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Interactions among irradiance, nutrients, and herbivores constrain a stream algal community

A.D. Rosemond

Department of Biology, Vanderbilt University, Nashville, TN 37235, USA

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Abstract. Using stream-side, flow-through channels, I tested for the effects of nutrients (NU) (nitrogen plus phosphorus), irradiance (L), and snail grazing (G) on a benthic algal community in a small, forested stream. Grazed communities were dominated by a chlorophyte (basal cells of *Stigeoclonium*) and a cyanophyte (*Chamaesiphon investiens*), whereas ungrazed communities were comprised almost entirely of diatoms, regardless of nutrient and light levels. Snails maintained low algal biomass in all grazed treatments, presumably by consuming increased algal production in treatments to which L and NU were increased. When nutrients were increased, cellular nutrient content increased under ambient conditions (shaded, grazed) and biomass and productivity increased when snails were removed and light was increased. Together, nutrients and light had positive effects and grazing had negative effects on biomass (chlorophyll *a*, AFDM, algal biovolume) and chlorophyll- and areal-specific productivity in ANOVAs. However, in most cases, only means from treatments in which all three factors were manipulated (ungrazed, +NU&L treatments) were significantly different from controls; effects of single factors were generally undetectable. These results indicate that all three factors simultaneously limited algal biomass and productivity in this stream during the summer months. Additionally, the effects of these factors in combination were in some cases different from the effects of single factors. For example, light had slight negative effects on some biomass parameters when added at ambient snail densities and nutrient concentrations, but had strong positive effects in conjunction with nutrient addition and snail removal. This study demonstrates that algal biomass and productivity can be under multiple constraints by irradiance, nutrients, and herbivores and indicates the need to employ multifactor experiments to test for such interactive effects.

Key words: Periphyton – Nutrients – Irradiance – Grazing – Stream

Many factors can constrain the productivity and biomass of primary producers in headwater streams. Among these constraints are low levels of light and streamwater nutrients, and removal of biomass by herbivores. The importance of each of these factors has been previously demonstrated (nutrients: Stockner and Shortreed 1978; Elwood et al. 1981; Peterson et al. 1983; Bothwell 1985; Grimm and Fisher 1986; Pringle 1987; Hart and Robinson 1990; irradiance; Triska et al. 1983; Steinman and McIntire 1987; Hill and Harvey 1990; Steinman 1992; herbivory: Lamberti and Resh 1983; Hart 1987; Hill and Knight 1987; Steinman et al. 1987; Power et al. 1988; Feminella et al. 1989). However, interactions among these factors may be more important than their singular effects. If several resources are low in availability and are non-substitutable (sensu Tilman 1982), simultaneous limitation by these resources (e.g., light, nutrients) can occur. In such cases, large increases in plant growth do not occur with the addition of one resource, but only when limitation by other factors has also been removed. For example, Gregory (1980), Triska et al. (1983), Lowe et al. (1986), and Hill and Knight (1988) found that nutrient addition resulted in greater increases in algal biomass in sunlit than shaded streams, suggesting simultaneous limitation by nutrients and light. Similarly, there can be simultaneous limitation of biomass by an abiotic factor, such as light, and control by biotic interactions, such as herbivory. This was shown by Feminella et al. (1989) and Steinman (1992) who found that algal biomass was increased to a greater extent under high light conditions only when grazing was suppressed. In this paper, I use the terms "limited" and "controlled" to describe sub-maximum periphyton productivity or biomass; limitation refers to a constraint that occurs from a deficit in resources required for growth and control as the extent to which periphyton is constrained by herbivory.

Studies previously conducted in Walker Branch (WB) suggested that the algal community of this small forested stream was potentially constrained by a combination of factors, namely, snail herbivory, streamwater nutrients, and irradiance. Studies showed that low algal biomass and productivity in this stream could be attributed in part to low concentrations of streamwater nutrients (Elwood et al. 1981) and high densities of grazing snails (Elwood and

Nelson 1972), alone, and together (Rosemond et al. 1993). In addition, Steinman (1992) showed increased algal productivity and biomass resulting from an experimental increase in irradiance during the late summer in WB when snails were excluded. Together, these results suggest that during the growing season, the algal community in this stream may be under simultaneous limitation and control by all three factors (nutrients, irradiance, and snail herbivory). To test for their effects, I manipulated levels of these three factors in flow-through channels, placed adjacent to Walker Branch, using a factorial design. This study is unique in that it simultaneously tests the effects of light, nutrients and herbivores on an algal community under field conditions (but see Winterbourn (1990) for indirect tests of light effects with manipulations of herbivores and nutrients and Summer and McIntire (1982) for an unreplicated laboratory study).

Materials and methods

Study site

This study was conducted for 7 weeks, from July 21, 1989, to September 8, 1989, in flow-through channels (102 cm long and 8 cm wide) placed next to a first-order reach of WB, located on the Department of Energy's Oak Ridge Reservation in eastern Tennessee. Water velocity in the channels was maintained at approximately 10–15 cm/s, which is characteristic of water velocity at baseflow in this spring-fed stream (Rosemond, pers. obs.). The stream flows over bedrock outcrops, cobble, and gravel. Riparian vegetation consists largely of deciduous trees (predominantly *Liriodendron tulipifera* L. and *Fagus grandifolia* Ehrh.) (Johnson and Van Hook 1989), which shade the stream from late April through mid-October. This study was conducted during the summer months, when the tree canopy above WB reduces incident light to the streamwater surface to $< 25\,\mu$mole quanta$\cdot m^{-2}\cdot s^{-1}$. The most abundant herbivore in this stream is the snail *Elimia clavaeformis* (Family: Pleuroceridae), which occurs at densities $> 1000/m^2$ year-round (Rosemond, unpub. data) and comprises $> 95\%$ of the invertebrate biomass in the stream (Newbold et al. 1983). Streamwater nutrient concentrations are low during all seasons (inorganic $N \leq 50$ µg/l, $P \leq 7$ µg/l), but are distinctly higher in the summer months than at other times of the year (Mulholland 1992).

Experimental

I tested the effects of light, nutrients, and snail herbivory using a complete factorial design; each factor was tested at two levels (light and nutrients at ambient and elevated levels and snails present or absent). All possible combinations of light, nutrients, and snails resulted in eight different treatment combinations that were randomly assigned among sixteen different flow-through channels. Unglazed ceramic tiles (upper surface area = 5.29 cm^2), which had been placed in WB several months prior to the study for algal colonization, were used as substrates in the channels. They were transferred to the channels approximately two weeks prior to the start of the experiment.

Snails were counted non-destructively along a 150 m reach of stream using an underwater viewer (250 cm^2) ($n = 40$) to determine approximate densities for experimental manipulations before the experiment was started and again, during and after the study. Grazing treatments consisted of 1) snails added at ambient densities (approx. $1100/m^2$) and 2) no snails. Ambient light conditions over 24 h periods were measured at the streamwater surface three times, before, during, and after the study was conducted on cloudless days, using ozalid paper light meters (Friend 1961) at twelve different locations along a 150 m reach of stream. To increase light in the stream-side channels, two lamps with 1000 watt metal halide bulbs (Sylvania Metalarc®) were suspended over them. Four layers of mosquito netting were placed over eight of the sixteen channels (shaded treatments) to maintain shaded conditions similar to those in the stream. Light levels in individual channels were also estimated at the beginning of the study using ozalid meters and instantaneous readings were periodically checked with a Li-Cor) sensor.

Nutrient concentrations were increased to roughly 10X the yearly average stream concentrations by dripping a concentrated nutrient solution at a constant rate from 50 L Mariotte bottles (Peterson et al. 1983) into the high nutrient treatments. Nutrient treatments consisted of 1) low or ambient (no addition) and, 2) high via addition of both inorganic N and P to 210 µg N/l as $NaNO_3$, 40 µg N/l as NH_4Cl, and 35 µg P/l as K_2HPO_4. To document nutrient treatments, water samples were taken weekly from the channels and filtered in the field through precombusted, washed glass-fiber filters (Gelman type A/E, pore size = 1 µm). Concentrations of soluble reactive phosphorus (SRP) were measured using the ascorbic acid method (American Public Health Association 1985), ammonium nitrogen (NH_4^+-N) by phenate colorimetry using an autoanalyzer (Technicon TRAACS 800), and nitrate plus nitrite nitrogen ($[NO_3^- + NO_2^-]$-N) by Cu-Cd reduction followed by automated colorimetric analysis (United States Environmental Protection Agency 1983).

Carbon fixation rates were determined from an average of five tiles from each channel at the beginning of the study, during weeks 1, 3, and 5, and at the end of the study. Tiles were placed in glass recirculating chambers containing 1 L of filtered (Gelman Type A/E filter) water from the treatment from which they were collected. Approximately 180-360 kBq of $NaH^{14}CO_3$ (specific activity 0.74 MBq/mmol) was added to the water in each chamber. Light levels during the incubation were maintained similar to treatment levels by illuminating "high light" chambers using a metal halide lamp and reducing incident light in "shaded" chambers using layers of mosquito netting. Following a 3 h incubation, tiles were removed from chambers, rinsed in stream water, placed in jars containing 10 ml dimethyl sulfoxide (DMSO), and samples were extracted in the dark overnight at room temperature. Subsamples of DMSO were then removed to measure chlorophyll *a* and ^{14}C-labeled photosynthate (Palumbo et al. 1987). Phaeopigment-corrected chlorophyll *a* was determined spectrophotometrically (Strickland and Parsons 1972) and ^{14}C uptake was measured by liquid scintillation on subsamples of the extract. Total inorganic carbon in the water in each chamber was measured by a total carbon analyzer (OI Model 700) to calculate total carbon fixation rates from ^{14}C incorporation measures. Carbon fixation rates were expressed on both an areal- and a chlorophyll-specific basis.

Ash-free dry mass (AFDM) of periphyton was estimated at the beginning and at the end of the study by collecting five tiles from each channel, placing the tiles with attached periphyton in aluminum pans, and determining differences between dry (at 60° C for 24 h) and ashed (at 500° C for 24 h) mass.

Five additional tiles were collected from each channel at the beginning of the study, during week 3, and at the end of the study to determine the response of algal community structure to experimental treatments. Algae were brushed from the tiles with a toothbrush and the resultant slurry was preserved in 2% glutaraldehyde. Samples were processed with a tissue homogenizer, as required, to break up large aggregations and then sonicated. Algal units (single cells or colonies, > 500/sample) were counted and identified at 400X using a Palmer-Maloney cell. Diatoms were lumped into groups or genera when counted at 400X. Identifications of diatoms to species were then made at 1000X from permanent slides (an oxidized subsample from each sample mounted in Hyrax®) and the groups or genera initially counted were further subdivided. For each taxon, length, width, and depth, or diameter of spherical cells (1-20/taxon) were measured with an ocular micrometer and biovolume was estimated using geometric formulae (Kellar et al. 1980).

N, P, and C contents of periphyton were determined on the final sampling date. Approximately 20 tiles were collected from each channel, scraped with a toothbrush into a beaker and dried at 60° C for several days. A subsample of dried periphyton from each channel (5–10 mg) was weighed and analyzed for carbon and nitrogen content using a Carlo Erba Model NA1500 CNS analyzer. An additional subsample of dried periphyton was weighed and ashed (500° C), and the ash was leached in 5 ml of hot 1N HCl for 30 min to extract P. The acid leachates were diluted to 100 ml with distilled water, and phosphorus concentrations were determined using the ascorbic acid method as described above.

Alkaline phosphatase activity (APA) was determined on an average of five tiles from each channel during week 3 and at the end of the experiment. Six small glass jars were filled with 20 ml filtered water collected from each channel. One tile was placed in each of five jars, and a sixth jar served as a stream water control. *P*-nitrophenyl phosphate (NPP; Sigma) was added to each jar to produce a final concentration of 3 mM. Following a 30 min incubation, the stream water/NPP solutions in the jars were filtered (Whatman GFF glass-fiber filters), and the pH raised to ≥ 10 by adding 0.05 ml of 1N NaOH. Absorbance of the filtrate was read at 410 nm on a spectrophotometer to determine hydrolysis of NPP. Tiles were then removed from the jars and placed in 10 ml DMSO to extract chlorophyll *a*. APA was calculated as the amount of NPP hydrolyzed (moles of nitrophenyl produced) per µg chlorophyll *a*.

Snail growth was determined as the AFDM gain of 90 snails from each channel during the experiment. Individual snail widths were initially measured with calipers to the nearest 0.01 mm and measured snails were marked with paint. Widths of each marked snail were remeasured at the end of each experiment. Percentage increases in AFDM [(final average − initial average)/initial average] were determined using a width: AFDM regression computed from snails previously taken from WB ($AFDM = 1.2332 \times 10^{-5}$ $(width)^{3.98363}$, $n = 50$, $R^2 = 0.96$).

Data were separated by week and analyzed by a three factor (nutrients, light, grazing) analysis of variance (ANOVA) using the ANOVA procedure of Statistical Analysis Systems (SAS Institute, Inc., 1985). Values used in the analyses for AFDM, chlorophyll *a*, areal-specific and chlorophyll-specific productivity were means from 5 tiles from each replicate channel. The model I used tested for main effects of nutrients (NU), light (L), and grazing (G), and all possible interactive effects (NU × G, L × G, L × NU, L × NU × G). Algal biovolumes were log-transformed and percentages (% algal biovolume and snail growth) were arcsin square-root transformed prior to analyses (Zar 1984). Snail growth data were analyzed using a two-factor (L, NU) ANOVA. Most parameters (except APA, and periphyton %C, %N, and %P) were measured before treatments were started and unless otherwise indicated, ANOVA of these data (to determine whether there were any pre-treatment differences) showed no initial differences among treatment groups. I report ANOVA effects as significant if $p < 0.05$ and marginally significant if $0.10 > p > 0.05$. I conducted post-ANOVA multiple comparison tests (if ANOVAs were significant) on most parameters using Ryan's *Q*-test (Day and Quinn 1989). Many parameters were measured at different times during the experiment (weeks 1, 3, 5, and 7) to determine how treatment effects changed temporally. However, I only present data from the last week of the experiment here to simplify the interpretation. Additional data can be found in Rosemond (1993).

Results

Light levels, nutrient concentrations, and snail densities

Mean snail densities (±SE) in WB, before, during and after the study was conducted (June 6: $1319/m^2 \pm 15$, August 2: $1288/m^2 \pm 16$, October 5: $1124/m^2 \pm 12$) were similar to densities used in the channels ($1100/m^2$). Mean light levels in shaded treatments were similar to ambient light at the stream water surface throughout the study period, whereas light was >10X ambient in high light treatments (Table 1). Ambient nutrient (SRP, NO_3-N, and NH_4-N) concentrations in the stream were low (3.8, 34.8 and 5.1 µg/l, respectively), and adding nutrients to create high nutrient treatments resulted in increases (approximately 10X) over ambient concentrations (Table 2).

Table 1. Mean (SE) light levels (mmole quanta $\cdot m^{-2} \cdot d^{-1}$) at the streamwater surface (Stream) ($n = 12$), in shaded channel treatments (Shaded) ($n = 8$), and in high light channel treatments (High Light) ($n = 8$). WEEK = # of weeks from when treatments were started in channels

Week	Date	Ambient Stream	Channel treatments Shaded	High light
1	17 July	601 (22)		
2	26 July	–	965 (34)	12,726 (241)
3	09 August	814 (34)		
7	08 September	731 (26)		

Algal biomass

All measures of biomass (chlorophyll *a*, AFDM, and total algal biovolume) were increased by combinations of removing snails and increasing nutrients and light. This was indicated by the significance of main treatment effects and interaction terms in ANOVAs (Table 3). For chlorophyll *a* and AFDM, post-ANOVA multiple comparison tests indicated that only means from treatments in which all three factors were simultaneously manipulated (snails removed, nutrients and light increased) were significantly different from control means or when one or two factors were manipulated (Fig. 1). Likewise, mean biovolume, another measure of biomass, was significantly higher in the ungrazed, +NU&L treatment than in other treatments. Biovolume was next highest in the ungrazed, +L treatment but lowest in the grazed, +L treatment (Fig. 2).

Algal taxonomic composition

Snail grazing strongly affected algal species composition. By the end of the experiment, all grazed communities were dominated by chlorophytes (primarily basal cells of *Stigeoclonium tenue* (Kuetz.)) and cyanophytes (primarily *Chamaesiphon investiens*), with some diatoms (primarily *Cocconeis placentula* (Ehr.) and *Achnanthes* (Bory) spp.) and a rhodophyte (*Audouinella* (Bory)) present to lesser degrees (Fig. 2a). Ungrazed communities were comprised almost entirely of a mixed assemblage of diatoms (Fig. 2b). The ungrazed, +NU&L treatment was dominated by one species of diatom, *Melosira varians* (Ag.); it comprised >90% of the biovolume in this treatment (Rosemond, unpub. data). The other ungrazed treatments had a somewhat richer diatom flora (see Table 4 for dominant species).

I found significant negative effects of grazing on the absolute and percentage biovolume of all diatom species tested as well as marginally significant negative effects on

Table 2. Mean (SE) nutrient concentrations (μg/l) in nutrient enriched stream-side channels ($n=8$) and in ambient stream water ($n=8$) at approximately the same time. Week = # of weeks after treatments were started. Mean (all dates) is a mean concentration over all weekly averages ($n=7$) during the experimental period. *$n=1$

Week	Date	Enriched treatments SRP	NO_3-N	NH_4-N	Ambient stream water SRP	NO_3-N	NH_4-N
1	24 July	34.1	248.5	36.4	4.9	39.2	7.6
		(6.2)	(48.4)	(5.7)	(0.1)	(0.3)	(1.0)
2	31 July	33.1	262.1	40.9	3.0	33.8	6.0
		(6.4)	(40.4)	(7.0)	(0.2)	(0.5)	(2.3)
3	07 August	45.5	302.4	45.6	3.9*	37.6	6.2
		(7.6)	(40.2)	(7.1)	(0)	(0.6)	(0.5)
4	14 August	18.6	48.2	13.7	3.6	34.9	0
		(1.7)	(8.8)	(1.4)	(0.2)	(1.3)	(0)
5	21 August	38.8	234.4	47.0	4.4	35.8	6.9
		(6.7)	(18.4)	(6.3)	(0.3)	(1.4)	(1.9)
6	29 August	40.4	293.3	45.8	3.8	33.1	5.9
		(6.1)	(40.4)	(7.1)	(0.1)	(0.3)	(1.4)
7	04 September	60.2	359.8	75.6	2.7	29.6	3.1
		(12.5)	(51.7)	(12.2)	(0.2)	(0.3)	(0.8)
Mean	(All dates)	38.7	249.8	43.6	3.8	34.8	5.1

Table 3. *F* values from ANOVA of chlorophyll *a* (CHL), ash-free dry mass (AFDM), and log total algal biovolume (BIOVOL) (week 7 only). $^{+}p<0.10$, $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$. Treatment effects: G = grazing, L = light, NU = nutrients. (+) = positive effects, (−) = negative effects, (+/−) = direction of effect is dependent on other treatment variables, otherwise significant interaction terms indicate that the magnitude of the effect changed, not the direction

Treatment	DF	CHL	AFDM	BIOVOL
G	1	(−) 12.97**	(−) 32.70***	(−) 69.80****
L	1	(+/−) 6.52*	(+) 21.41**	(+) 17.93**
NU	1	(+) 57.92****	(+) 14.92**	(+) 23.35***
G × L	1	18.50**	19.87**	20.01**
G × NU	1	14.30**	11.91**	8.77*
L × NU	1	41.45***	11.57**	5.39*
G × L × NU	1	12.37**	7.95*	4.93^{+}

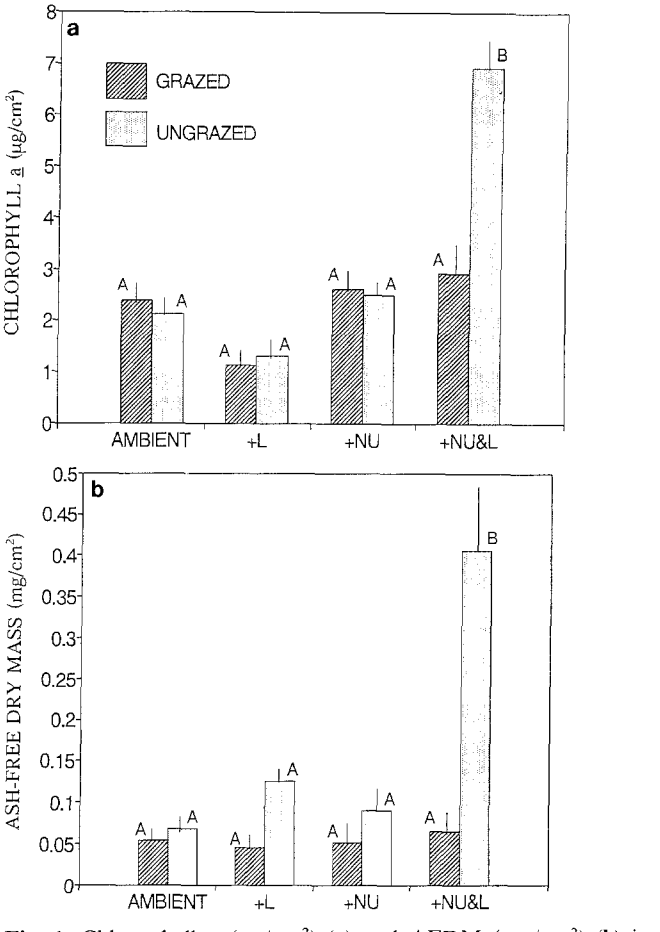

Fig. 1. Chlorophyll *a* (μg/cm²) (**a**) and AFDM (mg/cm²) (**b**) in grazed and ungrazed treatments during the last week of the study (week 7). *AMBIENT* = shaded, low nutrient treatments, *+NU* = shaded, high nutrient treatments, *+L* = high light, low nutrient treatments, *+NU&L* = high light, high nutrient treatments. *Bars* = ±1 SE. Means of bars having the same letter are not significantly different

one chlorophyte (*Rhizoclonium hieroglyphicum* (Ag.)) (Table 4). The absolute and percentage biovolume of two taxa were positively affected by grazing: *Stigeoclonium* (only marginally significant for absolute biovolume) and *Chamaesiphon*. Nutrient effects, when they occurred, were positive on the absolute biovolume of species of diatoms, but were negative on the proportion of several species of diatoms as well as on the proportion of *Chamaesiphon*. Light positively affected the proportion of some diatoms (*Amphipleura pellucida* (Kuetz.), *Melosira*, and *Peronia intermedium* (H.L. Sm.)) and the cyanophyte *Chamaesiphon*, but negatively affected the proportion of other diatoms (*Amphora ovalis* (Kuetz.), *Eunotia pectinalis* var. *minor*) and the chlorophyte, *Stigeoclonium* (marginal). In addition, there was a marginally significant negative effect of L on the absolute biovolume of *Stigeoclonium*.

Productivity

Similar to the effects on biomass, areal-specific productivity (ASP) was increased by a combination of removing snails and increasing nutrients and light. All main treatment effects and interaction terms were significant by ANOVA (Table 5), and only ASP from the ungrazed, +NU&L treatment was significantly higher than in other

Species/treatment	Absolute Biovolume	Percentage Biovolume
Chlorophyta		
Rhizoclonium hieroglyphicum		
G	(−) 4.61^{+}	ns
G × L × NU	ns	3.66^{+}
Stigeoclonium tenue (basal cells)		
G	(+) 5.03^{+}	(+) 34.97***
L	(−) 3.94^{+}	(−) 4.34^{+}
Chrysophyta (Class: Bacillariophyceae)		
Achnanthes (all spp.)		
G	(−) 69.25****	ns
L	(+) 7.28*	ns
NU	(+) 14.14**	ns
G × L	ns	4.35^{+}
Amphipleura pellucida		
G	(−) 9.06*	(−) 47.72****
L	ns	(+) 28.07***
NU	ns	(−) 17.09**
G × L	ns	28.07***
G × NU	ns	17.09**
L × NU	ns	32.99***
G × L × NU	ns	32.99***
Amphora ovalis		
G	(−) 22.88***	(−) 31.32***
L	(−) 7.44*	(−) 41.14***
G × L	4.43^{+}	8.41*
G × NU	5.60*	5.60*
L × NU	ns	6.10*
Cocconeis placentula		
G	(−) 132.53****	(−) 5.01^{+}
L	ns	(+/−) 7.13*
NU	ns	(−) 9.37*
G × L	ns	8.97*
G × NU	ns	7.11*
Eunotia pectinalis var. *minor*		
G	(−) 28.84***	(−) 9.34*
L	(−) 9.85**	(−) 8.54*
G × NU	8.40*	ns
L × NU	10.88**	5.07*
Melosira varians		
G	(−) 1031.86****	(−) 207.51****
L	(+) 1031.86****	(+) 207.51****
NU	(+) 23.75***	(+) 50.86****
G × L	1031.86****	207.51****
G × NU	23.75***	50.86****
L × NU	23.75***	50.86****
G × L × NU	23.75***	50.86****
Nitzchia linearis		
Peronia intermedium		
G	(−) 15.15**	(−) 34.29***
L	ns	(+) 5.64*
NU	ns	(−) 10.49**
G × L	ns	5.64*
G × NU	ns	10.49**
L × NU	ns	9.66**
G × L × NU	ns	9.66**
Cyanophyta		
Chamaesiphon investiens		
G	(+) 19.20**	(+) 429.55****
L	ns	(+) 12.54**
NU	(−) 5.23*	(−) 38.83***
G × L	ns	20.92**
G × NU	4.01^{+}	8.94*
Rhodophyta		
Audouinella sp.		
G × L	9.16*	5.46*
G × L × NU	ns	3.99^{+}

Table 4. *F* values (*p*) from ANOVA of log biovolume of different algal species. Treatments effects: G = grazing, L = light, NU = nutrients. $^{+}p<0.10$, $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$. A full model was tested in each case; to save space, only effects that were significant are listed. Data from week 7 only. (+) = positive treatment effects, (−) = negative treatment effects, (+/−) = direction of effect is dependent on other treatment variables, otherwise significant interaction terms indicate that the magnitude of the effect changed, not the direction. All taxa making up >5% average biovolume/treatment in >1 treatment/date are included. ns = no significant treatment effect

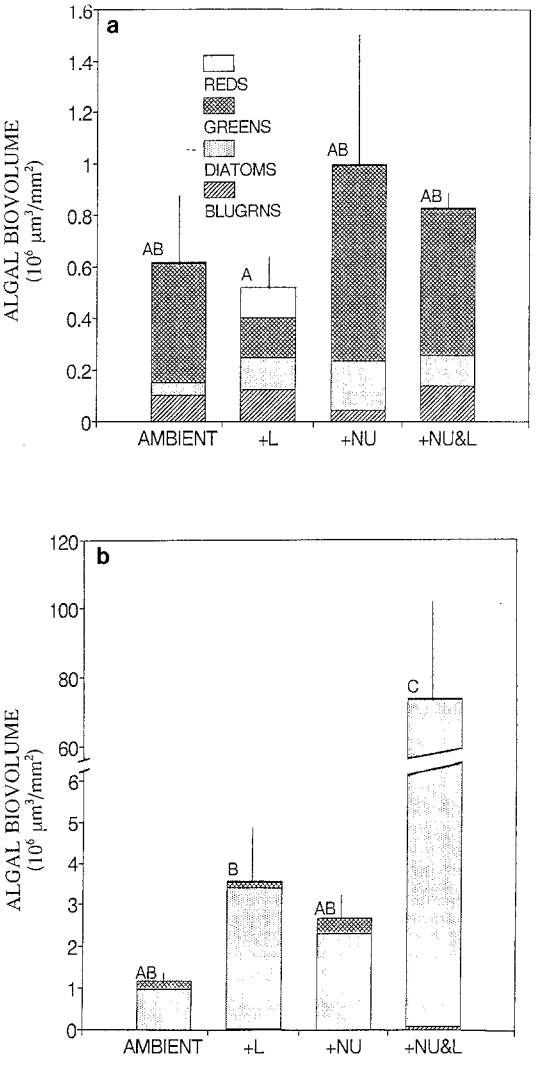

Fig. 2. Total algal biovolume (µm³/mm²) in grazed (**a**) and ungrazed (**b**) treatments during the last week of the study (week 7). *AMBIENT* = shaded, low nutrient treatments, *+NU* = shaded, high nutrient treatments, *+L* = high light, low nutrient treatments, *+NU&L* = high light, high nutrient treatments. *REDS* = biovolume of algae belonging to Division Rhodophyta, *GREENS* = biovolume of algae belonging to Division Chlorophyta, *DIATOMS* = biovolume of algae belonging to Division Chrysophyta, Class: Bacillariophyceae, *BLUGRNS* = biovolume of algae belonging to Division Cyanophyta. *Bars* = ± 1 SE. Means of bars having the same letter are not significantly different. Note that ordinates have different scales.

Table 5. *F* values from ANOVA of areal-specific productivity (ASP) and chlorophyll-specific productivity (CSP). $^{+}p<0.10$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$. Treatment effects: G = grazing, L = light, NU = nutrients. (+) = positive effects, (−) = negative effects

Treatment	DF	ASP	CSP
G	1	(−) 56.33****	(−) 39.64***
L	1	(+) 86.61****	(+) 176.46****
NU	1	(+) 103.57****	(+) 4.30^{+}
G × L	1	49.99****	13.51**
G × NU	1	41.50***	1.44
L × NU	1	79.99****	0.38
G × L × NU	1	35.50***	0.11

treatments (Fig. 3a), indicating that a significant response in ASP required the manipulation of all three factors.

Chlorophyll-specific productivity (CSP) was increased with light enhancement and decreased by grazing; effects of nutrients were only marginally significant (Table 5). A significant G × L interaction indicated that light effects were greater when herbivores were absent; the highest CSP occurred in ungrazed treatments where light was enhanced. Both with and without added nutrients (Fig. 3b).

Periphyton C, N, P content, C : N, and chlorophyll: AFDM

The addition of nutrients and presence of snails both increased %C, %P, and %N of periphyton and reduced C : N (Fig. 4). Increases in light resulted in reductions in %N and marginally significant increases in %P, but did not affect %C. C : N was positively affected by increases in light, presumably due to reductions in %N rather than increases in %C (Table 6). Generally, the highest %C, %N, and %P of periphyton were in grazed treatments in which nutrients, alone, or in combination with light, were increased (Figs. 4a–c). The C : N ratio was highest in ungrazed treatments in which light, alone, was increased (Fig. 4d). The chlorophyll : AFDM ratio was affected positively by grazing and nutrients, and negatively by increased irradiance (Table 6), resulting in the highest values in grazed treatments in which nutrients alone were increased (Fig. 4e).

Alkaline phosphatase activity

Alkaline phosphatase activity of periphyton was reduced with nutrient addition (Table 7). Effects of grazers and light addition were not significant by an ANOVA, although snails reduced and high light increased APA; APA was highest in the ungrazed, + L treatment (Fig. 5).

Snail growth

In an ANOVA on percentage increase of snail AFDM, I found no significant effect of nutrients ($F = 2.13$, $p = 0.20$), but a marginally significant effect of light ($F = 5.58$, $p = 0.06$). Snail growth rates were highest in treatments to which both light and nutrients were added but were not significantly different from growth under ambient conditions (Fig. 6).

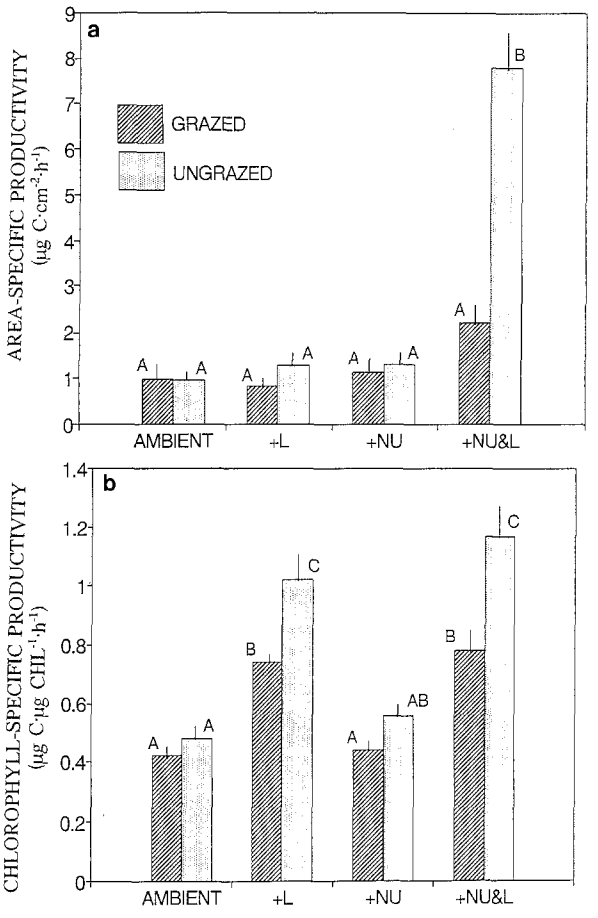

Fig. 3. Areal- (**a**) and chlorophyll- (**b**) specific productivity ($\mu g\ C \cdot cm^{-2} \cdot h^{-1}$ and $\mu g\ C \cdot \mu g\ CHL^{-1} \cdot h^{-1}$, respectively) in grazed and ungrazed treatments during the last week of the study (week 7). *AMBIENT* = shaded, low nutrient treatments, + *NU* = shaded, high nutrient treatments, + *L* = high light, low nutrient treatments, + *NU&L* = high light, high nutrient treatments. *CHL* = chlorophyll *a*. *Bars* = ± 1 SE. Means of bars having the same letter are not significantly different

Discussion and conclusions

Too often, ecologists have conducted experiments under the assumption that single factors limit the productivity of communities. In this study, algal biomass and productivity were constrained by multiple factors. As one resource was supplied (light, nutrients) or biomass was no longer controlled via consumption (by removing snails), little or no algal growth occurred, due to limitation by other factors. Nutrient addition, removal of snails, or enhancement of light, by themselves or in combination with one other factor, had no significant effect on algal biomass or productivity in most cases (Figs. 1a, b, 2a, b, and 3a). Without manipulating light in this experiment and testing for its limiting effect, the effects of nutrients and grazing would have been scarcely detectable. Likewise, effects of light and grazing or light and nutrients would have been

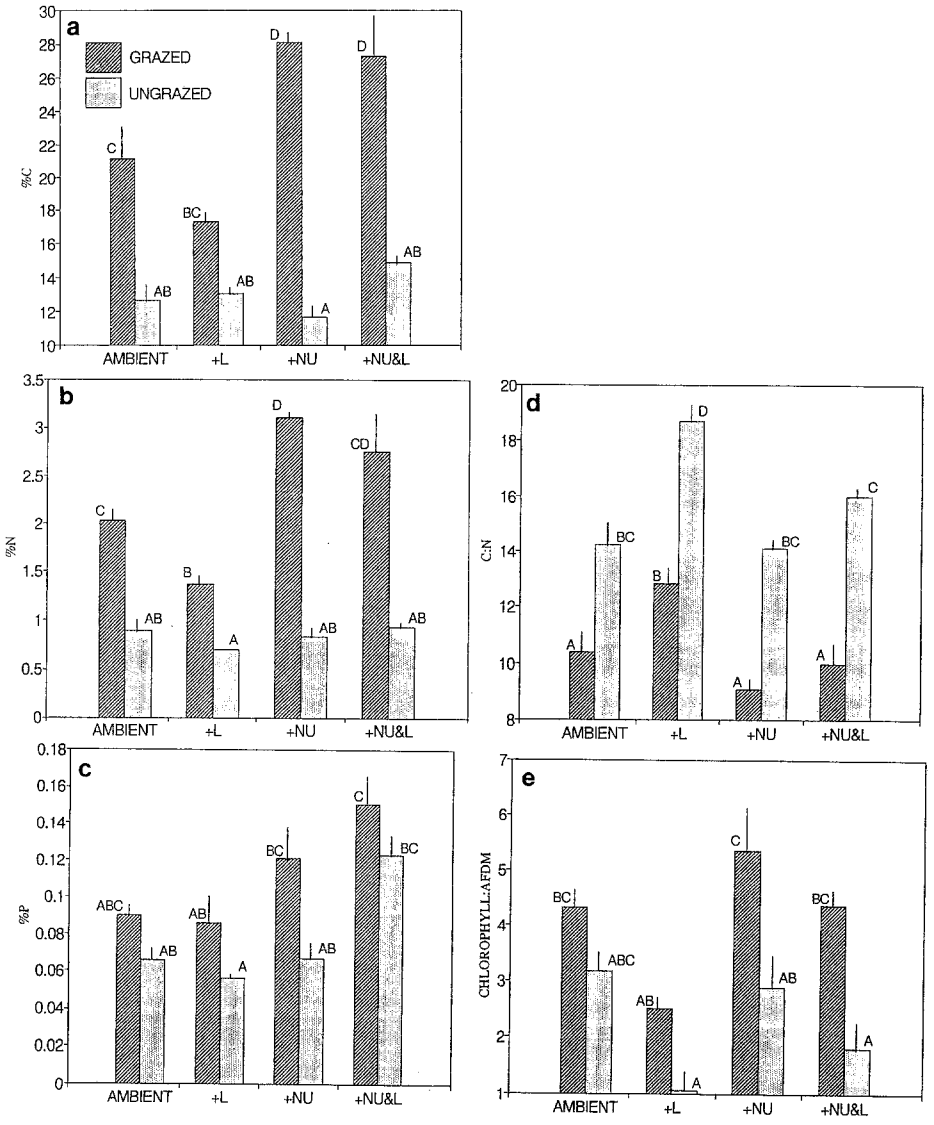

Fig. 4. (**a**) %C, (**b**) %N, (**c**) %P, (**d**) C:N, and (**e**) chlorophyll: AFDM of periphyton. *AMBIENT* = shaded, low nutrient treatments, *+NU* = shaded, high nutrient treatments, *+L* = high light, low nutrient treatments, *+NU&L* = high light, high nutrient treatments. *CHL* = chlorophyll *a*. Bars = ±1 SE. Means of bars having the same letter are not significantly different

Table 6. *F* values (*p*) from ANOVAs of periphyton nutrient content (%C, %N, and %P of dry wt.), C:N (mass ratio), and the ratio of chlorophyll *a*: ash-free dry mass (CHL:AFDM). $^{+}p<0.10$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$. Treatment effects: G = grazing, L = light, NU = nutrients. (+) = positive effects, (−) = negative effects

Treatment	%C	%N	%P	C:N	CHL:AFDM
G	(+) 177.19****	(+) 260.20****	(+) 20.12**	(−) 230.57****	(+) 37.56***
L	0.01	(−) 8.39*	(+) 4.62^{+}	(+) 50.98****	(−) 23.58**
NU	(+) 27.98***	(+) 40.21***	(+) 26.67***	(−) 25.82***	(+) 7.31*
G × L	7.66*	4.65^{+}	0.55	5.01^{+}	0.11
G × NU	22.13**	25.48***	0.29	0.93	3.85^{+}
L × NU	3.99^{+}	4.71^{+}	10.38*	8.61*	2.26
G × L × NU	0	0	1.70	0.64	0.02

Table 7. *F* values from ANOVA of alkaline phosphatase activity (APA). $^{+}p<0.10$, $^{***}p<0.001$. Treatment effects: G = grazing, L = light, NU = nutrients. (−) = negative effects

Treatment	DF	APA
G	1	2.76
L	1	1.02
NU	1	(−) 30.89***
G × L	1	0.02
G × NU	1	0.06
L × NU	1	4.02^{+}
G × L × NU	1	1.67

obscured if either nutrients or grazing had been left out of the experimental design. These results, by themselves, could suggest that algae in this stream was not controlled by these factors. However, the experimental design used here, which allowed for simultaneous testing of light, nutrient, and herbivore effects, indicated that changes in all three factors were required to achieve a significant increase in algal biomass and productivity in WB during the summer months, when this study was conducted. In all cases, the largest values of biomass and productivity were from ungrazed, +NU&L treatments. In contrast to some systems in which primary productivity may be limited by

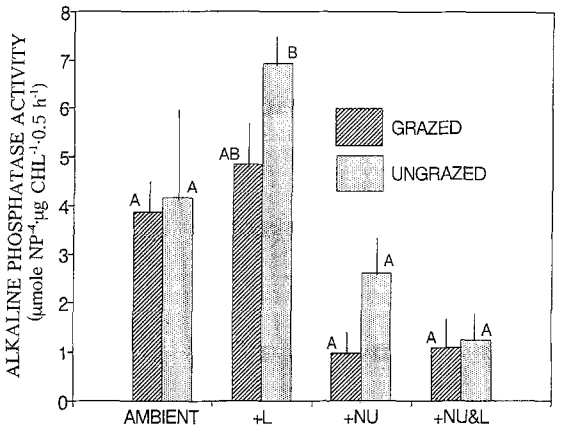

Fig. 5. Alkaline phosphatase activity (μmole $NP^{-4} \cdot \mu g\ CHL^{-1} \cdot 0.5\ h^{-1}$) in grazed and ungrazed treatments during the last week of the study (week 7). *AMBIENT*=shaded, low nutrient treatments, *+NU*=shaded, high nutrient treatments, *+L*=high light, low nutrient treatments, *+NU&L*=high light, high nutrient treatments. *NP*=nitrophenyl phosphate, *CHL*=chlorophyll *a*. Bars=±1 SE. Means of bars having the same letter are not significantly different

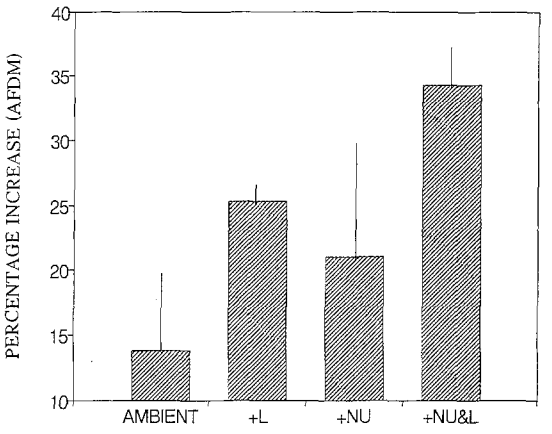

Fig. 6. Snail growth. *AMBIENT*=shaded, low nutrient treatments, *NU*=shaded, high nutrient treatments, *L*=high light, low nutrient treatments, *NU&L*=high light, high nutrient treatments. Bars=±1 SE. Means were not significantly different by Ryar's Q test

only one factor, other systems may be similar to WB, where there are several resources at growth-limiting levels and where herbivores play an important role. In these cases, to understand the true effects of even a single factor, studies must include tests for multiple effects, as illustrated here.

Some effects of factors that I tested were constant, having the same effects regardless of levels of other factors, whereas other effects changed in combination with other factors. For example, removing snails increased biomass and areal-specific productivity, but only to a significant degree when light and nutrients were at elevated levels. Otherwise, the potential for increased growth was stifled by a lack of resources. But when snails were present, they had similar effects among all grazed treatments, maintaining algal biomass at low levels despite nutrient and light availability. In other studies, increases in growth-limiting resources in streams resulted in increased periphyton biomass that outran consumption by herbivores (Stewart 1987; Lamberti et al. 1989; McCormick and Stevenson 1991). However, in this study, snails presumably ingested more algal biomass in +L, +NU, and +NU&L treatments as it became available. However, only L effects on snail growth were (marginally) significant. Other studies have similarly shown increased growth or density of stream herbivores due to increased irradiance (Lamberti et al. 1989; Triska et al. 1983), suggesting that both primary producers and herbivores can be limited by resources such as light in streams, and that the growth response of herbivores to increased primary productivity can be rapid.

Snails also changed the taxonomic composition of the algal community, from chlorophytes and cyanophytes in grazed treatments to a dominance by diatoms when snails were removed, regardless of resource level. Analyses by individual species indicated that all species of diatoms were negatively affected by grazing and that two other species, *Stigeoclonium* and *Chamaesiphon*, increased in relative abundance in grazed vs. ungrazed treatments. Basal cells of *Stigeoclonium* have been found to be a major component of algal communities in other streams in the southeastern U.S. that contain high densities of the same snail used in this study (McCormick and Stevenson 1989; Hill and Harvey 1990). When I experimentally removed snails, species that persisted under grazed conditions were overgrown by diatoms (Rosemond, pers. obs.). This was especially pronounced in the +NU&L treatment, in which a bloom of one diatom species, *Melosira varians*, occurred. Similarly, Power et al. (1988) showed that when grazers were experimentally excluded, a cyanophyte-dominated community was overgrown by diatoms, primarily, *Melosira*. In both of these studies, herbivores appeared to keep algal communities at an early successional stage, comprised of chlorophytes and cyanophytes. When herbivores were removed, this early successional stage was overgrown by diatoms, the degree to which, in this study, was dependent on resource availability.

These herbivore-driven changes in algal species composition, may have indirectly affected chlorophyll-specific rates of productivity. Although several studies have found positive effects of herbivores on chlorophyll- or biomass-specific productivity (Lamberti and Resh 1983; Lamberti et al. 1987; Stewart 1987; Hill and Harvey 1990), reductions in CSP have been found in other studies in which *Elimia* was the dominant grazer (Hill et al. 1992; Rosemond et al. 1993). Reductions in CSP can occur if the species that persist under heavily grazed conditions (e.g., *Stigeoclonium*) have inherently slower growth rates than those species that proliferate when grazers are removed (e.g., erect and filamentous diatoms). Grazing also increased periphyton %C, %P, and %N, reduced C:N ratios, and increased chlorophyll:AFDM ratios, such that grazed communities were higher in nutrients and chlorophyll *a* for a given biomass than ungrazed communities. There was some evidence that grazers also reduced phosphorus limitation in the algae, as APA values were lower (but not significantly so) in grazed vs. ungrazed treatments. Despite the higher nutrient and chlorophyll *a* content of

grazed communities, which should enhance their productive capacity, CSP was still lower in grazed vs. ungrazed treatments, possibly due to differences in algal species composition.

Whereas snails had negative effects, nutrients had positive effects on periphyton biomass and productivity. Three results indicate that WB algal communities were nutrient-limited: when nutrients were increased, 1) cellular %N and %P increased, 2) APA was reduced, and 3) biomass and areal-specific productivity increased. Only one of these parameters (periphyton nutrient content) would have indicated nutrient limitation under conditions that would be found in the stream naturally (shaded, grazed). Nutrient effects on biomass and productivity were only detectable when light was enhanced and snails were removed. Other studies have shown that increases in nutrient content and reductions in enzyme activity (APA), rather than increases in biomass, are typical responses of periphyton to increased nutrient concentrations in heavily grazed streams (Mulholland et al. 1991; Mulholland and Rosemond 1992). Therefore, these parameters may be the only indicators of nutrient limitation in cases where herbivores also control algal biomass and productivity.

Even when nutrients were added to, and snails were removed from, the stream-side channels, algal biomass and productivity were still constrained by the availability of light. There were positive effects of light on most biomass measures and ASP, but generally only when grazers were removed and/or nutrients were added, again, indicating simultaneous limitation by the three factors.

Under ambient nutrient conditions, effects of light addition, alone, were actually negative on most biomass measures. For chlorophyll *a*, this may have been due to lower chlorophyll *a*/cell in high light treatments, compared with low light treatments, which is a commonly observed physiological adaptation of the algae (Richardson et al. 1983). There were also slight reductions in the other two biomass measurements, AFDM and total algal biovolume, in grazed treatments when light was increased. This interaction between grazing and light effects may have resulted from taxonomic responses to these two factors. The relatively low values of chlorophyll *a*, total algal biovolume, and AFDM in the grazed, +L treatment were accompanied by a much lower relative abundance of *Stigeoclonium* (23% of total algal biovolume), compared with its abundance in other grazed treatments (65% in the +NU&L treatment, 62% in the +NU treatment, and 63% in the ambient treatment). This result is consistent with findings of Steinman (1992), who also reported dominance of *Stigeoclonium* in low light treatments but not in high light, ungrazed treatments. *Stigeoclonium* may not perform well under high light because of photoinhibition at the light level used in the +L treatments in this study (350 μmol quanta $\cdot m^{-2} \cdot s^{-1}$). Basal cells of this species, due to their morphology, may be more resistant to grazing, but are outgrown under high light conditions by other species (e.g., *Audouinella*, a filamentous rhodophyte, *Chamaesiphon*, an erect cyanophyte, motile diatoms). These other species may be more susceptible to snail grazing than *Stigeoclonium* (Steinman et al. 1992), resulting in reduced total biomass in grazed, +L treatments compared to other grazed treatments.

In summary, the biomass, productivity, and taxonomy of the periphyton community in this study were strongly limited by nutrients and light and controlled by herbivory. The addition of nutrients and light and the removal of snails resulted in a shift in the algal community from one composed of chlorophytes and cyanophytes, and characterized by low biomass, CSP, and ASP, to a community of filamentous diatoms, with high biomass, CSP, and ASP. Removal of herbivores, alone, resulted in taxonomic shifts towards diatoms, but with little increase in biomass or productivity. When nutrients were added alone, cellular nutrient content increased, APA was reduced, but, again, biomass and productivity were not strongly affected. Light addition by itself resulted in a reduction in the biomass of the dominant grazer-resistant species in this system, which may have contributed to slight reductions in biomass and ASP compared to controls. However, it was the interaction between all three factors tested that produced the greatest effects on biomass and productivity, indicating simultaneous limitation by light, nutrients, and herbivory during the summer in WB. These effects were much greater and, in some cases, different than the response to single factor manipulations (e.g., negative effects of light on biomass when added singly, strong positive effects when added in conjunction with other factors). Further, differential responses of individual algal species to each factor were important in defining overall interactive effects.

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