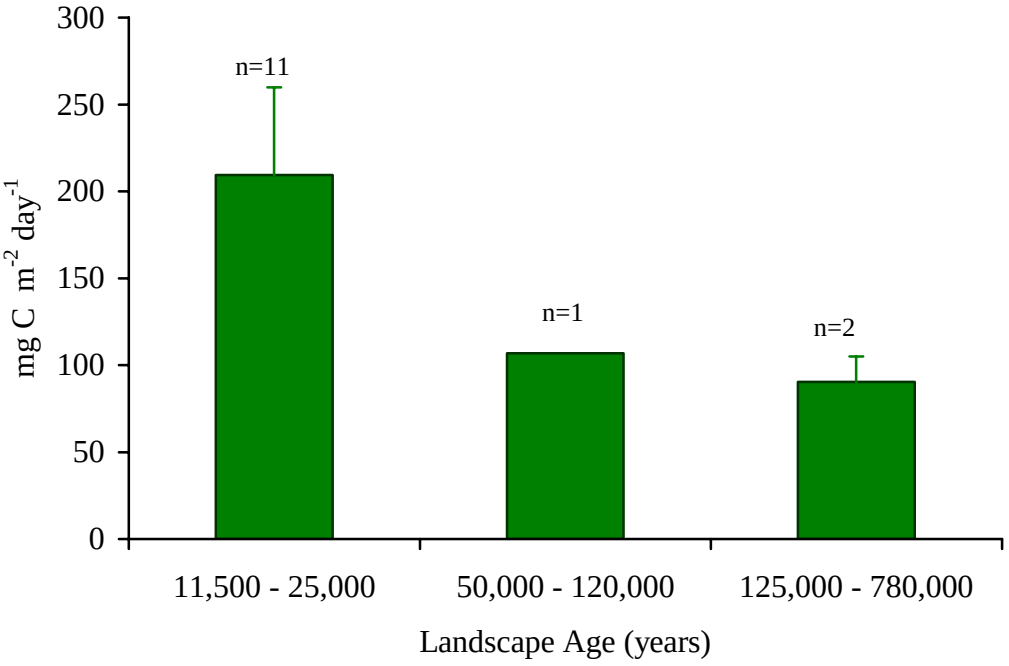

Figure A1.2. Compilation of benthic primary production measurements from LTER lakes (A. Giblin and G. Kipphut, unpublished data).

Method. In year 2002, measurements for benthic GPP, chlorophyll a, and N fixation were performed onver periods of light and dark, and N fixation was determined using acetylene red 3 intact sediment cores from 3 m depth in 3 lakes on each surface for a total of 9 lakes (See Chapter 1 for descriptions of these lakes). Rate measurements were performed in the incubation facility at Toolik Field station at ambient lake temperature and light conditions for each lake, which was 13^{o}C for all lakes but ranged between 20 – 180 μE $m^{-2}sec^{-1}$ for PAR. GPP was measured by documenting changes in oxygen o uction assay (ARA). Further details concerning methods for performing these measurements on intact sediment cores are described in Chapter 1.

In 2000, only N fixation was measured on sediment slurry samples from a total of 15 lakes (Table A1.1). Three slurry samples were collected at 1 m depth using a

specially adapted plastic 60 ml syringe (Polypropylene, Becton-Dickson) in which the tip was cut off at the zero mark to form a "mini-core." Mini-cores were collected by inserting the syringe into the sediment up to the 10 cc mark to collect a consistent volume of a known area of sediment from each lake. These samples were analyzed using acetylene reduction, and N fixation was expressed on an areal basis. The lakes included in this survey were not evenly distributed across the different aged surfaces, in part because lakes on older surfaces are less common (Gettel personal observation; Burkart 2006). Another important difference between the 2002 and 2000 datasets is that all of the 2000 survey samples were incubated at the same temperature and light conditions at 1 m depth in Toolik Lake at about 200 $\mu E\ m^{-2}sec^{-1}$ and $18^{o}C$. The locations of these survey lakes and the 2002 survey lakes are shown in Figure A1.3.

Table A1.1. Lakes characteristics sampled in the 2000 survey.

Lake	Surface	Lake Area (m^2)	Watershed Area (m^2)	Depth (m)	Conductivity ($\mu S\ cm^{-1}$)
Fog 2	Itkillik II	60,151	456,764	16	135.3
Fog 4	Itkillik II	20,624	260,987	3	75.0
S-6	Itkillik II	6,673	60,717	7	168.3
NE-9B	Itkillik II	1,785	138,255	7	335.0
N-1	Itkillik II	37,662	373,966	12	129.0
S-3	Itkillik II	36,018	187,793	1.5	NA
S-4	Itkillik II	3,885	81,236	1.5	NA
S-5	Itkillik II	4,254	185,563	1.5	NA
S-7	Itkillik II	6,443	102,322	4	205.5
GTH 11	Itkillik II	14,805	299,304	8	191.1
GTH 14	Itkillik II	2,860	171,137	2	129.0
Green Cabin	Itkillik I	208,561	2,433,455	14	38.7
E-5	Sagavanirktok	111,000	NA	12	11.5
E-6	Sagavanirktok	19,987	NA	3	11.5
GTH 86	Sagavanirktok	31,260	1,436,577	9	7.0

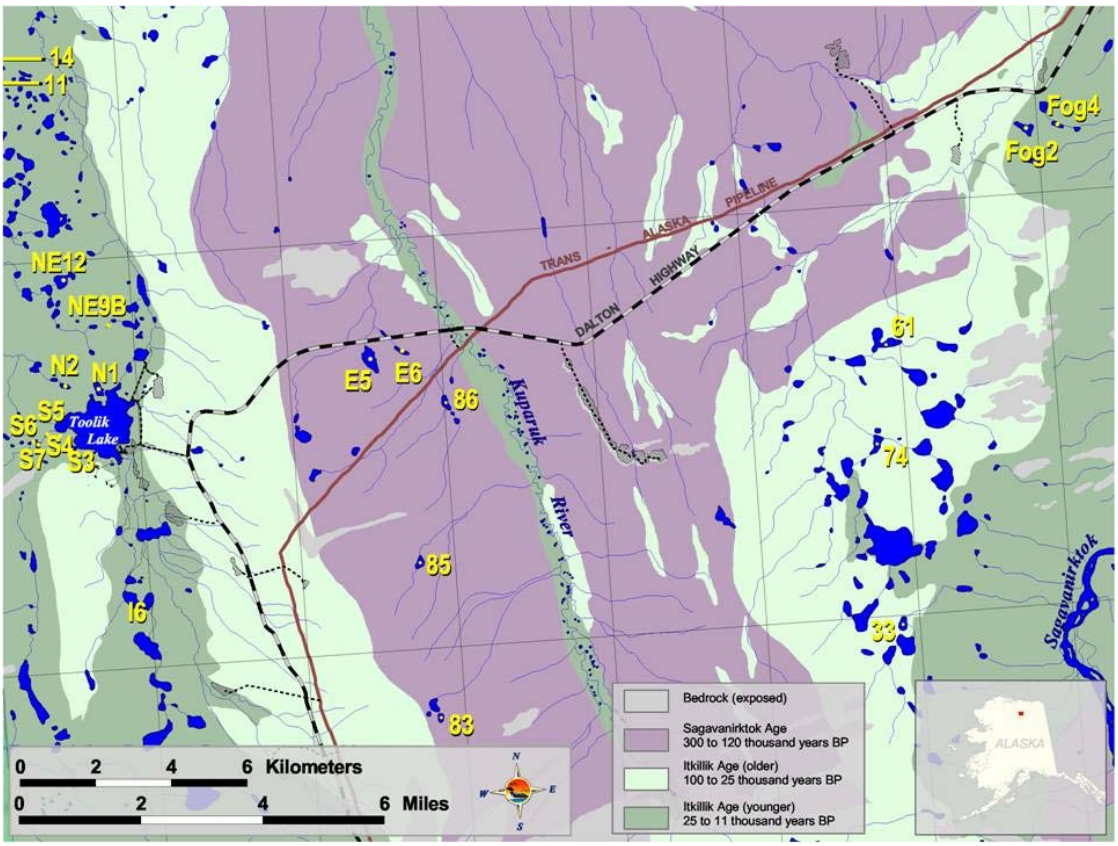

Figure A1.3. Map showing the locations of survey lakes in 2000 and 2003.

Results and Discussion. Benthic N fixation was detected in all lakes in 2000 and 2002. Results from the 2002 survey show that there is a suggested trend for higher rates of fixation in sediments with higher rates of benthic GPP (Figure A1.4A). With one outlier point removed (circled), there is a significant relationship between benthic N fixation and GPP ($y=0.0007x + 0.08$; $R^2=0.46$; $p=0.06$). Although there is no statistically significant relationship with benthic chlorophyll a ($p<0.05$; Figure A1.4B), this result is consistent with other studies in pelagic environments where higher rates of N fixation generally occur in more productive lakes (Howarth et al. 1988).

Benthic N fixation was not significantly predicted by PAR by linear regression (Figure A1.4C; $p>0.05$; statistics not shown). This result is consistent with results

shown in Chapter 2 in which light did not predict benthic N-fixation rates across lakes, even though it was a significant factor controlling N fixation within a given lake. N-fixation–light relationships may be difficult to discern when comparing across lakes because the relationship is non-linear and specific for each lake (Chapter 2). The light levels at which the 2002 surveys were incubated are probably on the linear part of the N-fixation–Irradiance response curve, which was 0 – 150 $\mu E\ m^{-2}sec^{-1}$ for four other Toolik-area lakes (Chapter 2), further complicating the ability to compare N-fixation rates among lakes when assays were performed at different light levels.

In the 2002 survey, benthic N fixation was not related to landscape age (Figure A1.4). However, the slurry samples in the 2000 survey show that N-fixation rates are higher on young surfaces than on older surfaces by >80%. (Figure A1.5). There may be several reasons for this disparity. First, I sampled more lakes on the young surface in 2000 than in 2002, which may increase the chances of measuring high rates of N fixation. Particularly high rates of fixation were measured in 2000 in small, productive lakes <2 m deep on the young surface (e.g., S-3, S-4, GTH 11), which are very uncommon (if not non-existent) on the old surface where lakes tend to be less abundant and more similar in their geomorphology (Gettel, personal observation; Burkart et al. In prep.). The sampling regime in 2000, therefore, captured the diversity of lake-types on the young surface better than the 2002 survey. Second, the slurry samples in 2000 were incubated at the same light level (200 $\mu E\ m^{-2}sec^{-1}$) rather than ambient lake light levels as they were in 2002. In four lakes in the vicinity of Toolik, N-fixation-Irradiance curves showed that 200 $\mu E\ m^{-2}sec^{-1}$ was at the asymptotic part of the response curve. Thus, the 2000 survey may have removed an important source of variability due to light that otherwise may confound the relationship between geologic surface and N fixation. These results indicate that some factors such as light

and geomorphology may be acting on individual lakes that swamp the landscape-scale pattern at small sample sizes as shown in the 2002 survey.

While the mechanism responsible for the landscape scale pattern of higher N fixation on younger surfaces remains unknown, experimental results from nutrient addition (Chapter 2) and grazing experiments (Chapter 3) corroborate these results. N fixation declined with increasing snail density by about 0.8 – 1.8% (Chapter 3), which suggests that N fixation should be lower if snails are more common on younger surfaces. However, a P addition to intact sediment cores from lake Fog 2 showed that N fixation was stimulated in response to increased P inputs by a much larger amount (38%; Chapter 2). Thus, nutrients may be a stronger control on landscape-scale patterns of N fixation than grazing. These patterns may be confounded by other factors such as lake geomorphology and light, and more work is needed to understand patterns in N fixation across the landscape.

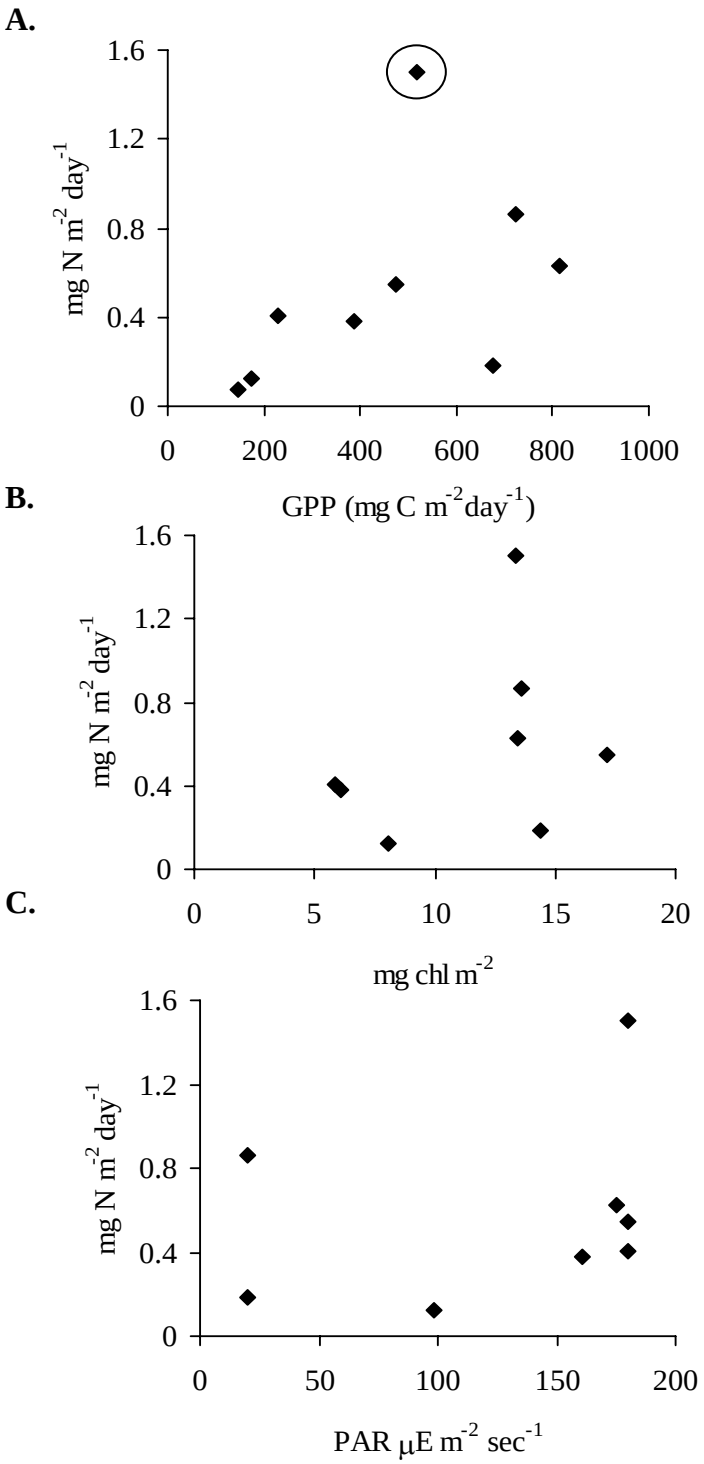

Figure A1.4. Relationship between benthic N fixation from 2002 survey and benthic GPP (panel A); benthic chlorophyll a (panel B); and light as photosynthetically active radiation (PAR; panel C). There was no statistically significant relationship among any of these variables except with the outlier point was removed in panel A. See text for details.

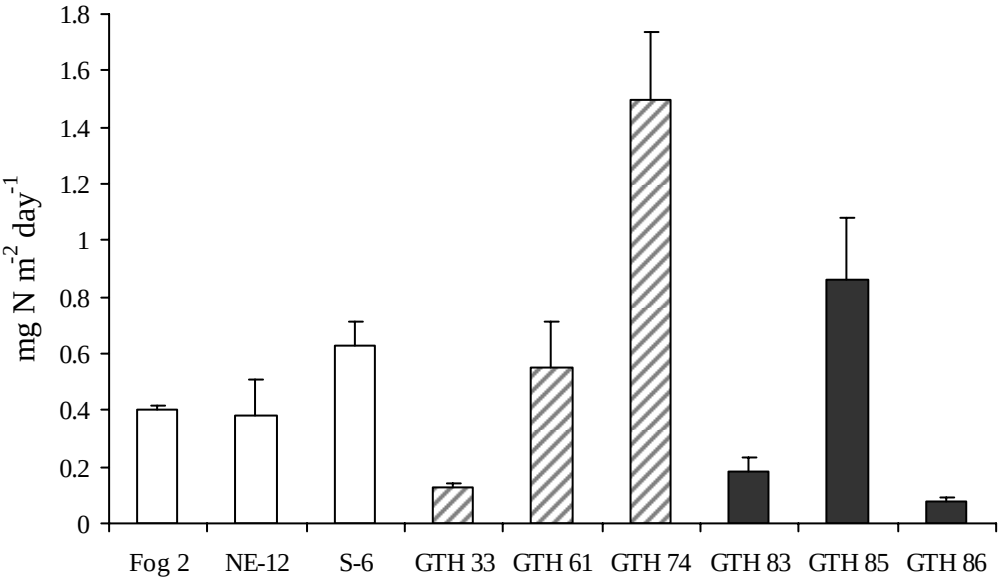

Figure A1.5. Results from 9-lake survey from year 2002, arranged from young surface lakes with clear bars, intermediate surface lakes in diagonal stripes, and old surface lakes are solid dark bars.

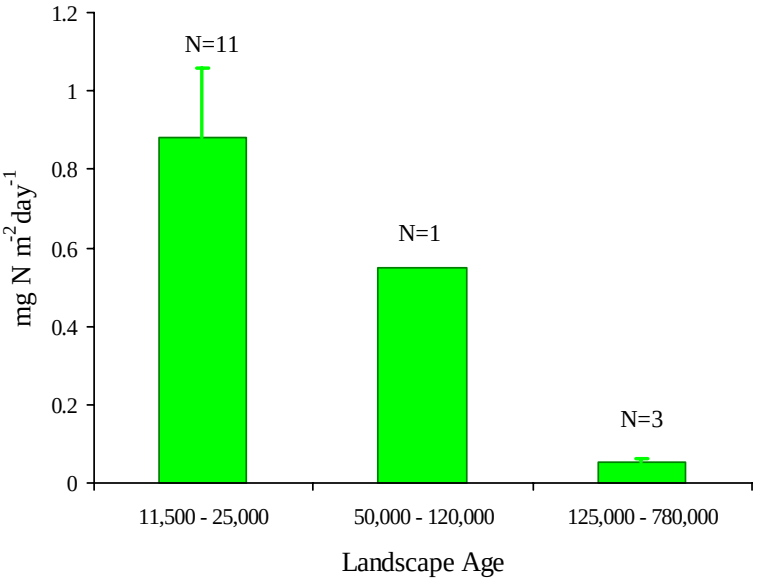

Figure A1.6. Benthic N fixation from a 15-lake survey from year 2000 along the chronosequence.

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APPENDIX 2. WATER COLUMN NITROGEN FIXATION IN YEARS 2002 – 2003.

1. Water-column N fixation for survey lakes.

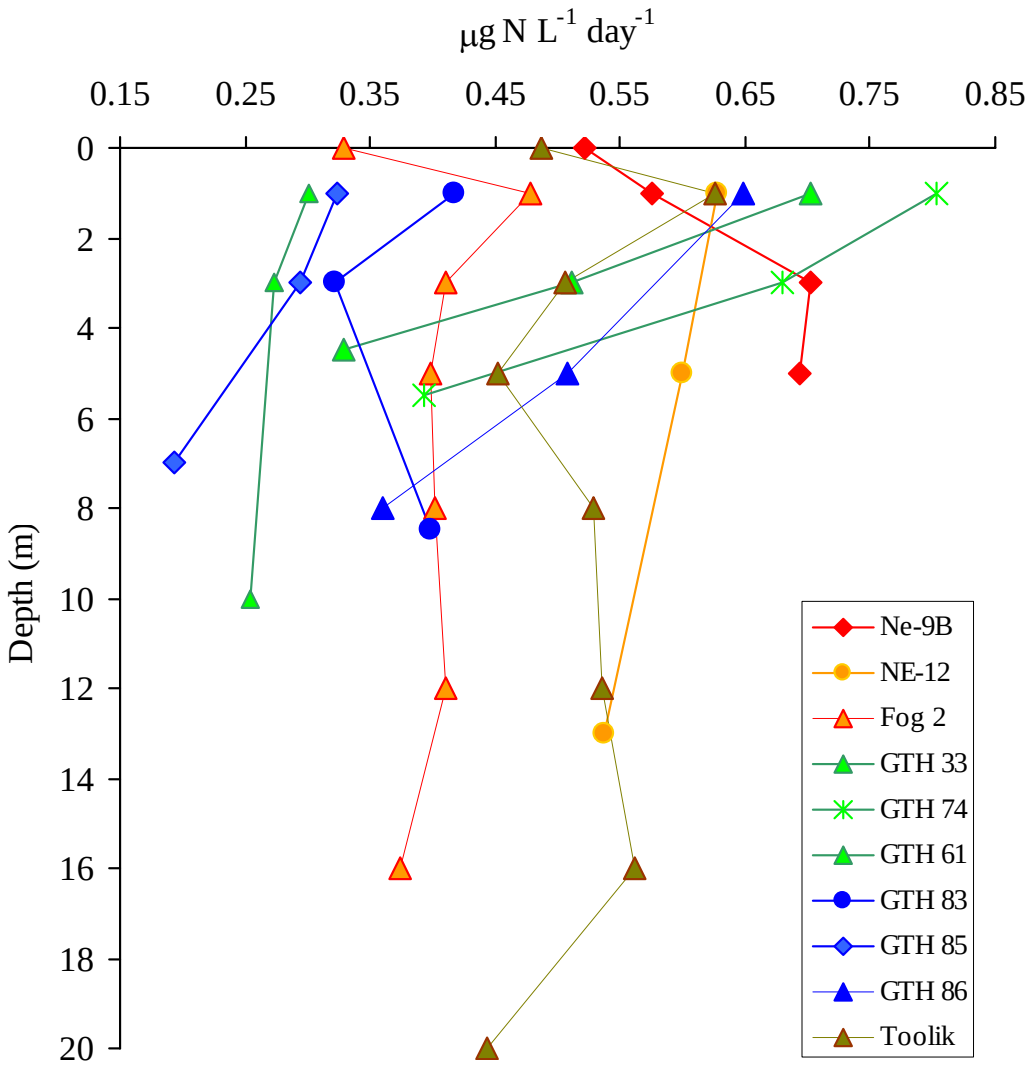

Figure A2.1. Depth profiles of water-column N fixation for lakes used in the benthic-water column comparison in Chapter 1.

Table A2.1. Water-column N fixation data used in the benthic-water column comparison in Chapter 1 and for Figure A2.1. ND signifies that N fixation was not detected. SE is standard error of the mean.

Lake	Date	Depth (m)	Light ($\mu E\ sec\ m^{-2}$)	Water Temp. (^oC)	Chlorophyll $\mu g\ L^{-1}$	N	N fixation ($\mu gN\ L^{-1}\ day^{-1}$)	SE
Ne-9B	1-Jul-03	0	750	16	1.77	3	0.521	0.168
Ne-9B	1-Jul-03	1	237	13	1.67	3	0.576	0.210
Ne-9B	1-Jul-03	3	108	8	2.56	3	0.703	0.134
Ne-9B	1-Jul-03	5	103	6	6.33	3	0.693	0.108
NE-12	26-Jul-02	1	300	13	0.97	3	0.627	0.045
NE-12	26-Jul-02	5	70	11	1.10	3	0.599	0.044
NE-12	26-Jul-02	13	20	8	2.21	3	0.537	0.075
GTH 33	27-Jul-02	1	75	13	1.94	3	0.302	0.003
GTH 33	27-Jul-02	3	20	11	1.94	3	0.274	0.034
GTH 33	27-Jul-02	10	10	8	0.76	3	0.254	0.032
GTH 74	26-Jul-02	1	300	13	1.15	3	0.803	0.010
GTH 74	26-Jul-02	3	180	11	1.08	3	0.679	0.070
GTH 74	26-Jul-02	5.5	20	8	2.65	3	0.392	0.056
GTH 61	26-Jul-02	1	300	13	2.29	3	0.702	0.029
GTH 61	26-Jul-02	3	180	13	2.09	3	0.511	0.021
GTH 61	26-Jul-02	4.5	20	8	2.27	3	0.328	0.165
GTH 83	27-Jul-02	1	180	13	3.00	3	0.418	0.049
GTH 83	27-Jul-02	3	20	11	2.98	2	0.323	0.003
GTH 83	27-Jul-02	8.5	10	8	2.22	3	0.399	0.092
GTH 85	27-Jul-02	1	180	13	1.24	3	0.323	0.033
GTH 85	27-Jul-02	3	20	11	1.28	3	0.294	0.008
GTH 85	27-Jul-02	7	10	8	0.78	2	0.194	0.274
GTH 86	26-Jul-02	1	180	13	2.68	3	0.649	0.140
GTH 86	26-Jul-02	5	20	8	2.47	3	0.508	0.049
GTH 86	26-Jul-02	8	20	8	0.75	3	0.360	0.080
Fog 2	31-Jul-02	0	1000	13	0.49	3	0.329	0.016
Fog 2	31-Jul-02	1	350	13	0.48	3	0.479	0.083
Fog 2	31-Jul-02	3	350	13	0.47	3	0.410	0.020
Fog 2	31-Jul-02	5	100	10	0.51	3	0.398	0.070
Fog 2	31-Jul-02	8	100	10	0.71	3	0.402	0.024
Fog 2	31-Jul-02	12	10	7	1.09	3	0.410	0.051
Fog 2	31-Jul-02	16	10	7	0.78	3	0.374	0.004
S-6	24-Jul-02	1	370	13	0.60	3	ND	-
S-6	24-Jul-02	3	250	11	1.39	3	ND	-
S-6	24-Jul-02	5	59	8	3.08	3	ND	-
Toolik	27-Jun-03	0	886	6	2.25	3	0.487	0.045
Toolik	27-Jun-03	1	298	5	1.45	3	0.626	0.188
Toolik	27-Jun-03	3	46	5	1.43	3	0.506	0.059
Toolik	27-Jun-03	5	13	5	1.52	3	0.452	0.082
Toolik	27-Jun-03	8	2	4	1.23	3	0.528	0.018
Toolik	27-Jun-03	12	1	4	0.76	3	0.535	0.029
Toolik	27-Jun-03	16	1	4	0.76	3	0.561	0.189
Toolik	27-Jun-03	20	1	4	-	3	0.444	0.068

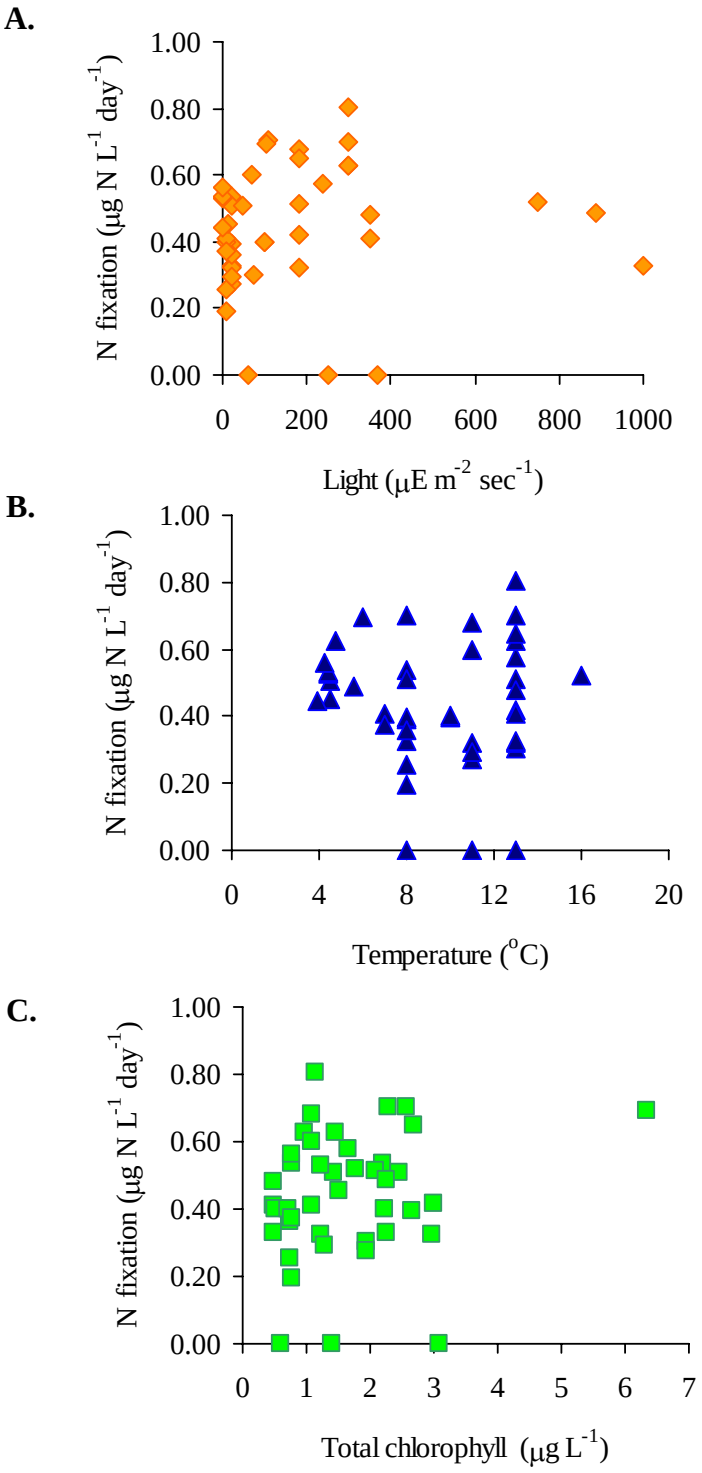

Figure A2.2. Relationship between water-column N fixation and light (panel A), temperature (panel B), and total chlorophyll (panel C). There was no statistically significant relationship among any of these variables and water-column N fixation.

2. Water column nitrogen fixation in Toolik Lake, 2003.

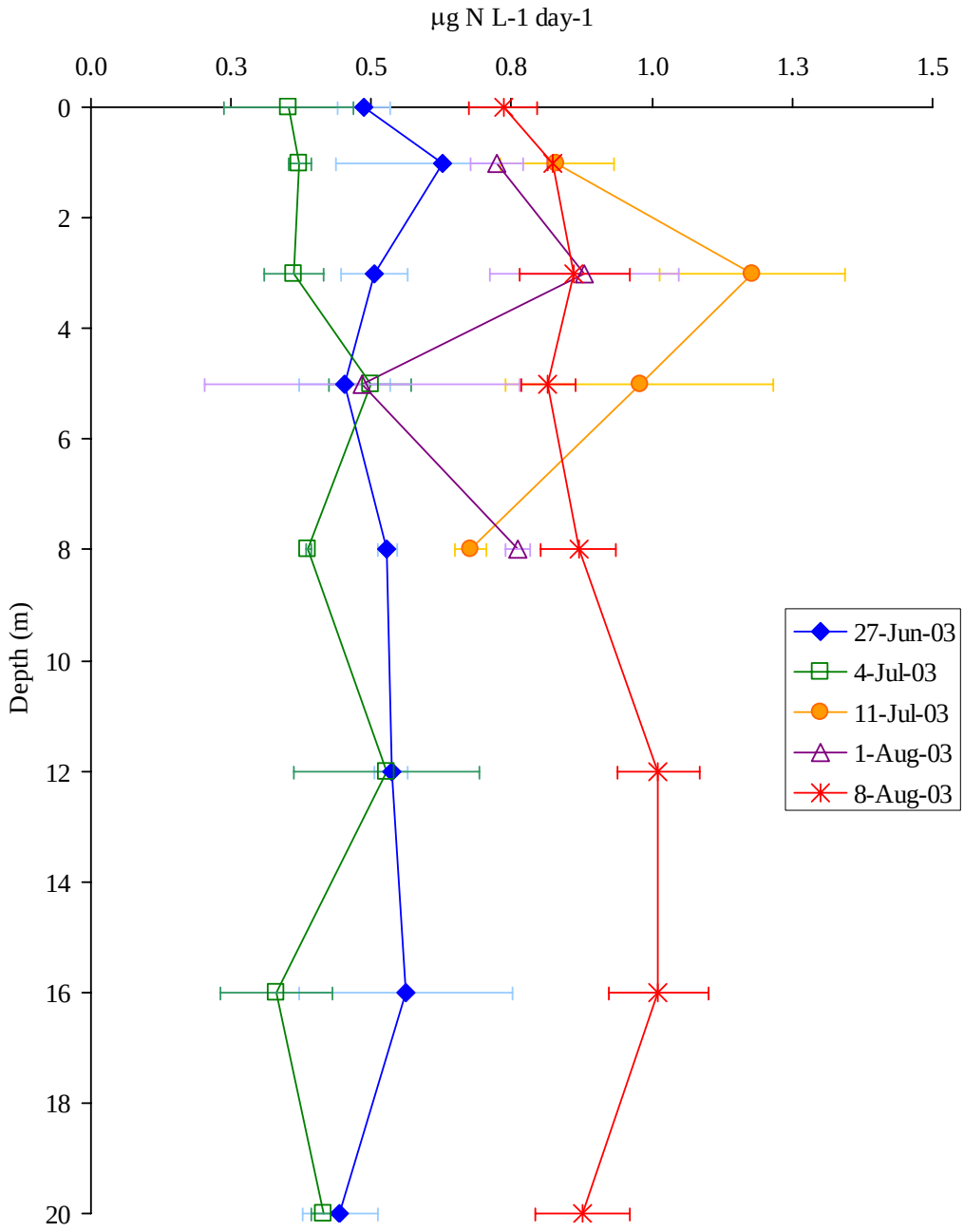

Figure A2.3. Depth profiles showing seasonal water-column N fixation in Toolik Lake in 2003.

Table A2.2 Summary of water-column N fixation in Toolik Lake, 2003.

Date	Depth (m)	Light ($\mu E\ m^{-2} sec^{-1}$)	Temp. (^oC)	N	N fixation ($\mu g\ N\ L^{-1}\ day^{-1}$)	SE
27-Jun-03	0	886	5.55	3	0.49	0.05
27-Jun-03	1	298	4.72	3	0.63	0.19
27-Jun-03	3	46	4.54	3	0.51	0.06
27-Jun-03	5	13	4.52	3	0.45	0.08
27-Jun-03	8	2	4.45	3	0.53	0.02
27-Jun-03	12	1	4.38	3	0.54	0.03
27-Jun-03	16	1	4.25	3	0.56	0.19
27-Jun-03	20	1	3.95	3	0.44	0.07
4-Jul-03	0	596	8.88	3	0.35	0.12
4-Jul-03	1	118	8.71	3	0.37	0.02
4-Jul-03	3	39	8.5	3	0.36	0.05
4-Jul-03	5	8	8.14	3	0.50	0.07
4-Jul-03	8	1	7.94	3	0.39	0.00
4-Jul-03	12	1	6.5	3	0.53	0.17
4-Jul-03	16	1	5.46	3	0.33	0.10
4-Jul-03	20	1	5.2	3	0.42	0.02
11-Jul-03	1	36	12.86	2	0.83	0.10
11-Jul-03	3	17	12.31	2	1.18	0.17
11-Jul-03	5	4	10.59	2	0.98	0.24
11-Jul-03	8	1	7.8	2	0.68	0.03
18-Jul-03	3		12.31	3	0.60	0.00
18-Jul-03	5		10.59	4	0.52	0.04
25-Jul-03	3	13	11.73	3	0.91	0.01
25-Jul-03	5	4	10.88	4	0.96	0.10
1-Aug-03	1	82	8.9	2	0.72	0.05
1-Aug-03	3	33	8.88	2	0.88	0.17
1-Aug-03	5	4	10.59	2	0.48	0.28
1-Aug-03	8	1	7.8	2	0.76	0.02
8-Aug-03	0	764	8.29	2	0.74	0.06
8-Aug-03	1	236	8.27	3	0.82	0.01
8-Aug-03	3	46	8.24	3	0.86	0.10
8-Aug-03	5	11	8.23	3	0.81	0.05
8-Aug-03	8	2	8.22	3	0.87	0.07
8-Aug-03	12	1	8.2	3	1.01	0.07
8-Aug-03	16	1	7.59	3	1.01	0.09
8-Aug-03	20	1	6.49	3	0.88	0.08

3. Temperature and light relationships for water-column N fixation in Toolik Lake, 2003.

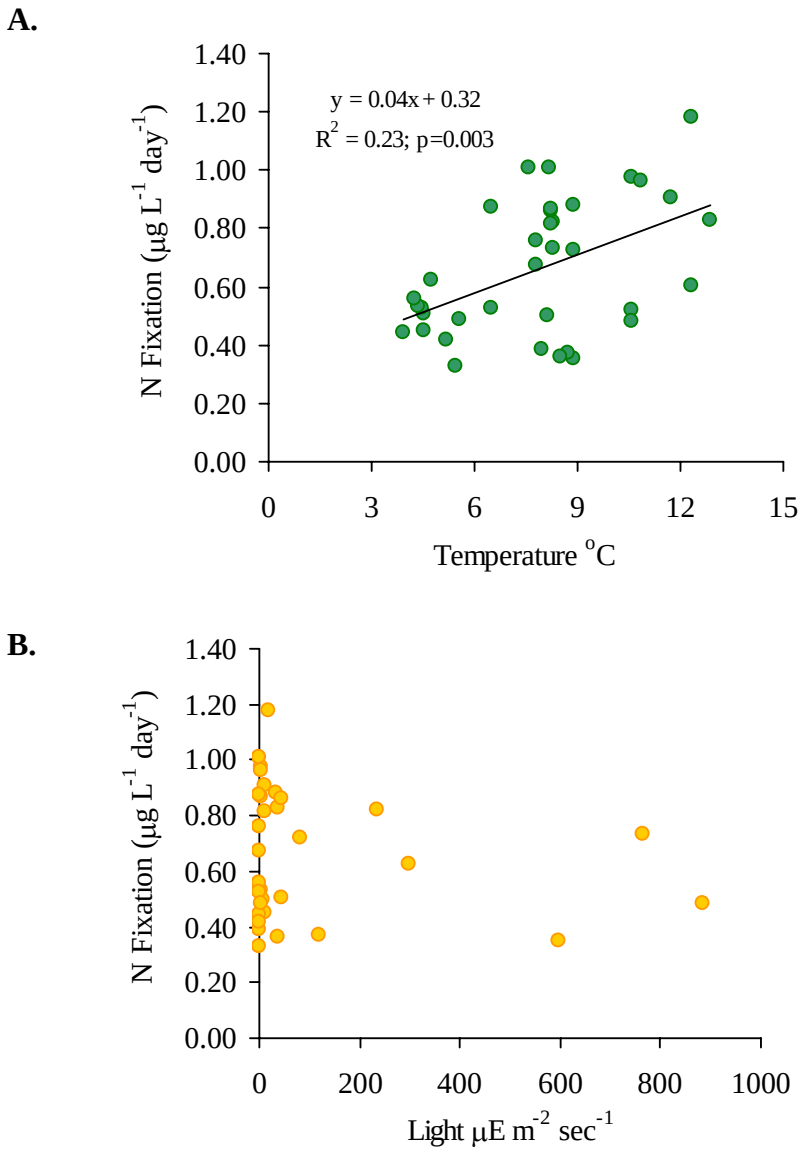

Figure A2.4. Relationship of water-column N fixation and temperature (panel A) and light (panel B) in Toolik Lake for all data in 2003. There was a significant relationship between temperature and water-column N fixation (statistics shown), but not between light and N fixation.

Table A2.3. Water-column N fixation for lake E-5. ND indicates that N fixation was not detected.

Date	Depth (m)	Light (μE sec m^{-2})	Water Temp. (^{o}C)	N	N fixation (μgN L^{-1} day^{-1})	SE
15-Jul-02	1	100	10	3	ND	-
15-Jul-02	2	100	10	3	ND	-
15-Jul-02	5	30	10	3	ND	-
15-Jul-02	8	30	10	3	ND	-
5-Aug-02	0	456	13	3	0.268	0.036
5-Aug-02	1	60	13	3	0.205	0.021
5-Aug-02	3	2	8	2	0.185	0.080
5-Aug-02	5	2	8	3	0.315	0.111
5-Aug-02	8	2	8	3	0.204	0.022
5-Aug-02	10	2	8	3	0.109	0.053
20-Jun-03	1	300	6	2	0.088	0.023
20-Jun-03	3	42	6	3	0.051	0.016
20-Jun-03	5	11	6	3	0.061	0.006
21-Jul-03	0	968	12	3	0.078	0.006
21-Jul-03	1	78	12	3	0.089	0.012
21-Jul-03	3	42	12	3	0.073	0.013
21-Jul-03	5	26	11	3	0.093	0.012
21-Jul-03	8	12	7	3	0.084	0.007
21-Jul-03	10	2	5	3	0.099	0.012

4. Water-column data for lakes E-5, E-6, Fog 2, and Fog 2 in years 2002 – 2003.

Table A2.4. Water-column N fixation for lake E-6. ND indicates that N fixation was not detected.

Date	Depth (m)	Light ($\mu E\ sec\ m^{-2}$)	Water Temp. (^{o}C)	N	N fixation ($\mu gN\ L^{-1}\ day^{-1}$)	SE
15-Jul-02	1	30	10	3	ND	-
15-Jul-02	2	30	10	3	ND	-
5-Aug-02	0	456	13	3	0.289	0.072
5-Aug-02	1	60	13	3	0.206	0.009
5-Aug-02	2	30	13	3	0.262	0.102
20-Jun-03	0	1000	11	3	0.065	0.009
20-Jun-03	1	500	8	3	0.056	0.012
20-Jun-03	2	72	8	3	0.063	0.002
21-Jul-03	0	968	12	3	0.082	0.003
21-Jul-03	1	12	12	3	0.069	0.001
21-Jul-03	2	12	12	3	0.081	0.003

Table A2.5. Water-column N fixation for lake Fog 2.

Date	Depth (m)	Light (μE sec m^{-2})	Water Temp. (^{o}C)	N	N fixation (μgN L^{-1} day^{-1})	SE
11-Jul-02	1	220	11	3	0.044	0.027
11-Jul-02	3	220	11	3	0.028	0.027
11-Jul-02	5	220	11	3	0.039	0.029
11-Jul-02	8	66	11	3	0.062	0.041
11-Jul-02	12	66	11	3	0.275	0.169
31-Jul-02	0	1000	13	3	0.329	0.016
31-Jul-02	1	350	13	3	0.479	0.083
31-Jul-02	3	350	13	3	0.410	0.020
31-Jul-02	5	100	10	3	0.398	0.070
31-Jul-02	8	100	10	3	0.402	0.024
31-Jul-02	12	10	7	3	0.410	0.051
31-Jul-02	16	10	7	3	0.374	0.004
26-Jun-03	1	283	11	2	0.592	0.216
26-Jun-03	3	130	7	3	0.425	0.333
26-Jun-03	5	130	7	3	0.391	0.211
26-Jun-03	8	20	4	3	0.640	0.462
12-Jul-03	0	695	11	2	0.107	0.017
12-Jul-03	1	400	11	3	0.103	0.004
12-Jul-03	3	200	11	3	0.079	0.003
12-Jul-03	5	130	11	3	0.076	0.009
12-Jul-03	8	60	6	3	0.080	0.036
12-Jul-03	12	10	6	3	0.101	0.018
12-Jul-03	16	10	6	3	0.104	0.018
31-Jul-03	0	564	9	3	0.856	0.018
31-Jul-03	1	82	9	3	0.745	0.097
31-Jul-03	3	33	9	3	0.867	0.025
31-Jul-03	5	8	9	3	1.050	0.121
31-Jul-03	8	2	9	3	1.005	0.226
31-Jul-03	12	1	8	3	0.897	0.010
31-Jul-03	16	1	7	3	0.938	0.055

Table A2.6. Water-column N fixation for lake Fog 4.

Date	Depth (m)	Light ($\mu E\ sec\ m^{-2}$)	Water Temp. (^oC)	N	N fixation ($\mu gN\ L^{-1}\ day^{-1}$)	SE
10-Jul-02	1	66	11	3	0.397	0.206
10-Jul-02	2	66	11	3	0.226	0.178
31-Jul-02	0	1000	13	3	0.547	0.049
31-Jul-02	1	350	13	3	0.473	0.090
31-Jul-02	2	100	10	3	0.411	0.006
31-Jul-03	0	525	8	3	0.570	0.016
31-Jul-03	1	525	8	3	0.703	0.214
31-Jul-03	2	9.8	8	3	0.562	0.036

Table A2.7. Water-column N fixation for Toolik Lake. N fixation was measured along the same depth profile but not detected on 26 Jun 2002.

Date	Depth (m)	Light ($\mu E\ sec\ m^{-2}$)	Water Temp. (^oC)	N	N fixation ($\mu gN\ L^{-1}\ day^{-1}$)	SE
2-Aug-02	0	820	15	3	0.435	0.053
2-Aug-02	1	350	14	3	0.376	0.049
2-Aug-02	3	90	13	3	0.395	0.045
2-Aug-02	5	10	12	3	0.211	0.130
2-Aug-02	6.5	10	12	3	0.328	0.019
2-Aug-02	8	3.5	7	3	0.445	0.018
2-Aug-02	12	3.5	7	3	0.381	0.102
2-Aug-02	16	3.5	7	3	0.341	0.052
2-Aug-02	22	3.5	7	3	0.335	0.038

APPENDIX 3. ALGAL COMPOSITION DATA FOR SNAIL GRAZING EXPERIMENT.

Algal community composition was enumerated in sediment samples collected from control and snail enclosures as described in Chapter 3. In a manner similar to samples collected for chlorophyll a analysis, one core 2.5 cm in diameter was collected from each enclosure, and the top 5 cm were removed and homogenized. A 5 ml sub-sample was removed and stored in 10 ml of lake water and preserved with 1 μL of gluteraldehyde. Permanent slides were made using Tafts Syrup Medium according to protocol described in Stevenson and Bahls (1999) Three slides were made for each enclosure, and a total of 8 fields were counted on each slide after plotting taxonomic diversity against the number of fields and determining that 7 fields reached the asymptotic part of the relationship. Algal cells and colonies were counted and measured at 100X and 400X on a Wild M-40 inverted microscope.

A random-coefficients model was used to analyze the relationship between snail density and the number and size (length) of filamentous cyanobacterial colonies present. Details of the statistical analysis are identical to the random-coefficients model described in Chapter 3. Similar analyses were performed on the genera *Nostoc sp.*, which are known to fix nitrogen, and the identical conclusions were reached (statistics not shown). This appendix shows data tables for algal diversity in all counted samples as well as the average length and standard deviation for filamentous cyanobacterial groups present.

Table A3.1. Algal counts for grazing experiments described in Chapter 3. Counts are in #/ml of sediment.

Lake	Year	Block	Replicate	Snail Treatment	Snail Density (#/m^2)	Araphid	Biraphid	Centric Diatom	Coccoid Cyanobacteria or Green algae	Colonial Cyanobacteria	Craticula	Cyclotella Chain	Cyclotella (single cell)	Desmid	Filamentous Cyanobacteria	Filamentous Cyanobacteria or Green algae	Filamentous Green	Fragilaria	Merismopedia	Monoraphid	Nostoc	Oscillatoria	Pediastrum	Pennales Diatom	Scenedesmus	Snowella	Spirulina	Staurodesmus	Stastrum	Xanthidium
Fog 2	2001	A1	1	Zero	0	0	10	1	1	16	0	27	1	0	11	0	28	3	0	0	8	0	0	61	1	0	0	1	0	0
Fog 2	2001	A1	1	Control	1.3	0	7	1	1	13	0	25	1	0	8	0	37	4	0	0	5	1	1	62	1	0	0	0	0	1
Fog 2	2001	A1	1	Low	12	0	9	1	1	10	0	26	1	0	8	0	30	3	0	0	5	1	1	95	1	0	0	1	0	0
Fog 2	2001	A1	1	High	60	0	5	1	1	8	0	45	1	1	10	0	16	2	0	0	15	0	1	82	1	0	0	0	0	0
Fog 2	2001	A1	2	Zero	0	0	6	1	1	16	0	50	1	0	8	0	36	3	0	0	4	0	0	69	1	0	0	0	0	0
Fog 2	2001	A1	2	Control	1.3	0	12	1	1	8	0	20	1	0	9	0	28	2	0	0	6	0	0	52	1	0	0	0	0	0
Fog 2	2001	A1	2	Low	12	0	7	1	1	25	0	27	1	1	13	0	15	0	0	0	2	0	1	61	1	0	0	0	0	0
Fog 2	2001	B2	1	Zero	0	0	10	2	2	12	0	40	2	1	25	0	43	3	0	0	8	0	1	111	2	0	0	0	0	1
Fog 2	2001	B2	1	Low	12	0	7	1	1	10	0	28	1	0	22	0	13	4	0	0	7	1	1	56	1	0	0	0	1	0
Fog 2	2001	B2	1	High	36	36	16	1	1	47	0	18	1	1	10	4	32	2	0	0	22	0	0	57	1	2	0	0	0	0
Fog 2	2001	B2	2	Zero	0	0	13	1	1	14	0	43	1	1	8	0	29	3	0	0	10	2	1	86	1	0	0	0	0	0
Fog 2	2001	B2	2	Control	1.3	0	3	1	1	23	0	32	1	1	8	0	20	4	0	1	13	0	0	86	1	0	0	0	0	0
Fog 2	2001	B2	2	Low	12	0	13	1	1	9	0	26	1	0	6	0	31	1	0	0	7	1	0	76	1	0	0	0	1	0
Fog 2	2001	B2	2	High	36	0	4	1	1	12	0	17	1	0	10	0	14	1	0	1	4	2	0	69	1	0	0	0	0	0
Fog 2	2001	C3	1	Zero	0	0	0	1	1	9	0	11	1	1	9	0	13	0	0	0	6	1	0	49	1	0	0	0	0	0
Fog 2	2001	C3	1	Control	1.3	0	3	1	1	9	0	22	1	1	9	0	29	3	0	0	3	1	1	68	1	0	0	0	0	0
Fog 2	2001	C3	1	Low	12	0	7	1	1	11	0	12	1	0	4	0	30	0	0	0	7	1	0	60	1	0	0	0	0	0
Fog 2	2001	C3	1	High	36	0	3	1	1	11	0	15	1	0	7	0	31	3	0	0	6	2	1	64	1	0	0	0	0	0

Table A3.1. (Continued).

Lake	Year	Block	Replicate	Snail Treatment	Snail Density (#/m^2)	Araphid	Biraphid	Centric Diatom	Coccoid Cyanobacteria or Green algae	Colonial Cyanobacteria	Craticula	Cyclotella Chain	Cyclotella (single cell)	Desmid	Filamentous Cyanobacteria	Filamentous Cyanobacteria or Green algae	Filamentous Green	Fragilaria	Merismopedia	Monoraphid	Nostoc	Oscillatoria	Pediastrum	Pennales Diatom	Scenedesmus	Snowella	Spirulina	Staurodesmus	Stastrum	Xanthidium
Fog 2	2003	A1	1	Zero	0	0	10	1	1	4	0	80	1	0	2	0	31	1	0	0	4	0	0	70	1	0	0	0	0	0
Fog 2	2003	A1	1	Control	2.6	22	7	1	1	19	1	35	1	1	16	45	5	1	1	12	9	0	1	84	1	1	0	0	0	0
Fog 2	2003	A1	1	Low	36	0	13	1	1	23	0	35	1	1	17	0	12	2	0	0	8	0	0	106	1	0	0	0	0	0
Fog 2	2003	A1	1	High	72	0	9	1	1	22	0	42	1	1	11	0	25	2	0	0	1	4	0	77	1	0	0	0	0	0
Fog 2	2003	A1	2	Zero	0	0	7	1	1	19	0	38	1	0	10	0	22	4	0	0	6	4	0	72	1	1	0	0	0	0
Fog 2	2003	A1	2	Control	2.6	0	4	1	1	17	0	38	1	0	5	0	10	4	0	0	7	0	1	65	1	0	0	0	0	0
Fog 2	2003	A1	2	Low	36	0	11	1	1	12	0	41	1	1	10	0	22	5	0	0	2	0	0	101	1	0	0	0	0	0
Fog 2	2003	A1	2	High	72	0	11	1	2	30	0	40	1	0	14	0	38	4	0	0	6	1	0	101	2	0	0	0	0	0
Fog 2	2003	B2	1	Zero	0	0	1	1	1	6	0	31	1	0	10	0	18	1	0	0	3	0	1	43	1	0	0	0	0	0
Fog 2	2003	B2	1	Control	2.6	0	4	1	1	6	0	37	1	1	15	0	30	1	0	1	2	0	0	64	1	0	0	0	0	0
Fog 2	2003	B2	1	Low	36	0	7	1	1	7	0	48	1	1	5	0	44	4	0	0	9	2	0	98	1	0	0	0	0	0
Fog 2	2003	B2	1	High	72	0	6	1	1	6	0	43	1	0	5	0	21	4	0	0	4	1	0	81	1	0	0	0	0	0
Fog 2	2003	B2	2	Zero	0	0	11	1	1	12	0	58	1	1	8	0	36	5	0	0	7	3	0	129	1	0	0	0	0	0
Fog 2	2003	B2	2	Control	2.6	0	5	1	1	7	0	36	1	1	10	0	35	1	0	0	3	0	0	51	1	0	0	0	0	0
Fog 2	2003	B2	2	Low	36	0	11	1	1	12	0	50	1	0	16	0	30	3	0	0	8	0	1	98	1	0	0	0	0	0
Fog 2	2003	B2	2	High	72	0	5	1	1	11	0	49	1	0	9	0	26	2	0	0	4	0	0	102	1	0	0	0	0	0

Table A3.1. (Continued).

Lake	Year	Block	Replicate	Snail Treatment	Snail Density (#/m^2)	Araphid	Biraphid	Centric Diatom	Coccoid Cyanobacteria or Green algae	Colonial Cyanobacteria	Craticula	Cyclotella Chain	Cyclotella (single cell)	Desmid	Filamentous Cyanobacteria	Filamentous Cyanobacteria or Green algae	Filamentous Green	Fragilaria	Merismopedia	Monoraphid	Nostoc	Oscillatoria	Pediastrum	Pennales Diatom	Scenedesmus	Snowella	Spirulina	Staurodesmus	Stastrum	Xanthidium
S-6	2002	A1	1	Zero	0	0	10	1	1	33	0	16	1	0	33	0	25	1	0	0	21	8	1	74	1	0	0	0	0	1
S-6	2002	A1	1	Control	5.6	2	8	1	1	22	0	12	1	1	21	0	29	1	0	0	20	11	1	89	1	0	0	0	0	1
S-6	2002	A1	1	Low	24	13	12	1	1	53	1	16	1	0	61	0	24	0	0	2	27	0	0	58	0	0	0	1	0	0
S-6	2002	A1	1	High	60	3	6	1	1	18	0	12	1	1	21	0	22	3	0	0	13	4	0	59	0	0	0	0	0	0
S-6	2002	C3	1	Zero	0	10	4	1	1	32	0	4	1	0	58	0	9	10	0	0	44	5	1	87	1	0	0	0	0	0
S-6	2002	C3	1	Control	5.6	12	11	1	1	16	0	6	1	0	27	0	7	8	0	1	26	8	0	70	1	1	1	0	0	0
S-6	2002	C3	1	Low	24	3	3	1	1	8	0	4	1	0	41	1	12	7	0	1	30	4	0	64	0	0	0	0	0	0
S-6	2002	C3	1	High	60	10	13	1	0	0	0	8	1	1	55	0	6	1	0	1	41	0	0	128	0	0	0	0	0	0

Table A3.2. Average length of filamentous cyanobacterial colonies (microns) in snail grazing experiment described in Chapter 3.

Lake	Year	Block	Replicate	Snail Density	Average Length (mm)	Standard Deviation
Fog 2	2001	A1	1	0	10.84	5.19
Fog 2	2001	A1	1	1.3	20.07	24.80
Fog 2	2001	A1	1	12	15.57	11.69
Fog 2	2001	A1	1	60	15.64	12.10
Fog 2	2001	A1	2	0	19.17	30.32
Fog 2	2001	A1	2	1.3	21.27	16.92
Fog 2	2001	A1	2	12	16.07	7.59
Fog 2	2001	B2	1	0	19.85	10.21
Fog 2	2001	B2	1	12	17.00	12.52
Fog 2	2001	B2	1	36	17.72	24.88
Fog 2	2001	B2	2	0	11.25	9.16
Fog 2	2001	B2	2	1.3	15.19	13.78
Fog 2	2001	B2	2	12	29.79	29.58
Fog 2	2001	B2	2	36	29.00	34.26
Fog 2	2001	C3	1	0	18.56	14.15
Fog 2	2001	C3	1	1.3	33.77	44.39
Fog 2	2001	C3	1	12	15.75	8.28
Fog 2	2001	C3	1	36	23.53	16.70
Fog 2	2003	A1	1	0	8.17	3.06
Fog 2	2003	A1	1	2.6	55.84	52.64
Fog 2	2003	A1	1	36	25.60	27.57
Fog 2	2003	A1	1	72	48.13	82.35
Fog 2	2003	A1	2	0	27.40	44.67
Fog 2	2003	A1	2	2.6	12.33	9.37
Fog 2	2003	A1	2	36	13.83	8.57
Fog 2	2003	A1	2	72	28.14	30.75
Fog 2	2003	B2	1	0	28.08	38.99
Fog 2	2003	B2	1	2.6	17.65	11.79
Fog 2	2003	B2	1	36	13.13	11.93
Fog 2	2003	B2	1	72	19.00	16.97
Fog 2	2003	B2	2	0	19.83	17.69
Fog 2	2003	B2	2	2.6	31.15	28.62
Fog 2	2003	B2	2	36	18.75	25.68
Fog 2	2003	B2	2	72	15.69	10.46
S-6	2002	A1	1	0	45.31	40.20
S-6	2002	A1	1	5.6	41.06	38.78
S-6	2002	A1	1	24	36.85	31.39
S-6	2002	A1	1	60	56.84	100.68
S-6	2002	C3	1	0	42.92	80.17
S-6	2002	C3	1	5.6	53.89	61.48
S-6	2002	C3	1	24	49.11	44.00
S-6	2002	C3	1	60	50.71	96.74

Table A3.3. Results from a random coefficients model to test the relationship between snail density and the total number of filamentous cyanobacterial colonies in grazing experiments conducted in lakes Fog 2 and S-6 in years 2001 – 2003. Snail density was not significant.

Effect	Numerator DF	Denominator DF	F-Value	p-value
Snail Density	1	32	0.00	0.97
Year	2	4	23.26	0.01
Snail Density*Year	2	32	0.35	0.71

Table A3.4. Results from a random coefficeints model to test the relationship between snail density and the length of filamentous cyanobacterial colonies in lakes Fog 2 and S-6 in grazing experiments in years 2001 – 2003. Snail density was not significant.

Effect	Numerator DF	Denominator DF	F-Value	p-value
Snail Density	1	32	0.82	0.37
Year	2	4	10.39	0.03
Snail Density*Year	2	32	0.26	0.78

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