

The effects of grazing by the snail, *Lymnaea elodes*, on benthic N₂ fixation and primary production in oligotrophic, arctic lakes

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Abstract

This study assessed whether grazing by the snail, *Lymnaea elodes*, limits benthic dinitrogen (N₂) fixation and primary production in nitrogen (N)-limited oligotrophic lakes near Toolik Field Station on the North Slope of Alaska. We also tested whether snail excretion increased N and the ratio of N and phosphorus (P) supply ratio to benthic algae, which could indirectly affect production and the N₂ fixation rate. We performed in situ, randomized-block experiments in two lakes in 3 years in which snail density was manipulated and compared to open cage controls. Snails significantly decreased areal rates of N₂ fixation in both lakes in all years ($p < 0.05$), but did not appear to cause a reduction in cyanobacterial abundance or filament size ($p > 0.05$). Snails did not significantly affect measures of benthic production, including gross primary production, respiration, net ecosystem production, and chlorophyll biomass ($p > 0.05$). Snail-induced declines in N₂ fixation probably did not result from snail excretion. The molar N:P excretion ratio of ammonium (NH₄⁺) and phosphate (PO₄³⁻) was very low (4.8), indicating that snails likely exacerbated N limitation, a response that would tend to favor enhanced rather than reduced N₂ fixation. Furthermore, the excretion rate of N-NH₄⁺ was several orders of magnitude lower than the N₂ fixation rate (0.002–0.02 mg N m⁻² day⁻¹ vs. 0.1–0.4 mg N m⁻² day⁻¹, respectively) and met almost none (<<1%) of the N demand by primary producers. Although the mechanism by which *Lymnaea elodes* caused a decline in N₂ fixation is unknown, the effect was small, and accounted for a reduction of N inputs of only 0.12 mg N m⁻² summer⁻¹ or by 0.85–1.8% at ambient snail densities. Because N₂ fixation is a new N input able to support new production, this effect may be important across long time scales or where densities of *L. elodes* are higher.

Nitrogen (N) fixation of atmospheric N₂ by free-living cyanobacteria is common in aquatic environments, and it represents a new source of N that can support new

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production. In lakes of moderate to high productivity, N₂ fixation generally compensates for N limitation of net primary production (e.g. Schindler 1977; Hendzel et al. 1994); however, N₂ fixation does not appear to compensate for N limitation in many lakes of low productivity, and they remain N-limited, 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; Elser et al. 1990; Levine and Whalen 2001). We have yet to identify the main factors that limit N₂ fixation and to determine whether these factors help maintain N limitation in oligotrophic lake ecosystems (Howarth et al. 1988a; Vitousek et al. 2002).

Grazing of free-living cyanobacteria is increasingly recognized as a possible controlling factor of N₂ fixation in N-limited aquatic systems. In pelagic freshwater and estuarine environments, grazing can limit the filament size, heterocyst production, and growth rate of filamentous cyanobacteria (Chan et al. 2004; Chan et al. 2006). As a result, grazing helps maintain N limitation prevalent in most estuarine ecosystems (Howarth et al. 1999; Marino et al. 2002). In benthic environments, results are more variable. Direct consumption by snail grazers caused

a reduction of cyanobacterial biomass in two freshwater lake mesocosm experiments (McCollum et al. 1988; Tuchman and Stevenson 1991), whereas fish caused an increase of cyanobacterial biomass in two freshwater streams (Power et al. 1988; Flecker 1996).

In addition to community-level effects caused by direct consumption, grazers may also affect N₂ fixation rates indirectly through the excretion of inorganic nutrients (N and phosphorus [P]). Grazers can increase inorganic nutrient availability to primary producers (e.g., Grimm 1988; Hall et al. 2003) and thereby alleviate nutrient limitation (Hillebrand et al. 2004) and reduce the competitive advantage of fixing N₂ (Elser 1999). The N:P ratio in snail excretion may also determine the identity of the limiting nutrient (Elser et al. 2000). If the N:P excretion ratio is below that of optimal growth for benthic algae, it is likely that N limitation is exacerbated and N₂ fixation is favored; conversely, if the N:P excretion ratio is above the optimal ratio of benthic algae, N limitation is alleviated, and the energetically-expensive N₂ fixation process is no longer advantageous. To our knowledge, no studies have tested the effects of grazer excretion on N₂ fixation rate or compared N supply from excretion relative to inputs of new N via N₂ fixation.

Autotrophic N₂-fixing filamentous cyanobacteria are common on sediment surfaces in oligotrophic lakes because water-column production is low and light penetration is high (Moeller and Roskoski 1978; Loeb and Reuter 1981). Because littoral zones in oligotrophic lakes can comprise a significant portion of lake area (e.g., Wetzel 1964; Vadeboncoeur et al. 2003), inputs of new N through autotrophic benthic N₂ fixation is important to whole-lake N budgets and productivity in several oligotrophic lakes (e.g., Mirror Lake, New Hampshire, Moeller and Roskoski 1978; Lake Tahoe, Reuter et al. 1986; Northwest Territories Lakes, Canada, Bergmann and Welch 1990; and Lake Malawi, Higgins et al. 2001). Howarth et al. (1988a) hypothesized that N inputs via benthic N₂ fixation are important in oligotrophic systems and that N₂ fixers could be most limited by grazers because reducing conditions generally increase P and iron availability in the sediments.

More studies have examined the effect of benthic grazing on cyanobacterial abundance than on N₂ fixation rate, and very few have examined both (for exceptions see Wilkinson and Sammarco 1983; Williams and Carpenter 1997). However, N₂ fixation rate can be affected by other factors, such as nutrient availability, that cannot be detected by enumerating community structure changes alone. As far as we know, only a few studies have examined the consequences of grazing for N₂ fixation and cyanobacterial biomass in oligotrophic aquatic ecosystems (Wilkinson and Sammarco 1983; Williams and Carpenter 1997), and no published studies have addressed the relationship between grazing and N₂ fixation in oligotrophic lakes.

Here we report results of in situ grazing experiments in N-limited, oligotrophic lakes on the North Slope of Alaska near Toolik Field Station, Arctic Long Term Ecological Research (LTER) site. We examine the effects of grazing by the dominant snail, *Lymnaea elodes*, on N₂ fixation,

cyanobacterial abundance and filament size, chlorophyll biomass, and benthic production. We also quantify the rate of N excretion and its N:P ratio to assess the likelihood that snails affect primary production and N₂ fixation indirectly.

Methods

Site description—The study lakes were located near the Toolik Field Station in the northern foothills of the Brooks Mountain Range in arctic Alaska (68°37'N, 149°35'W; Fig. 1). The area is underlain by continuous permafrost, and the landscape is rolling tundra terrain, with a large number of shallow (3–15 m) glacial kettle lakes. Ice-free season is mid-June to mid-September, and maximum epilimnetic temperatures range from 13°C to 18°C. Lakes in this region are dimictic and ultra oligotrophic (Miller et al. 1986); 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 primary production measured with radiocarbon (¹⁴C) ranges from 12–16 g C m⁻² year⁻¹ (Miller et al. 1986). In general, lake bottoms consist of extremely fine-grained, unconsolidated sediment. The N₂-fixing cyanobacterium, *Nostoc* sp., forms balls as large as 2 cm in diameter and is visibly prevalent on sediment surfaces in many lakes, including the lakes in this study (G. M. Gettel personal observation). Sediments also contain *Anabaena* sp. and other genera of filamentous N₂-fixing cyanobacteria (G. M. Gettel personal observation).

Experiments were carried out in two lakes: Fog 2 and S-6. Lake Fog 2 is a headwater lake (G. M. Gettel surface area = 0.01 km²; maximum depth = 20 m) on a young glaciated surface originating from the recent advance of the Itkillik Phase II glaciation 12,000–25,000 years ago (Hamilton 2003). Benthic production in Fog 2 is high relative to many other lakes in the region (up to 400 mg C m² day⁻¹), and nutrient-bioassay experiments on water column production show the strongest responses to N addition followed by N and P addition, indicating primary N limitation and secondary P limitation (Gross unpubl. data). Fog 2 contains arctic char, which prey upon *L. elodes* and likely control its size, distribution, and density (Hershey et al. 1999). Lake S-6 (surface area = 0.011 km²; maximum depth = 8.5 m), is also on a glaciated surface originating from the Itkillik Phase II advance. S-6 has higher benthic production than Fog 2 (up to 750 mg C m⁻² day⁻¹). S-6 does not contain fish species that prey on *L. elodes* and was expected to have high snail densities (Merrick et al. 1991).

Experimental design—The experiment was a randomized block design in which snail density was manipulated in cages in situ above the thermocline at 3–4.5-m depth. This experiment was replicated in Fog 2 in multiple years (2001, 2002, and 2003) and in S-6 in 2002. Each block had three snail density treatments, and there were 2–3 blocks and 1–2 replicates per treatment depending on the year (Table 1). Each experiment included a snail exclusion (zero snails) treatment, but low- and high-density treatments varied between years, from 12 snails m⁻² for the low-density

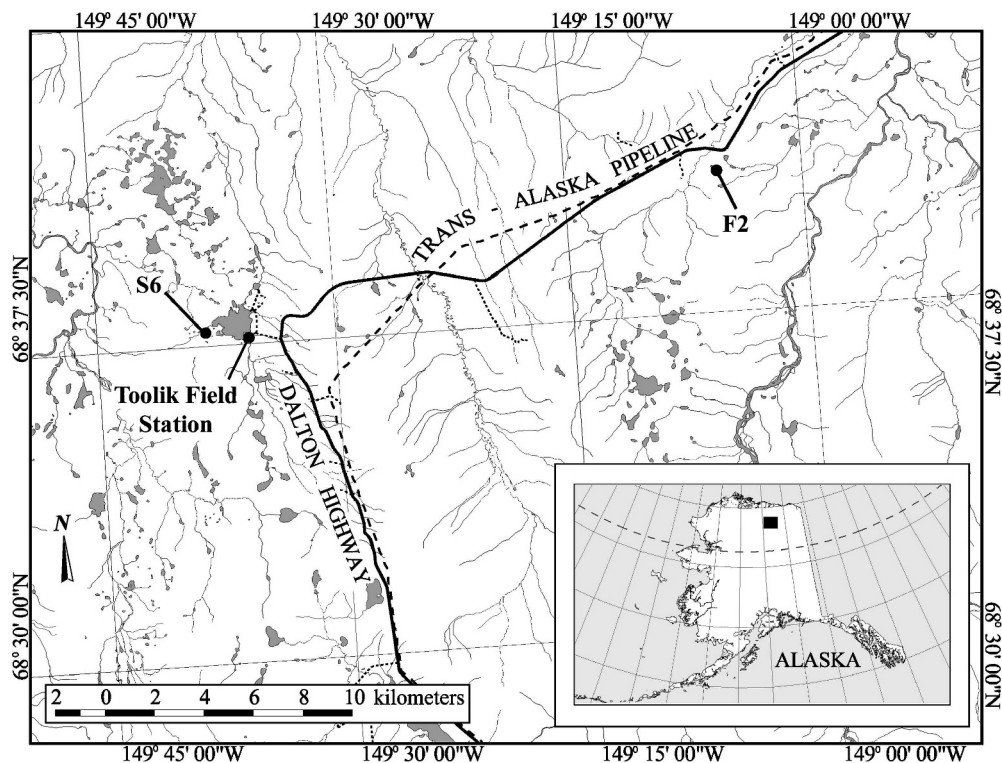


Fig. 1. The grazing experiment was conducted in lakes Fog 2 (labeled F2) and S-6, at Toolik Field Station on the North Slope of Alaska, located 150 miles north of the Arctic circle, off the Dalton Highway (68°37'N, 149°35'W).

treatment in 2001 to 72 snails m^{-2} for the high-density treatment in 2003 (Table 1).

Cages were constructed by cutting off the bottoms of 18.9-L plastic buckets (0.25 m^2 in area) and inserting the buckets into the soft sediment. The tops were covered with

Table 1. Experimental information for in situ randomized block design grazing experiment in lakes Fog 2 and S-6 in 2001–2003 to examine the effect of snail density on benthic N_2 fixation and primary production.

Year	Experimental design			
	2001	2002	2003	2003
Lake	Fog 2	Fog 2	S-6	Fog 2
Number of blocks	3	3	2	2
Replicates/treatment	1	1	1	2
Year	Snail density treatments (no. m^2)			
	2001	2002	2003	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
Year	Ambient conditions			
	2001	2002	2003	2003
Lake	Fog 2	Fog 2	S-6	Fog 2
Temperature ($^{\circ}C$)	10	13	13	12
Light ($\mu mol m^{-2} s^{-1}$)	268	180	180	201

plastic mesh screen with 4-mm diameter. In 2001 and 2003, windows were cut in the sides of the buckets and covered with mesh to prevent shading and to enable water exchange through the cage. In 2002, we chose not to use windows to reduce the buoyancy of the cages; however, this bucket design exhibited some cage effects (Table 2; *see below*), so windows were used again in 2003. To control for cage effects, which included a 25% reduction in light, a cage control was used in which open windows enabled free access to the sediment by snails at ambient densities. Ambient snail density was determined three times throughout the course of the experiment. Three 10-m transect lines were placed with SCUBA at 3–5-m depth on the lake bottom, and the number of snails within 1 m to one side of the transect line was counted.

In mid-July, the cages were deployed using SCUBA, and snails were added and allowed to graze for approximately 3 weeks. To normalize for snail biomass within treatments, snails with shells between 19.5 mm and 22 mm in length were placed in the cages. These snails weighed 0.8–1.07 g by length-mass regression ($\ln(g) = \ln(mm) \times 2.79 - 8.53$; $R^2 = 0.76$; $p < 0.001$; $n = 30$). At the end of the experiment in early August, one small (2.7-cm diameter) and three large (9.5-cm diameter) intact sediment cores were collected by SCUBA from each cage and transported from the field to Toolik Field Station via helicopter in a water-filled dark cooler. Any snails visible in the cores were removed, and these cores were then used to make measurements of N_2 fixation, primary production, chlorophyll biomass, and algal composition as described next.

Table 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 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)			ER (mg C m ⁻² day ⁻¹)			Total chlorophyll (μg m ⁻² day ⁻¹)		
	A	C	<i>t</i> p	A	C	<i>T</i> p	A	C	<i>df</i> p	A	C	<i>t</i> p	A	C	<i>df</i> p
2001															
Fog2	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	-113 (17)	-93 (11)	5 -0.9 0.4	NA	NA	NA NA
2002															
Fog2	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	-270 (6)	-204 (17)	5 -4.1 0.01	310 (54)	350 (47)	5 3.23 0.9
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	-304 (12)	-384 (31)	5 2.75 0.4	708 (83)	671 (109)	5 0.27 0.8
2003															
Fog2	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	-161 (31)	-147 (12)	14 -0.3 0.75	426 (25)	459 (39)	12 -0.8 0.5

Algal composition, chlorophyll a, and phaeophytin analysis—Upon return to Toolik Field Station, the small core was immediately sectioned for chlorophyll and algal composition analysis. The top 2 centimeters were removed and homogenized, and two 5-mL subsamples were collected for chlorophyll and algal composition. Chlorophyll samples were frozen at -80°C, and algal composition samples were stored in 10 mL of lake water and preserved with 1 μL of glutaraldehyde.

Chlorophyll samples were transported to the Ecosystems Center, Woods Hole and analyzed the following fall. Samples were extracted in acetone and analyzed on a spectrophotometer according to the Arctic LTER protocol, which was adapted from Lorenzen (1967). Because the degradation of chlorophyll *a* (Chl *a*) to phaeophytin pigments can be rapid in sediments (Bianchi et al. 1991) and when exposed to high grazing pressure, total chlorophyll as the sum of Chl *a* and phaeophytin was used in the analysis reported here. Statistical outcomes were the same if the analysis was performed on Chl *a* and phaeophytin alone.

Algal cells and colonies were counted and measured in a 5-mL counting cell at 100× and 400× on a Wild M-40 inverted microscope. Seven fields per sample were counted, which we demonstrated was sufficient to capture the taxonomic diversity present in the samples. The length of all cyanobacterial filaments was measured using an ocular micrometer. Algal composition was not enumerated in Fog 2 in year 2002 because significant cage effects were shown (see below).

Rates of N₂ fixation—Measurements of N₂ fixation were performed in an incubation facility at Toolik Field Station for N₂ fixation measurements using the acetylene reduction assay (ARA), which quantifies the reduction of acetylene (C₂H₂) to ethylene (C₂H₄) by the nitrogenase enzyme (Hardy et al. 1968). Chillers provided constant temperature control, and light level was controlled by Hydrofarm Radiant System lamps with AgroSun Metal Halide and Sodium 1,000-W bulbs. Light and temperature were set to ambient lake conditions at the time of sampling as described in Table 1. One core was used for the ARA, and two cores were used to correct for C₂H₄ consumption by heterotrophic bacteria and C₂H₄ production that can occur from diatom stress (Lee and Baker 1992). In our study, C₂H₄ was rarely produced in the absence of C₂H₂, but C₂H₄ consumption was more common, ranging from 5% to 15%. Corrections were linear and did not change qualitative results.

The cores maintained an intact sediment–water interface and thus were used as incubation chambers for the ARA. Cores were 30-cm tall and contained about 10 cm of sediments with ~1 liter of overlying water and a 100-mL gas headspace. Cores were placed between two clear polycarbonate plates (1-cm thickness), which had gas-tight, O-ring fittings and were held tightly together by nylon threaded rods. The top plate had a bulkhead-style septum port to enable sampling of the headspace, and the water–gas interface had an externally operated magnetic stirring apparatus, which gently stirred the water and maintained the water and gas phases in equilibrium.

Table 3. *F*-values and *p*-values for the significant factors for the randomized coefficient analysis, which related snail density, year, and lake to measured variables. These factors resulted in the most parsimonious models and therefore include only significant factors (see text).

		Numerator df	Denominator df	<i>F</i> -value	<i>p</i> -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
N ₂ fixation per chlorophyll biomass	Grazer density	1	8	5.74	0.044
	Grazer density	1	6	15.41	0.004
Portion N demand met by N ₂ fixation	Grazer density	1	6	15.41	0.004
Cyanobacterial filament count (no. mL ⁻¹)	Lake	1	9	86.36	<0.0001
	Lake	1	9	39.71	<0.0001
Total chlorophyll (mg m ⁻²)	Lake	1	5	5.31	0.069
	Lake	1	6	29.37	0.002
GPP (mg C m ⁻² day ⁻¹)	Lake	1	6	18.34	0.003
	Year	2	6	18.34	0.003
	Year	2	6	11.11	0.016
NEP (mg C m ⁻² day ⁻¹)	Lake	1	6	11.11	0.016
	Year	2	6	22.26	0.002
	Year	2	6	22.26	0.002
ER (mg C m ⁻² day ⁻¹)	Lake	1	6	51.59	<0.001
	Year	2	6	12.68	0.007
	Year	2	6	12.68	0.007

GPP, gross primary production; NEP, net ecosystem production; ER, ecosystem respiration.

A concentration of 10% C₂H₂ was achieved by replacing 10% of the overlying water with C₂H₂-saturated water. C₂H₂ was bubbled through lake water for about 25 minutes while on a stir plate to ensure saturation and to eliminate the C₂H₄ contamination present in many C₂H₂ tanks (see Marino et al. 2003 for details). In addition, C₂H₂ was first bubbled through a 10% sulfuric acid solution before bubbling it through the lake water to reduce the substantial ammonia contamination (Groffman et al. 1999).

Incubations lasted 4–6 hours over which C₂H₄ production was linear, and measurements of N₂ fixation in the overlying water in the mud cores proved negligible. C₂H₄ samples were analyzed on a Shimadzu GC 8-A Flame ionization detector using a Porapak N column, mesh size 80/100. The total amount of C₂H₄ produced was calculated using Henry's Law and the temperature–solubility relationship presented in Sander (1999). Moles of C₂H₄ produced were converted to moles of N₂ fixed by assuming a 3:1 conversion factor. Although the relationship between moles of C₂H₄ produced and moles of N₂ fixed is not always fixed at the assumed 3:1 value, measured conversion factors for autotrophic cyanobacteria from benthic environments are reasonably constrained, ranging from 1.9 to 5.4 (Howarth et al. 1988b).

Gross primary production, net ecosystem production, and respiration—After the ARA incubations, metabolism measures were made on the two cores used for C₂H₄ controls. Previous work showed that these controls did not affect measures of metabolism. Incubation chambers were similar to the ARA chambers, except that the chambers were completely filled with water, and the top plate was changed to enable the introduction of a data-logging O₂ probe (WTW Oxi 340i). Changes in oxygen concentration were recorded every 15 minutes during periods of dark and ambient light levels, each lasting approximately 12 h at ambient lake conditions (Table 1). In addition to the magnetic stirrer in the core, the oxygen probe had a small magnetic stirrer attached to the tip. This stirrer and the floating stirrer were approximately 15 cm above the surface

of the sediment and were operated by the external magnetic stirrer as described above.

Net ecosystem production (NEP) was calculated as the rate of O₂ production during the light period of the incubation and multiplied by 24 h (although light levels vary, there are 24 h of light in an arctic summer). Ecosystem respiration (ER) was measured as the rate of O₂ consumption during the dark period of the incubation and multiplied across 24 h, and gross primary production (GPP) was calculated as the sum of NEP and ER.

Snail excretion N:P ratio—Snails were collected from the sediments of Fog 2 and immediately placed in clean bottles containing 0.2- μ m-filtered lake water. Bottles with snails were left for 10 h in the incubation facility at ambient lake conditions (light = 180 μ mol m⁻² s⁻¹ and temperature = 12°C). Excretion rate (expressed as μ mol time⁻¹ snail biomass⁻¹) was calculated by measuring ammonium (NH₄⁺) and phosphorus (PO₄⁺) concentrations in the lake water before and after the incubation. The incubation period was long to ensure that we were able to measure changes in ammonium concentrations, which remained near the detection limit; however, the lack of food availability during the incubation period may underestimate the excretion rate. To account for this possible source of error, a range of estimates was used in which an upper estimate was calculated assuming that all of the N and P was excreted in the first hour.

Data analysis—All data were analyzed using a randomized-coefficient, linear-regression model in Proc Mixed SAS (2002), which accounts for unbalanced sampling design and provides for random effects. Snail density (as a continuous variable), lake, year, and interactions among those variables were treated as fixed effects; Block and Block \times Year \times Lake were treated as random effects. 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 Akaike information criterion (AIC) value. Data and model fit were checked for

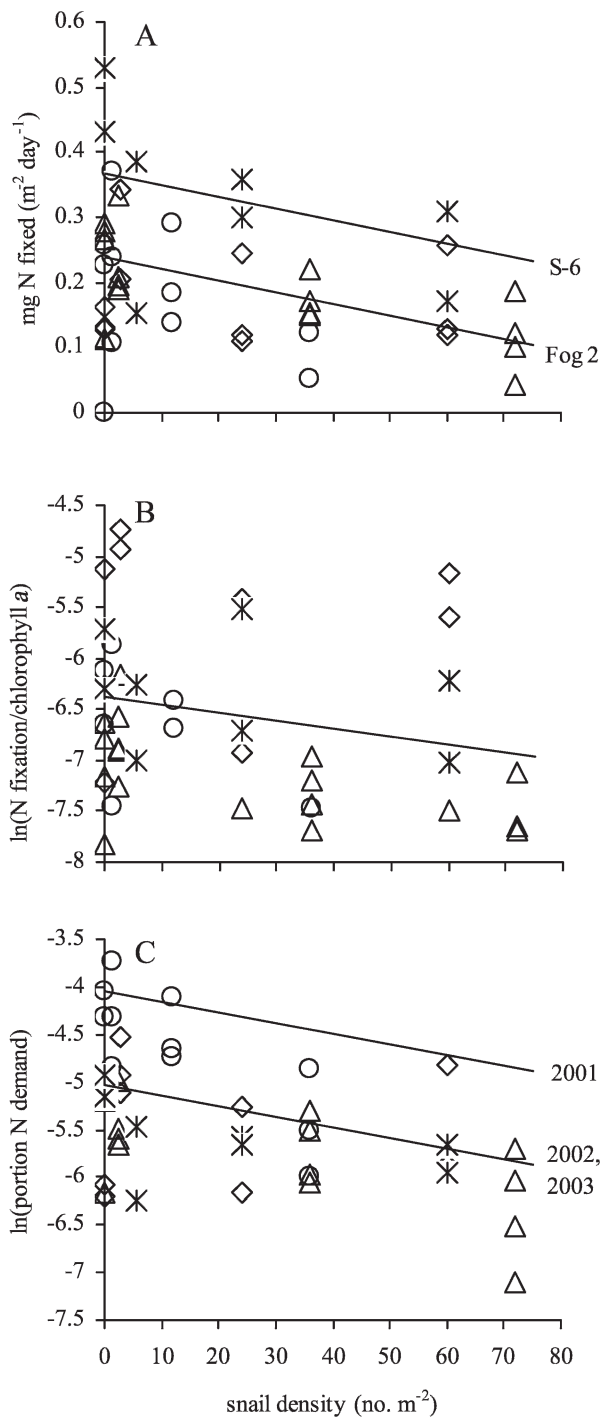


Fig. 2. Regression generated from a random-coefficient analysis relating snail density to measures of N₂ fixation. The statistical analysis takes into account block, lake, and year variability, and the points depicted are data points from all blocks and all years (asterisk = Lake S-6; all other points are from Lake Fog 2; circles = 2001; diamonds = 2002; triangles = 2003). (A) N₂ 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) N₂ fixation per Chl *a* biomass declines with increasing snail density. There was no significant difference between years or between lakes. (C) The proportion N-demand met by N₂ fixation declines with increasing snail density.

the assumption of normality, and two of the N₂-fixation variables (biomass-specific N₂ fixation and N fixed as a portion of N demand) were natural-log transformed.

Some data were eliminated from the analysis for logistical and weather-related reasons. In 2002, cores from block 2 in S-6 were tipped over in transport, which caused significant sediment disturbance. In 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°C colder. No snails were recovered, possibly because they bury themselves in the sediment during the winter (Eisenberg 1966).

Results

Snail density—Ambient snail density in Fog 2 was lower in 2001 (1.3 ± 0.7) than in 2002 and 2003 (2.9 ± 0.51 and 2.6 ± 0.41 , respectively; $p < 0.05$ by ANOVA and Tukey's post-hoc test). S-6 had about a two-fold higher snail density than Fog 2 of $5.6 \pm 1.7 \text{ m}^{-2}$ ($p < 0.05$).

Cage effects—In 2001 and 2003, measurements of N₂ fixation, production, and biomass variables did not differ between ambient cores and control cages (t -tests; $p > 0.05$; Table 2). However, in 2002 in Fog 2, areal rates of N₂ fixation and respiration were lower in control cages than ambient measurements ($p < 0.05$; Table 2). Also in 2002 in S-6, NEP was lower in control cages than ambient cages in S-6 ($p = 0.07$; Table 2). Differences between control cages and ambient measurements in 2002 probably reflect the cage design, which did not include screened windows (*see Methods*). However, including 2002 data in the randomized coefficient analysis did not change the statistical outcomes or the relationship between snail density and the response variables. Thus, 2002 data were included in the analyses except for filamentous cyanobacteria in Fog 2, which were not enumerated (*see Methods*).

The effect of grazing—Snail density was a significant factor affecting measures of N₂ fixation (Table 3). Area-specific and biomass-specific N₂ fixation as well as the proportion of N demand met by N₂ fixation declined significantly with increasing snail density in both Fog 2 and S-6 and across years ($p < 0.05$; Table 3; Fig. 2).

Area-specific N₂ fixation ranged from $0.05 \text{ mg N m}^{-2} \text{ day}^{-1}$ to $0.52 \text{ mg N m}^{-2} \text{ day}^{-1}$ across all years and both lakes. These rates are generally similar to or lower than rates measured in other oligotrophic lakes in the Arctic and lower latitudes (Moeller and Roskoski 1978; Loeb and Reuter 1981; Bergmann and Welch 1990; Higgins et al. 2001). There was no significant year-to-year variation in Fog 2 (Table 4; Fig. 2A), and snail density affected areal

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This relationship was not different between lakes, but was significantly different in 2001 as compared to 2002 and 2003.

Table 4. Linear regression parameters and standard errors relating snail density to N₂ fixation variables. Other factors (lake or year) are shown if they were significant in the randomized coefficient regression model. For areal rates of N₂ fixation, S-6 served as a dummy variable and for the variable describing the portion N demand met by N₂ fixation, year 2003 served as a dummy variable. For mass-specific N₂ fixation, there was no significant lake or year effect.

		Estimate	Standard error	df	<i>t</i> -value	<i>p</i> -value	Meaning of the <i>p</i> -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 Fixed mg Chl ⁻¹ 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.

rates of N₂ fixation in S-6 and Fog 2 in a statistically similar manner so that when N₂ fixation was regressed against snail density, both lakes have parallel slopes (Table 4; Fig. 2A). However, the intercept was significantly higher in S-6 than in Fog 2 by about two-fold (Table 4; Fig. 2A), showing that S-6 has naturally higher rates of N₂ fixation even though it has a two-fold higher ambient snail density. These results are corroborated by the fact that S-6 is more productive than Fog 2 (*see below*), and may have stronger positive bottom-up (nutrient) influences on N₂ fixation.

Biomass-specific N₂ fixation ranged from 0.0002 mg N mg⁻¹ chlorophyll to 0.008 mg N mg⁻¹ chlorophyll and was also negatively related to snail density. Neither lake nor year was a significant factor, and one regression line was generated from all data pooled across both lakes and across all years (Table 4, Fig. 2B). This relationship results from the fact that Chl *a* biomass did not vary significantly between years and was not very different between lakes (Table 3).

We also expressed N₂ fixation rate as a proportion of N demand by primary production. N demand was calculated by assuming that net primary production (NPP) was 75% of measured GPP (Sundback et al. 2004) and the carbon (C):N molar ratio for benthic algae was 17:1 (Hillebrand and Sommer 1999). These assumptions are simply linear scaling factors and therefore do not affect the ability to compare among treatments. N₂ fixation provided 0.2–2.5% of N demand by primary production across snail density treatments, and this proportion declined significantly with increasing snail density ($p < 0.05$; Table 4; Fig. 2C). This relationship was similar between Fog 2 and S-6. GPP (and hence NPP) was lower than in 2001 in Fog 2 than in subsequent years, resulting in a higher proportion of N demand that was met by N₂ fixation and a higher intercept in Fig. 2C.

Snail density was not a significant factor controlling benthic chlorophyll biomass in Fog 2 or S-6 in any year ($p > 0.05$) and was therefore not included in the most parsimonious model in the randomized coefficient analysis (Table 3). Similarly, snail density did not significantly affect measures of benthic metabolism (GPP, NEP, or ER) or measures of the cyanobacterial filament community (count or size) ($p > 0.05$). As a result, the randomized coefficient models for these response variables included significant factors of lake and/or year (Table 3; *see below*).

Lake and year comparisons—Because snail density did not affect the cyanobacterial community, chlorophyll biomass, or measures of ecosystem metabolism, the most parsimonious randomized coefficient model resulted from pooling data across snail density treatments and comparing these parameters among lakes and years (Table 5; Figs. 3 and 4). In Fog 2, cyanobacterial count and length averaged ~17 filaments mL⁻¹ and ~22 μm in length and were not significantly different between 2001 and 2003 ($p = 0.44$; $p = 0.31$ respectively; Fig. 3). The cyanobacterial population was significantly different between S-6 and Fog 2, and both the abundance and the length of cyanobacterial filaments was higher in S-6 at 72 mL⁻¹ and 47 μm, respectively ($p < 0.0001$; Table 5; Fig. 3). These results are consistent with the higher measured rates of N₂ fixation in S-6 and the higher intercept in Fig. 2A.

Consistent with patterns of higher N₂ fixation and cyanobacterial biomass in S-6, other measures of benthic production were also higher. S-6 had higher chlorophyll biomass than Fog 2 at 575 mg m⁻² versus an average across years of ~404 mg m⁻² for Fog 2 ($p = 0.07$; Tables 3 and 5; Fig. 4). Benthic GPP was also significantly greater in S-6, averaging more than 600 mg C m⁻² day⁻¹ versus 150–400 mg C m⁻² day⁻¹ in Fog 2 ($p = 0.002$; Tables 3 and 5; Fig. 4). Similarly, ER was greater in S-6 ($p < 0.001$; Table 3)

Table 5. LS Means estimate of net ecosystem production (NEP), gross primary production (GPP), ecosystem respiration (ER), total chlorophyll, and filamentous cyanobacterial abundance and average length for Lake Fog 2 and S-6 for 2001–2003.

	Estimate	Standard error
GPP (mg C 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 m⁻² day⁻¹)		
Lake Fog 2		
2001	38	23
2002	157	23
2003	262	24
Lake S-6		
2002	277	28
ER (mg C 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 m⁻²)		
Lake Fog 2		
2001	335	103
2002	398	83
2003	478	99
Lake S-6		
2002	575	102
Filamentous cyanobacteria abundance (no. mL⁻¹)		
Lake Fog 2		
2001	19.13	2.43
2003	16.15	2.68
Lake S-6		
2002	72.38	12.38
Filamentous cyanobacteria average length (μm)		
Lake Fog 2		
2001	19.61	2.51
2003	23.73	2.69
Lake S-6		
2002	47.08	2.21

at 376 mg C m⁻² day⁻¹ versus 91–203 mg C m⁻² day⁻¹ for Fog 2 (Table 5; Figure 4). NEP was significant, but only slightly higher in S-6 than Fog 2 ($p = 0.016$; Table 3) at 277 mg C m⁻² day⁻¹ versus 38–262 mg C m⁻² day⁻¹ (Table 5; Figure 4). The NPP rates we calculate from gross benthic primary productivity for these two lakes are within the range of epilimnetic production values reported by Vadeboncoeur et al. (2003) for oligotrophic lakes in Greenland using ¹⁴C techniques at ~20 mg C m⁻² h⁻¹.

In addition to the differences between lakes, there were interannual differences in metabolism measurements in Fog 2. Specifically, the epilimnetic temperature in 2001 was

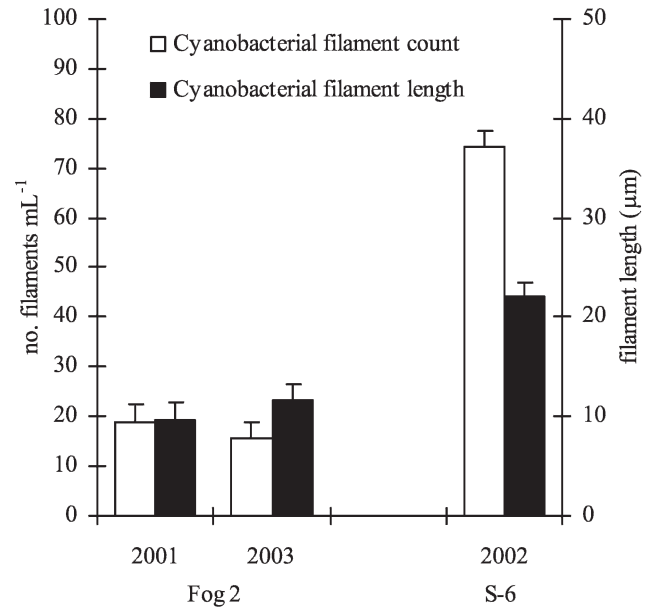


Fig. 3. Filamentous cyanobacterial counts and average length. There were fewer and shorter filaments in Fog 2 than S-6; however, there was no significant effect of snail density.

colder than in subsequent years (10°C vs. 12–13°C; Table 1), and GPP, NEP, and CR were all significantly lower than in 2002 and 2003 ($p < 0.007$ for all variables; Table 3). Although metabolism measurements were different among years in Fog 2, there was no significant year effect on total chlorophyll biomass, which ranged from 335 mg m⁻² to 478 mg m⁻² (Table 5; Fig. 4).

Snail excretion—For an average snail of 20 mm, our range of estimated excretion rates for P ranged from 2.8×10^{-4} to 3.1×10^{-3} mg P-PO₄⁻ day⁻¹ and N from 4.4×10^{-4} to 5.2×10^{-3} mg N-NH₄⁺ day⁻¹ ($n = 16$). The molar N-NH₄⁺:P-PO₄⁻ excretion ratio was very low, averaging 4.8 ± 1.42 (SE) ($n = 16$).

Discussion

Grazing by the snail, *L. elodes*, caused a decline in areal and per-biomass rates of N₂ fixation (Fig. 2). However, grazing did not cause declines in measures of ecosystem metabolism (NEP, GPP, or ER), nor did snails cause a decline in total chlorophyll biomass (Table 3). 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 across time (years) and in multiple lakes.

How can snails cause changes in N inputs without causing changes in primary production or algal biomass? One possibility is that cyanobacteria are more sensitive to direct grazing pressure than other species of algae and recover more slowly from a grazing disturbance. Despite the fact that some species of cyanobacteria have adaptations that reduce their susceptibility to certain grazers (Dodds and Castenholz 1987; Reynolds 1987), many benthic studies indicate that grazing can have negative

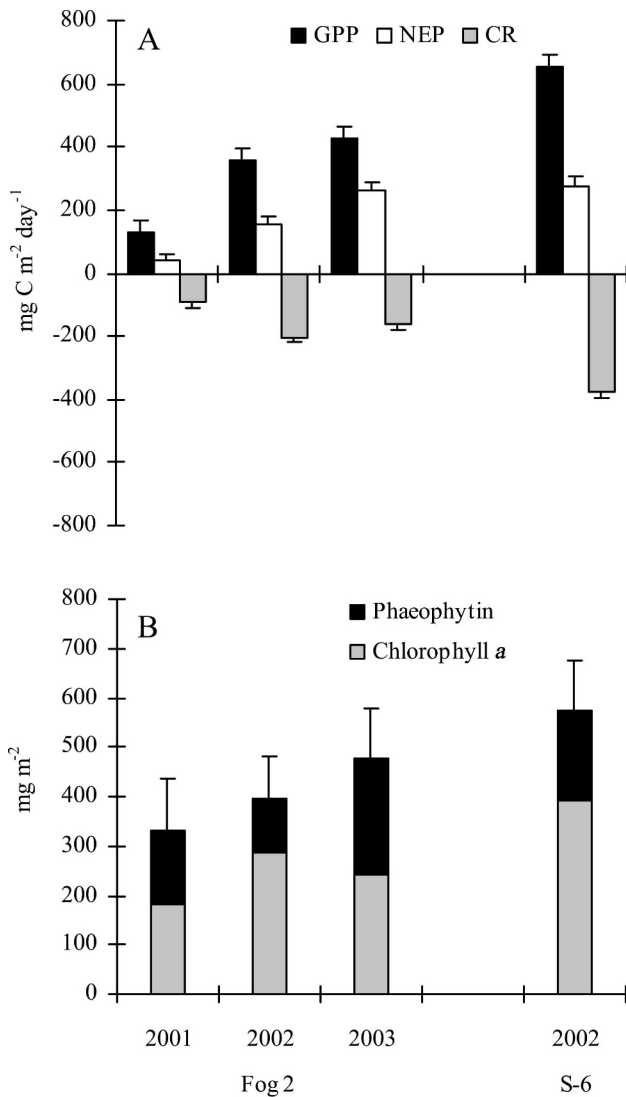


Fig. 4. (A) Gross primary production (GPP), net ecosystem production (NEP), and ecosystem respiration (ER) for lakes Fog 2 and S-6 for 2001–2003. (B) Total chlorophyll biomass for Lake Fog 2 and S-6 for 2001–2003.

effects on cyanobacterial biomass, especially when snails are the grazer involved (Table 6). Other studies in pelagic environments have shown that the size of cyanobacterial filaments was reduced in response to grazing, which in turn led to slower growth rates and lower N_2 fixation rates (Howarth et al. 1999; Chan et al. 2004; Chan et al. 2006). In this study, neither the abundance nor the length of cyanobacterial filaments were reduced by grazers (Table 3), suggesting an alternative mechanism linking *L. elodes* and reduced N_2 fixation rates.

The effects of grazer species on algal community structure, N_2 fixation, and benthic production are not entirely clear. Whereas results from this and other studies have demonstrated a N_2 fixation reduction with increased grazer density, this contradicts some studies—most of which involved grazing fish—that have shown a positive response of benthic N_2 fixation and/or cyanobacterial biomass to grazing (Table 6; Wilkinson and Sammarco 1983; Power et

al. 1988; Flecker 1996; Gettel and Flecker unpubl. data). The variability in N_2 -fixer responses may be a result of differences among modes of grazing by different species. Large, scraping invertebrate grazers such as snails may be less selective grazers than fish, and the susceptibility of algal and cyanobacterial species to snails may be based on morphology rather than food quality (Cattaneo and Kalff 1986; Lowe and Hunter 1988) such that filamentous cyanobacteria are more vulnerable to grazing than prostate species of algae (McCollum et al. 1988; Hillebrand et al. 2000). In a review of grazing effects in freshwater benthic environments, Steinman (1996) also noted that whenever benthic production did not respond to grazers—a pattern we also observed in our study—snails were the herbivore involved. He hypothesized that snails may exert low grazing pressure relative to other groups because of their slower movements, lower consumption rates, and lower mobility.

The small grazing effect by snails may also depend on the degree to which primary production and algal turnover rates associated with different substrates can compensate for a grazing disturbance. The majority of the snail studies in Table 6 were conducted on rock substrates, whereas this study was conducted on soft sediment. Coker (1983) showed that on rock substrates in Toolik Lake, *L. elodes* reduced Chl *a* biomass and primary production by about 20% mg^{-1} snail, contradicting our results that snails caused no change in production and chlorophyll biomass on sediment. The epilithic production in these lakes is two orders of magnitude smaller ($0.4 g m^{-2} yr^{-1}$; Yeakel 1978) than epipelagic production ($18 g C m^{-2} yr^{-1}$; our data assuming a 60-day growing season). Calculations of the algal turnover rate (our data) in sediments compared to grazing rates from Coker (1983) further support the hypothesis that epipelagic production may be sufficiently high to compensate for grazing by *L. elodes*.

Feedback between snails and N_2 fixation and primary production may occur if snail excretion increases the supply rate of inorganic N and/or alters the N:P supply ratio (Elser 1999; Hillebrand et al. 2004). Our data suggest that snail excretion is not an important factor in explaining the lack of response in production or the reduction in N_2 fixation. First, *L. elodes* excreted a very low N:P molar ratio (~ 4.8), which is well below the optimal 17:1 molar ratio for benthic algae (Hillebrand and Sommer 1999). Few data exist for the N:P excretion ratio of other snails, but Evans-White and Lamberti (2005) and Mulholland et al. (1991) showed that the snail *Elimia* sp. excretes a higher N:P ratio ranging from 7–50. Their studies 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—a condition that would favor increased N_2 fixation rates.

Second, the $N-NH_4^+$ supply rate via snail excretion meets almost none of the N demand by primary producers. One snail supplies 0.001–0.017% of the demand per day and supports very little production (0.003 – $0.03 mg C m^{-2} day^{-1}$ of NPP). Even in the highest density treatment ($72 snails m^{-2}$), the excretion rate is small (0.1–1.23% of the primary producer N demand) and supports only 4.8–

Table 6. Comparison of results from benthic grazing studies that enumerated the response of N₂-fixing cyanobacteria or measured N₂ fixation.

Study	Benthic grazer	Ecosystem type	Cyanobacteria response	N ₂ fixation
This Study	Snail (<i>Lymnaea elodes</i>)	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 (<i>Cerithidea californica</i>)	In situ, tidal flats, Mugo Lagoon, Southern California	Cyanobacterial pigment zeaxanthin declined when snails were present	N ₂ fixation not measured
Cattaneo and Kalf 1986	Snail (<i>Annicola</i> sp.) and meiofauna assemblage including chironomids, oligochaetes, and cladocerans	In situ, Lake Memphremagog, Quebec	Filamentous cyanobacteria increased when snails were present, especially <i>Gloeotrichia pisum</i>	N ₂ fixation not measured
Dodds and Castenholz 1987	Snail (<i>Vorticifex effusa</i>)	In situ, Mare's Egg Spring, Oregon	Epiphytes were removed from <i>Nostoc pumifforme</i> colonies, increasing growth rate	N ₂ fixation not measured
Evans-White and Lamberti 2005	Snail (<i>Lymnaea livescens</i>)	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 (<i>Physa integra</i>)	In situ, Spring Lake, northern Michigan	Filamentous cyanobacteria <i>Schizothrix</i> sp. and other genera of cyanobacteria reduced in high density treatment	N ₂ fixation not measured
McCollum et al. 1998	Snail (<i>Physella heterostropha</i>)	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 (<i>Elimia livescens</i>)	Flow-through mesocosms near Douglas Lake, MI and Kentucky Lake, Kentucky	Filamentous cyanobacteria <i>Schizothrix calicicola</i> and <i>Phormidium tenue</i> were reduced when snails were present	N ₂ fixation not measured
Rosemond et al. 1993	Snail (<i>Elimia claviformis</i>)	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	Detritivorous fish (<i>Prochilodus mariae</i>)	In situ, tropical stream (Rio Las Marias, Venezuela)	Number of <i>Calothrix</i> sp. filaments was higher on rock substrates exposed to grazing.	N ₂ fixation not measured
Gettel and Flecker unpubl. data	Armored catfish (<i>Ancistrus triradiatus</i>)	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 (<i>Campostomoa anomalum</i>), minnows, and snails	In situ, stream in Ozark Mountains	Filamentous cyanobacterial felts comprised of <i>Calothrix</i> 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 (<i>Diadema antillarum</i>)	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 (<i>Hemiglyphidodon plagiometopon</i>) and native grazing fish	In situ, Britomart Reef, Great Barrier Reef, Australia	Percentage substrate containing cyanobacteria was higher on grazed substrate	N ₂ fixation increased when grazers were present

55.7 mg C m⁻² of NPP. These results suggest that N inputs from direct snail ammonium excretion are not sufficient to reduce the need to fix atmospheric N₂ and are consistent with previous experiments at Toolik Lake, which showed that excretion by *L. elodes* did not increase algal production of periphyton communities (Cuker 1983). They are also consistent with the results of Evans-White and Lamberti (2005) who found that Chl *a* biomass did not increase in snail grazing treatments in a recirculating stream experiment designed to examine the effect of *Elimia livescens* excretion on periphyton.

One possible explanation for our results could be that cyanobacteria remain viable in the snail gut. Cuker (1983) found that *L. elodes* egest viable cells that photosynthesize at 80% the rate of ungrazed substrates and hypothesized that these viable cells may take up inorganic N within the gut. This process would lead to the low concentrations of inorganic N observed in excretion and could result in a lowered N demand and a reduced need to fix N₂. Furthermore, the egestion of viable cells could help explain our observed lack of change in production and chlorophyll biomass.

Previous investigators have identified important top-down controls on *L. elodes* in these arctic lakes and demonstrated that its abundance is strongly determined by the presence or absence of fish predators (Merrick et al. 1991; Hershey et al. 1999). Our results confirm this pattern; Fog 2 contains a key predator (arctic char), whereas S-6 does not contain predators and has a two-fold higher ambient snail density (Fig. 2). Our results, however, suggest that this top-down control does not comprise a trophic cascade extending down to the level of benthic primary production, but that such cascades do have the potential to make small changes in rates of benthic N₂ fixation.

In these arctic lakes, the reduction of N₂ fixation by snails at ambient densities is quite small. Each snail reduced N₂ fixation by 0.002 mg N m⁻² day⁻¹ or, during the course of a 60-day growing season, 0.12 mg N m⁻² summer⁻¹. This reduction comprises only 0.85–1.8% of the ambient N₂-fixation rate. These results suggest that grazing by the snail *L. elodes* is probably unimportant in maintaining N limitation of oligotrophic arctic lakes for short time scales, but snail grazing may be important where natural densities are higher, as has been observed in some temperate lakes and reservoirs where densities can reach 100s per m⁻² (Eisenberg 1966). Because N₂ fixation represents an input of new N that can support new production, the reduction of N₂ fixation by *L. elodes* may have a stronger influence on N limitation from the perspective of ecosystem evolution, which occurs across much longer time scales.

We found that grazing by the snail, *L. elodes*, reduces benthic N₂ fixation in oligotrophic arctic lakes without affecting cyanobacterial abundance or benthic production. It is unlikely that these patterns result from recycling of nutrients via snail excretion, but may result from the egestion of viable cells. These results contrast with studies where grazing by fish and larger invertebrates were found to increase N₂ fixation; however, few grazing studies have directly measured the responses of both benthic N₂ fixation and cyanobacterial abundance. Although the mechanism causing our observed patterns is unknown, the effect of *L.*

elodes on N₂ fixation is relatively small at densities commonly found in these arctic lakes, and more work is needed to fully understand the degree to which grazers can maintain N limitation in oligotrophic lakes.

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