Resistance of Lotic Ecosystems to a Light Elimination Disturbance: A Laboratory Stream Study

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Resistance of lotic ecosystems to a light elimination disturbance: a laboratory stream study

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Black plastic was placed over eight laboratory stream channels for 92 d to examine the resistance of lotic ecosystems to light elimination. Prior to the disturbance, four treatments (high grazing-recirculated flow, high grazing-once-through flow, low grazing-recirculated flow, low grazing-once-through flow) were imposed on the streams, resulting in systems with different biomass levels and recycling indices. Light elimination resulted in significant declines of all functional and most structural properties associated with the streams, irrespective of treatment. Declines in species diversity and number were greater in high grazed than low grazed streams. However, high grazed streams appeared more resistant than low grazed streams with respect to autotrophic biomass and carbon fixation. Nutrient levels had little influence on resistance. The relatively small effect of the treatments on system resistance compared with the large influence of light elimination suggests that resistance may be more dependent on the qualities of the disturbance (e.g. magnitude and duration) than those of the system.

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Introduction

Ecosystem stability can be partitioned into numerous components (DeAngelis et al. 1989a), but most ecosystem-oriented studies have viewed stability in terms of either resistance or resilience (e.g. Webster et al. 1975, Vitousek et al. 1981). Resistance refers to the ability of a system to withstand displacement by a disturbance, whereas resilience refers to the rate at which a system recovers following a perturbation. This partitioning has improved our conceptual understanding of ecosystem stability by recognizing that certain attributes may contribute to resistance (see below), whereas others contribute to resilience (e.g. the input rates [DeAngelis et al. 1989b] and control of losses of limiting nutrients [Vitousek et al. 1981]).

Several mathematical models have been devoted to the study of resistance and resilience, but of the two concepts, resilience is better understood mathematically (Harrison and Fekete 1980). Resistance has proven to be a more complex concept than resilience to define usefully in models of ecological systems because the level of some steady state variable (e.g. population level) can be "changed by a stroke of the modeller's pen" (Harrison and Fekete 1980). Hence, resistance to a direct change in the level of that variable has no useful meaning. Therefore, it is customary to measure the resistance of a model state variable with respect to imposed changes in some selected inputs (e.g. nutrients, radiation, temperature), losses (e.g. biomass, nutrients), or transfer rates (e.g. decomposition) to the system. Empirical studies that address resistance from the viewpoint of changes in input, loss, and transfer rates are compatible with models of this type and will allow future models to address this concept in a less arbitrary fashion.

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Factors that may influence an ecosystem's resistance include (1) size of the system's standing crop: O'Neill et al. (1975) suggested that resistance to a perturbation of live biomass was increased by a large organic matter pool because this pool turns over very slowly and can provide a buffer to disturbance by releasing nutrients that promote the regrowth of biomass. The larger the biomass compartment, the greater the buffering capacity in theory; (2) species diversity: Margalef (1963) suggested that higher species diversity could lead to greater ecosystem stability, although this concept has been the focus of considerable debate in ecology (see Webster et al. 1983); (3) structural and functional redundancy: Cairns and Dickson (1977) suggested that among other things, inertia (= resistance) was a function of how well other species in the ecosystem could assume similar structural or functional roles of species that were lost following a disturbance. In addition, they hypothesized that resistance was influenced by how accustomed the native organisms were to variable environmental conditions, how close the system was to a major ecological transitional threshold, and in the particular case of running waters, the cleansing capacity and chemical buffering capacity of the water body.

In the present study, experiments were conducted to examine the resistance of laboratory stream ecosystems to the elimination of light. Prior to the elimination of light, these streams had been exposed for 8 months to treatments of high vs low grazing levels and once-through vs recirculated flow, resulting in systems with different biomass levels and nutrient recycling indices. We hypothesized that streams with low biomasses and species diversities would be less resistant than streams with high biomasses and species diversities (cf. Margalef 1963, O'Neill et al. 1975).

Materials and methods

Experimental design

Eight laboratory streams were made of fiberglass and housed indoors. Each channel was 0.3 m wide, 20 m long and U-shaped, with the bend at 10 m. Water was supplied by a groundwater-fed pond, and total flow was maintained at 0.64 $l \cdot s^{-1}$ in each stream, resulting in an approximate water depth of 3 cm and current velocity of 22 $cm \cdot s^{-1}$. In-line sand filters removed most particulate matter. Irradiance was supplied by 400-watt metal halide lamps that provided ca. 150 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ when measured with a Li-Cor Li-188B quantum meter at the water surface at midchannel. Daily photoperiod was set at 8L:16D.

Unglazed ceramic cylinders (1.6 cm × 1.6 cm) were placed at the bottom of each stream (18,000 per stream) and served as substrates for periphyton colonization and units for sampling. To inoculate the streams, rocks were collected from a second-order reach of Walker Branch, a stream on U.S. Department of Energy's Oak Ridge reservation. At the time of light elimination, periphyton had been growing for over twelve months in the streams.

Beginning in March, 1988 four treatments were imposed on the laboratory channels, giving two replicates per treatment: (1) high grazing, once-through flow; (2) low grazing, once-through flow; (3) high grazing, recirculated flow; and (4) low grazing, recirculated flow. Streams with once-through flow had all incoming water leave the channel after making its 20 m circuit. In the recirculated streams, 95% of the water was recirculated back to the upstream end after making its 20 m circuit, resulting in a continuous mix of 5% new water and 95% recirculated water. Each pipe that carried recirculated water back to the channel's upstream end was surrounded by a cold water "jacket" that cooled the recirculated water down to the water temperature in the once-through streams. Incoming water from the pond varied in temperature from 10 to 13°C during the course of the experiment, and temperatures of recirculated streams generally were within 1°C of those of once-through streams.

High grazing was imposed by adding the snail *Elimia clavaeformis* Lea to four of the streams at a density of 1000 $\cdot m^{-2}$, a density commonly found in Walker Branch (Burris et al. 1990, Amy Rosemond, pers. comm.). Although the other streams contained no snails, chironomid larvae and microfauna were present in all streams, so even the streams without *Elimia* were exposed to a low level of grazing.

On 24 October 1988, black plastic was placed over each stream. Only the most upstream 1 m was left uncovered. We left this area uncovered so it might serve as inoculum for future (i.e. at the end of the light elimination period) growth of autotrophs in the event that light elimination resulted in the death of all covered autotrophs. The lamps were kept on at a daily photoperiod of 8L:16D during the light elimination in order to irradiate the uncovered section of each stream. With the lights on, irradiance levels under the black plastic were < 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The plastic was removed from all streams on 24 January 1989, leaving the downstream 19 m of each stream without light for 92 d.

Sampling design and analytical methods

Ten cylinders were randomly selected (five upstream, five downstream) from each stream 49 and 5 d before the plastic was placed over and the day before the plastic was removed from the streams to measure ash-free dry mass (AFDM). Cylinders with periphyton attached were dried for 24 h at 60°C, weighed, ashed at 475°C, and reweighed. AFDM was calculated as the difference in mass between dried and ashed matter.

Eight cylinders were randomly selected (four upstream, four downstream) from each stream 49 and 5 d before the plastic was placed over, and on the day the plastic was removed from the streams to measure chlo-

rophyll a and adenosine triphosphate (ATP). In addition, five cylinders (three upstream, two downstream) were randomly selected from each stream on d 23, 36, 56, and 71 of the experiment to measure chl a. Cylinders with attached periphyton were placed in test tubes at room temperature for 24 h containing 10 ml of dimethyl sulfoxide (DMSO) to which phosphoric acid had been added (and the DMSO pH raised back to > 8.5 by addition of NaOH) to prevent ATP hydrolysis by phosphatase enzymes produced by periphyton (Palumbo et al. 1987). Extracts were analyzed spectrophotometrically before and after acidification for determination of chlorophyll a and total phaeophytin (Shoaf and Lium 1976). Subsamples of the extract from each cylinder were frozen for subsequent ATP analysis. Samples were analyzed within 8 wk of freezing. For ATP analysis, the subsamples were thawed and diluted with 0.02 M Tris buffer and ATP was determined using a Lumac Biocounter (Model M20) with purified Lumac reagents (Palumbo et al. 1987). Internal standards were used with every sample.

Twelve cylinders were randomly selected (six upstream, six downstream) from each stream 5 d before the plastic was placed over and on the day the plastic was removed from the streams to enumerate the algal component of the attached periphyton community. The attached periphyton was scraped from each cylinder with a toothbrush and the resulting 12 slurries were pooled into four samples per stream consisting of three scraped cylinders each. Scraped periphyton was immediately fixed in a modified Lugol's solution. For microscopic examination, samples were gently homogenized with a teflon tissue grinder and a 1 ml subsample was added to a cover slip with 1 drop of 100% Taft's syrup medium (TSM). A minimum of 500 cells per slide were examined at 1000X magnification. Burn mounts were used to identify problematic small diatoms.

Four cylinders were randomly selected (two upstream, two downstream) from each stream 49 and 5 d before the plastic was placed over, and the day before the plastic was removed from the streams to measure protozoan numbers. Cylinders were placed in vials containing 8 ml of stream water and placed on ice until further processing (usually within 3 h). All material was scraped off the cylinder with a scalpel, fixed in Bouin's fixative, and stained with protagol silver stain (Lee et al. 1985). Permanent slides were made using stained material. Slides were counted on a Nikon compound microscope and scanned at magnfications of 300–800X. Protozoa were classified as amoebae (with or without diatoms and as cysts), ciliates (bacterivorous or carniverous), or rotifers.

Attached bacteria were enumerated using the acridine orange direct count technique (AODC) (Hobbie et al. 1977). Bacterial numbers were sampled at the same time and from the same cylinders as those collected for algal enumeration. A 1 ml subsample of material scraped from each cylinder (i.e. 12 cylinders per stream) was used for bacterial enumeration. Subsamples were sonicated for 2 min and then 0.1 ml was placed in a vial containing 0.9 ml of filtered (0.2 μm pore size) distilled water. The diluted sample was sonicated for 2 min more to disperse the bacteria. 0.5 ml of the final dilutant was counted after staining with acridine orange and filtration onto a prestained black nuclepore filter (0.2 μm pore size). The number of bacteria on 20 fields was counted for each sample.

Eight cylinders were randomly selected (four upstream, four downstream) from each stream 48 and 4 d before the plastic was placed over, and the day the plastic was removed from the streams to measure autotrophic carbon fixation. Cylinders were placed in recirculating, glass chambers with one liter of filtered water from their stream of origin. Each chamber was connected to a submersible pump and chambers were placed in a large tank that served as a temperature-controlled water bath. A metal halide lamp, suspended over the tank, provided irradiance to the chambers. One layer of green screening was placed between the lamp and the chambers to make the irradiance level reaching the chambers similar to the level measured at the stream water surface (ca. 175 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Approximately 183 kBq of $NaH^{14}CO_3$ was added to each chamber. After a 3 h incubation, individual substrates were removed from each chamber, gently rinsed in fresh stream water, and placed in test tubes with 10 ml of DMSO for simultaneous extraction of chlorophyll a and ^{14}C-labelled photosynthate (Palumbo et al. 1987). Carbon fixation was calculated in terms of both areal and chlorophyll-specific production.

Tritiated thymidine incorporation into DNA was measured to estimate production by the attached bacteria, using methods similar to those described by Findlay et al. (1984) and Palumbo et al. (1989). Nine randomly selected cylinders were taken from each stream 48 and 4 d before the plastic was placed over and the day the plastic was removed from the streams, and placed into 50 ml plastic centrifuge tubes containing 10 ml of water from the same stream. A total of 1, 4, and 7 nmoles of unlabeled thymidine were added (each concentration was added to three of the cylinders from each stream) and one concentration of tritiated ($\approx$ 0.13 nmoles) thymidine (NET-250), specific activity $\approx$ 78 $Ci \cdot mmol^{-1}$ (2.886×10^{12} $Bq \cdot mmol^{-1}$), was added to each tube. The cylinders were incubated for 30 min within two degrees of the stream temperature by using a water bath. After incubation, 0.5 ml of 37% formaldehyde was added to the tubes to stop thymidine uptake. Killed controls, in which formaldehyde was added before incubation, were used at each concentration.

DNA was extracted from the tiles using the methods of Findlay et al. (1984) except that after centrifugation, the precipitate was collected on Gelman 0.45 μM pore size filters. The filters were placed back into the rinsed centrifuge tubes, 5 ml of 5% TCA was added, and the filters were extracted for 30 min at 100°C. One ml

Tab. 1. Dissolved nutrient concentrations of incoming water in experimental streams. Values represent averages over a 3-wk period prior to light elimination (n = 3); all units are μg l^{-1} except DOC (mg l^{-1}). SRP = soluble reactive phosphorus, DOC = dissolved organic carbon.

Treatment	Rep	SRP	NH_4-N	NO_3-N	DOC
Once-through,	a	8.4	< 2	81.3	0.75
high grazed	b	9.0	< 2	80.9	0.74
Recirculated,	a	4.9	< 2	17.6	0.98
high grazed	b	5.3	< 2	23.6	0.78
Once-through,	a	9.5	< 2	83.1	0.68
low grazed	b	9.4	< 2	81.0	0.72
Recirculated,	a	3.7	< 2	12.5	0.88
low grazed	b	4.6	< 2	10.4	0.81

subsamples of the 5% TCA extracts were analyzed for radioactivity by liquid scintillation using external standardization to correct to DPM. Significant amounts of labeled thymidine appeared in the non-DNA fractions, but were not routinely quantified. The uptake reported is the incorporation into the DNA fraction only.

Data were transformed and analyzed to determine the degree of isotope dilution (Findlay et al. 1984). The data reported are corrected for isotope dilution effects which were very large, but are not corrected for incomplete recovery of DNA. In past experiments recovery of DNA has been about 75%.

Phosphatase activity was analyzed on eight randomly selected cylinders (four upstream, four downstream) from each stream one week before the plastic was placed over the streams. Each cylinder was placed in a glass jar with 20 ml of filtered stream water. Paranitrophenyl phosphate (NPP) was added to each jar to produce a final concentration of 3 mM NPP. Jars were incubated at room temperature for 30 min, contents filtered (Whatman GFF) and the pH raised back to 10 by addition of 1N NaOH. Filtrates were analyzed spectrophotometrically at 410 nm to determine hydrolysis of NPP. Cylinders were then removed and immersed in 10 ml of DMSO to extract ATP (see earlier).

Water samples were collected at the upstream and downstream ends of each stream every week for 6 wk before the plastic was placed over the streams and on the day the plastic was removed. Samples were filtered through pre-combusted and washed glass fiber filters (Gelman type A/E, pore size 1 μm). Concentrations of soluble reactive phosphorus (SRP), ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), and dissolved organic carbon (DOC) were measured in the filtrate. SRP was measured using the ascorbic acid method (Anon. 1985). NH_4-N was measured by phenate colorimetry using a Technicon autoanalyzer (TRAACS 800). NO_3-N was analyzed by the copper-cadmium reduction followed by automated colorimetric analysis (Anon. 1983). DOC concentrations were measured by high temperature persulfate oxidation and detection of evolved CO_2 by infrared spectrophotometry (OI Corporation Model 700 Total Carbon Analyzer).

Statistical analysis

A robust test for resistance requires knowledge of the system's reference level at pre-disturbance steady state. This can be problematic in lotic ecosystems because they are notorious for their apparent lack of a steady state (Fisher 1983). Although greater control over environmental variation can be obtained with laboratory channels than in natural streams, they too can be subject to oscillations (Lamberti et al. 1989, A. D. Steinman, pers. obs.). In order to obtain as robust an index of steady state values as possible, pre-disturbance reference levels for each variable of interest were based both on measurements taken 2–3 d before the disturbance (time 2 = t_2) and another set of measurements taken prior to those (time 1 = t_1). Most of the t_1 samples were taken 6 wk before the plastic was placed on the streams, although some were taken only one week before the disturbance.

For all data except the algal community structure, a two-way repeated measures analysis of variance using the "mean" statement was conducted on all variables (SAS 1988). The "mean" statement allowed us to generate a contrast between post-disturbance observations and the mean of the two pre-disturbance observations. Prior to statistical analysis, tests for assumption of homogeneity of variance were conducted. If variances were heterogeneous, data were log-transformed (except for nutrient data, where no transformations were done). The 2-way ANOVA allowed us to determine if: (1) grazing or nutrient level had a significant effect on system resistance and (2) variables significantly differed from zero over time (i.e. did their values change significantly after light was eliminated). If grazing or nutrient had a significant effect, a multiple comparison test was used (Student-Newman-Keuls) to determine where differences among the streams resided. Untransformed data are presented in the text.

Algal community structure was evaluated for each stream by calculation of species diversity (Shannon and Weaver 1949) and number. Comparisons of taxonomic composition between assemblages were made with the SIMI index of similarity (Steinman and McIntire 1986). The measure ranges from 0 to 1, where a value of 0 indicates that a given pair of assemblages have no taxa in common, while a value of 1 indicates that the two assemblages have identical species compositions and proportional abundances. All analyses were based on numerical count data.

Results

Prior to light elimination, dissolved concentrations of SRP and NO_3-N were significantly higher in once-through (OT) than in recirculated (REC) channels for both high-grazed (HG) (SRP: $P < 0.01$; NO_3-N: $P < 0.005$) and low-grazed (LG) (SRP: $P < 0.01$; NO_3-N: $P < 0.001$) treatments (Tab. 1). No significant differences

Tab. 2. Indices of recycling in experimental streams prior to light elimination. Values are means (n = 2) ± 1 SD. Gross uptake rates were calculated from ^{33}P uptake studies; net uptake rates were calculated from upstream-downstream differences in SRP multipled by flow rate.

Treatment	Gross:net uptake ratio	ATP-specific phosphatase activity (μg NP μg^{-1} ATP h^{-1})
Once-through, high grazed	2.64 ± 1.97	0.052 ± 0.046
Recirculated, high grazed	6.86 ± 2.62	0.020 ± 0.012
Once-through, low-grazed	2.50 ± 0.64	0.168 ± 0.078
Recirculated, low-grazed	5.48 ± 1.50	0.374 ± 0.038

existed between OT and REC channels for DOC ($P > 0.05$), irrespective of grazing treatment (Tab. 1).

Differences in SRP concentrations due to recirculation translated into differences in recycling indices as well. Prior to light elimination, gross:net P uptake ratios were significantly greater in REC compared with OT streams ($P < 0.05$) in both grazing treatments, whereas ATP-specific phosphatase activities were significantly greater in REC vs OT streams in low grazed treatments only ($P < 0.05$; Tab. 2). Both these indices indicate that recycling of phosphorus was greater in REC than OT streams.

The net upstream-downstream differences in NO_3-N and SRP concentrations were significantly greater before light was eliminated ($P < 0.001$ and $P < 0.01$, respectively) compared with the difference measured on the day the black plastic was removed (Fig. 1). Hence, 92 d of darkness resulted in either decreased rates of nutrient uptake by biota or increased rates of nutrient regeneration in the channels. With respect to treatments, LG streams had marginally greater net differences than HG streams for both NO_3-N ($P < 0.06$) and SRP ($P < 0.08$), and OT streams had significantly greater net differences compared to REC streams for NO_3-N ($P < 0.002$) but not for SRP ($P > 0.66$) over the 92 d period. Net upstream-downstream differences in DOC were relatively constant over the experimental period and were not significantly affected by treatments (Fig. 1).

Chlorophyll a declined in all streams over time (Fig. 2), although percent declines were greater in REC (HG = 48.5%; LG = 59.8%) than OT (HG = 38.9%; LG = 37.3%) streams. Rate of chlorophyll decline was highest in the LG-REC streams (–0.43% d^{-1}), but this was not significantly different from rates measured in the other streams.

AFDM also exhibited significant declines in all streams ($P < 0.001$; Fig. 3). Declines were greater in LG than HG and REC compared with OT streams, although these differences were not significant ($0.05 < P < 0.10$). Bacterial numbers declined in all streams after 13 wk of darkness (Fig. 3), but these declines were not related to differences in nutrient levels ($P > 0.52$) or grazing regimes ($P > 0.83$). Protozoan communities were dominated by amoebae in all streams, although numbers were very low throughout the study. Protozoan numbers increased over the experimental period in

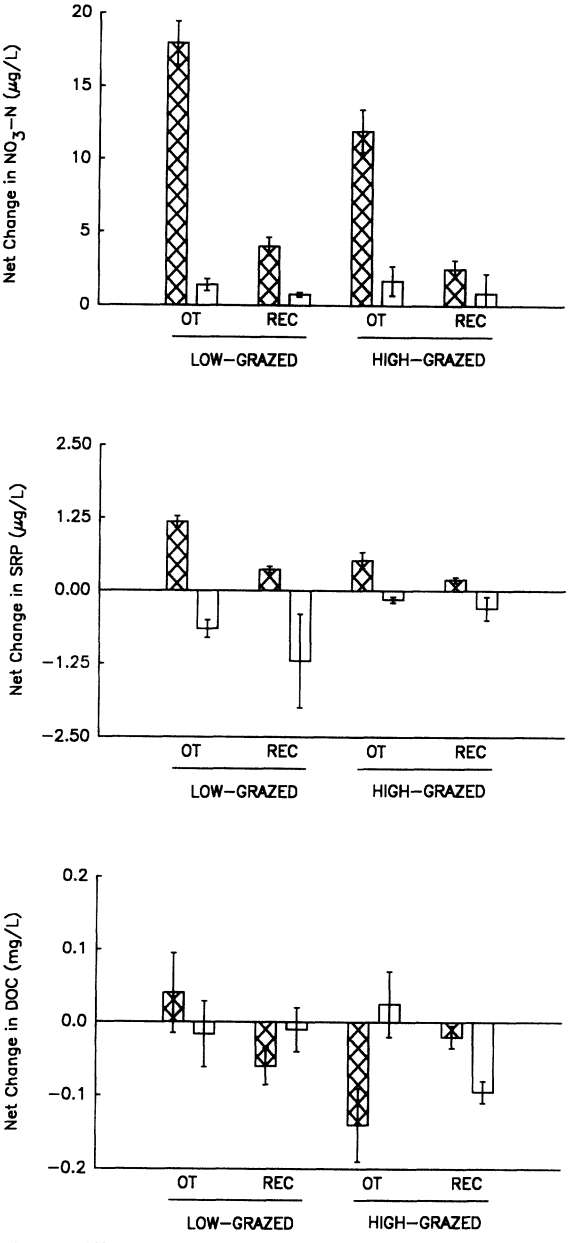

Fig. 1. Difference in concentration of nitrate, soluble reactive phosphorus, and dissolved organic carbon between upstream and downstreams sites for low grazed-once-through, low grazed-recirculated, high grazed-once-through, and high grazed-recirculated streams before black plastic was placed over the streams (hatched bars; n = 4) and after plastic was removed (open bars; n = 2). Positive values indicate net decline in concentration and negative values denote net increase (i.e. regeneration) of nutrient. Error bars represent ± 1 SE.

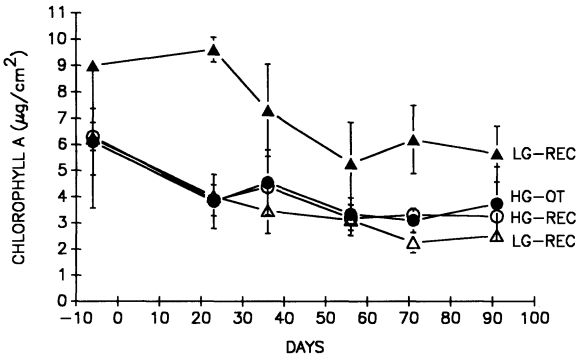

Fig. 2. Chlorophyll a densities in streams over time. Black plastic was placed over streams on day 0. LG = low grazed; HG = high grazed; OT = once-through flow; REC = recirculated flow. Error bars as in Fig. 1.

all streams except HG-OT, where no protozoa were observed either before or after the plastic was placed over the streams (Fig. 3). As with bacteria, numerical changes of protozoa were not significantly influenced by nutrient ($P > 0.52$) or grazing level ($P > 0.25$). ATP levels declined significantly after 13 wk of darkness ($P = 0.001$; Fig. 3); declines did not significantly differ between streams with different nutrient regime ($P > 0.41$) or grazing pressure ($P > 0.94$).

Rates of carbon fixation (areal and chlorophyll-specific) and thymidine incorporation all significantly declined over the experimental period ($P < 0.005$ in each case; Fig. 4). Although LG streams had slightly greater declines in areal carbon fixation than HG streams ($P < 0.06$), declines were not related to nutrient level ($P > 0.10$). Decreases in both chlorophyll-specific carbon fixation and thymidine incorporation did not significantly differ between nutrient level ($P > 0.79$ and $P > 0.24$, respectively) or grazing level ($P > 0.60$ and $P > 0.16$, respectively).

Fresh weight of *Elimia* did not significantly change during the period of darkness (Fig. 5), irrespective of nutrient level ($P > 0.40$).

The elimination of light reduced algal species diversity and the number of species in all streams (Tab. 3). These declines were particularly large in HG streams, where only basal cells of *Stigeoclonium* were observed after the plastic was removed. LG streams also exhibited declines in species diversity and number, but in both HG and LG streams the dominant species did not change as a result of light elimination. This is reflected in the SIMI values, which were all quite high, suggesting that taxonomic compositions changed very little as a result of light elimination. Because the SIMI index gives greater weight to dominant taxa, the high SIMI values indicate that the taxa that were dominant during the

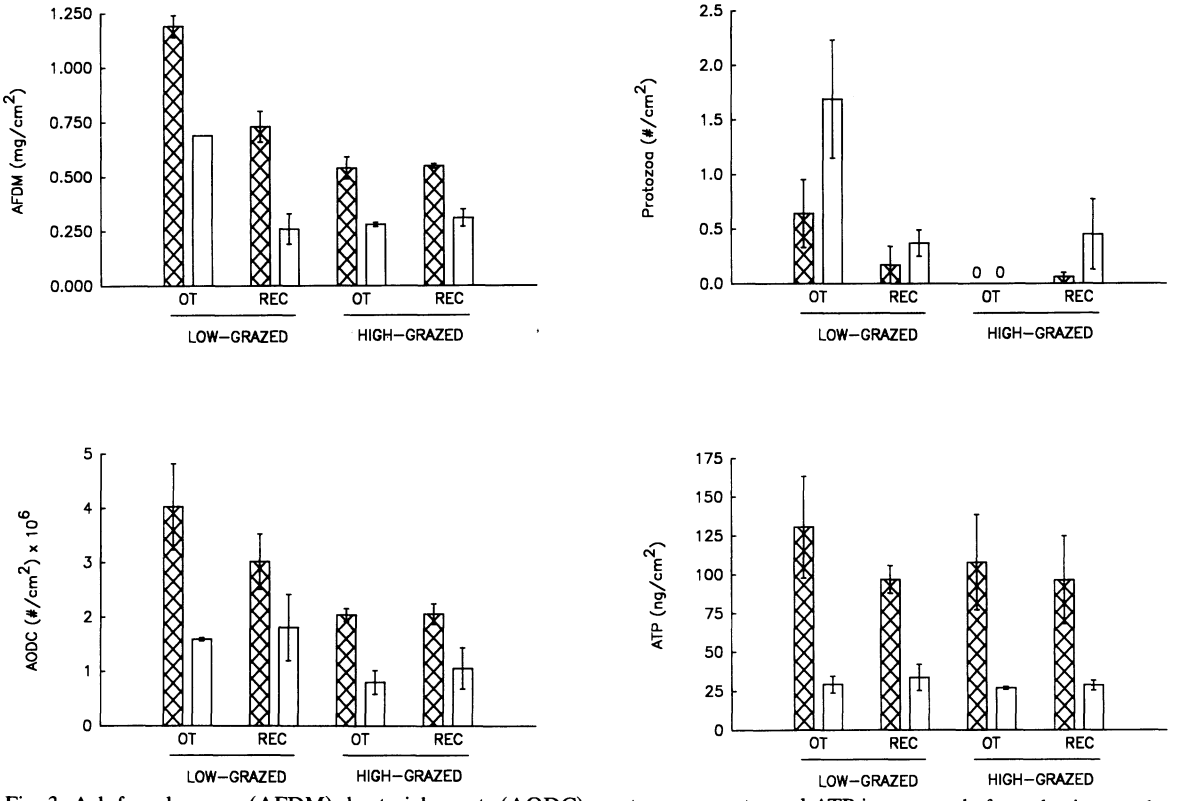

Fig. 3. Ash-free dry mass (AFDM), bacterial counts (AODC), protozoan counts, and ATP in streams before plastic was placed over streams (hatched bars; n = 4) and after plastic was removed (open bars; n = 2). Abbreviations and error bars as in Fig. 1.

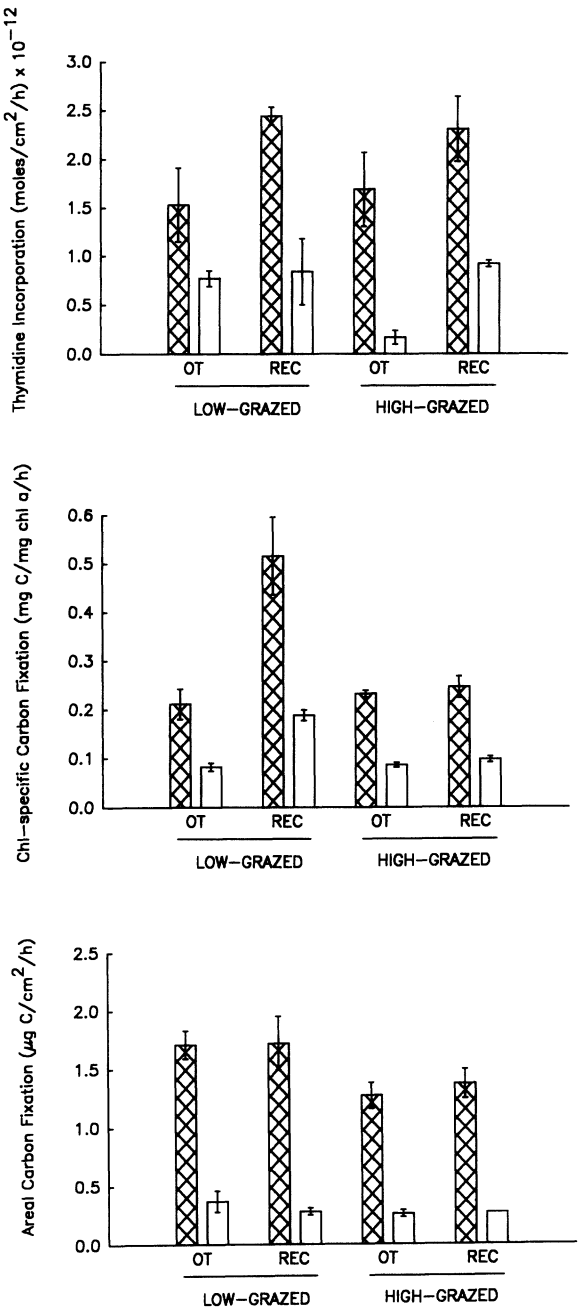

Fig. 4. Rates of thymidine incorporation, chlorophyll-specific carbon fixation, and areal carbon fixation in streams before plastic was placed over streams (hatched bars; n = 4) and after plastic was removed (open bars; n = 2). Abbreviations and error bars as in Fig. 1.

pre-disturbance period remained dominant after the disturbance. Consequently, the declines in species diversity and number were attributable to changes in less common species.

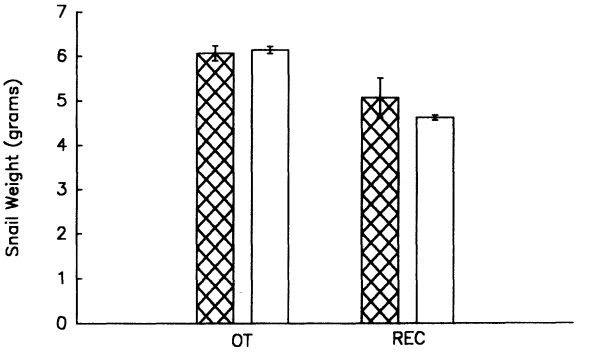

Fig. 5. Fresh weight of snails *(Elimia)* based on 8 samples of 25 snails from each stream before plastic was placed over streams (hatched bars) and after plastic was removed (open bars). Snails were blotted dry before weighing. Abbreviations and error bars as in Fig. 1.

Discussion

The elimination of light resulted in a significant decline in most variables. Declines in chlorophyll a and AFDM ranged from ca. 40% in OT streams to 60% in LG-REC streams. Fuller et al. (1986), however, reported that chlorophyll a declined > 99% in a darkened (black plastic) riffle of a second order stream compared with a (non-darkened) downstream riffle after 10 wk. In contrast, Bothwell and Jasper (1983) showed that chlorophyll a increased at a linear rate for 2–3 wk in complete darkness in experimental troughs, but attributed this accrual to passive settlement of colonists. In the present study, downstream settlement of algae from the unshaded one meter section at the upstream end of each stream may have replenished degrading chlorophyll a levels. However, given the low amounts of biomass in the seston (< 1 mg $\cdot$ l^{-1}) and the relatively small lighted area of stream to provide inoculum (especially com-

Tab. 3. Species diversity, richness (number), and similarity indices (SIMI) for algal communities sampled before (pre) and after (post) light was eliminated. SIMI values compare pre- and postlight elimination taxonomic structures within the same stream. Values range from 0 to 1, with 1 denoting the two communities have identical species compositions and proportional abundances and 0 indicating that a given pair of communities have no taxa in common.

Treatment	Rep	Diversity Pre	Diversity Post	Species # Pre	Species # Post	SIMI
Once-through, high grazed	a	0.185	0	7	1	> 0.999
	b	0.251	0	10	1	> 0.999
Recirculated, high grazed	a	0.613	0	10	1	0.992
	b	0.491	0	9	1	0.993
Once-through, low grazed	a	1.622	0.239	24	8	0.935
	b	1.874	0.296	28	8	0.945
Recirculated, low grazed	a	1.756	0.469	29	10	0.968
	b	1.296	0.690	20	9	0.983

Tab. 4. Chlorophyll a values on ceramic cylinders taken from two laboratory streams the day after black plastic was removed and placed under black plastic in a local, second order stream. Values are means ± 1 SD. Different superscripts within a treatment indicate significant differences at the 0.05 level (Scheffe's multiple comparison test).

	Chlorophyll a ($\mu g\ cm^{-2}$)		
	Initial (n = 8)	4 wk (n = 5)	8 wk (n = 5)
Once-through, low grazed	0.36^a ± 0.12	0.11^b ± 0.03	0.04^c ± 0.02
Recirculated, high grazed	0.25^a ± 0.08	0.26^a ± 0.09	0.08^b ± 0.03

pared with natural streams where the entire upstream catchment can act as a source of colonists), it is unlikely that passive settlement was responsible for the relatively high resistance of autotrophic biomass to light elimination.

An alternative explanation for resistance of autotrophic biomass was the absence of spates in our laboratory streams. Periphyton mats may have been susceptible to sloughing after a long period in the dark, but high flows were not present to remove the biomass. To test this possibility, ten cylinders were transported from a HG-REC and a LG-OT stream on the day after the black plastic was removed to a darkened (black plastic), second order riffle in a local stream (Walker Branch), where they were exposed to variable flow and potential scour events. Chlorophyll a values were measured after 4 and 8 wk. After 4 wk, chlorophyll a had declined 69% and not at all on cylinders removed from the LG-OT and HG-REC streams, respectively (Tab. 4). Although declines were greater after 8 wk of darkness in the natural stream (21 wk altogether), they still did not approach the dramatic changes reported by Fuller et al. (1986). These results suggest that constancy of flow and lack of abrading material in the laboratory streams may have helped retain biomass in the system relative to natural streams, but resistance still was relatively high irrespective of where substrates were located.

Of additional interest, however, was the fact that after 4 wk of darkness in the natural stream, no significant declines in chlorophyll a were detected from cylinders removed from the HG-REC stream (Tab. 4), although significant decreases were noted from cylinders taken from the LG-OT stream. AFDM values from the laboratory streams indicated that high grazed and once-through streams were more resistant to light elimination than those that were low grazed or exposed to recirculated flow. High grazing pressure should lead to greater resistance of periphyton to scour events because the resultant community is often dominated by a thin layer of cells with prostrate growth forms (Steinman et al. 1987, Lowe and Hunter 1988). This community will be better protected by a benthic boundary layer, and hence be exposed to less shear stress. Indeed, Power and Stewart (1987) reported that short turfs of *Spirogyra* were more resistant to flood events than longer, mature filaments of *Spirogyra*. Mechanistically, it is unclear why heavily grazed periphyton communities should also be more resistant to light elimination. Perhaps high grazing pressure results in a community of generally stress-tolerant organisms (sensu Grime 1979).

AFDM and chlorophyll a results from the laboratory streams suggested that recirculated streams (particularly LG) were somewhat less resistant than OT streams. Since net upstream-downstream differences of SRP and NO_3-N decreased over time, suggesting less demand for inorganic nutrients, it seems surprising that resistance may have been linked to relative nutrient levels. However, DeAngelis et al. (in press) also found that periphyton communities in recirculated laboratory streams were less resistant (to a scour disturbance) than those in once-through streams, suggesting that nutrient level may have an important influence on system resistance. This is particularly true when the disturbance is relatively long lasting, such as in the present case, for the organisms may have time to respond to the disturbance. Periphytic organisms stressed by limiting nutrients may result in relatively weak attachment to the substrate or may affect cellular control of buoyancy (Bothwell et al. 1989), and contribute to greater levels of sloughing or emigration.

Whereas nutrient levels apparently had relatively little influence on system resistance, we suspect that they may have important effects on system resilience. This is because in systems where production is limited by nutrient availability, recovery rate following disturbance is related to input rates and recycling of nutrients within the system (Grimm and Fisher 1986, DeAngelis et al. in press). As a consequence, disturbance type and magnitude may be critical factors dictating resilience; disturbances that remove living biomass or detritus from the stream channel (e.g. high discharge events) may further retard recovery rate because of the time it would take for sufficient organic matter to accumulate to facilitate recycling (O'Neill 1976, Mulholland et al. submitted).

Bacterial numbers, ATP levels, and rates of carbon fixation and thymidine incorporation all declined significantly in the absence of light. Bacterial numbers and activity decreased presumably because the rate, absolute amount, or quality of DOC excreted from autotrophs declined after light was eliminated and photosynthesis ceased (Brock and Clyne 1984). Increases in bacterial numbers and metabolism might be expected if DOC was released from degrading autotrophic cells in the dark (Cole et al. 1982). However, based on chlorophyll data, there was no obvious crash of autotrophic biomass. In addition, DOC concentrations did not increase in the streams (Fig. 1), although rapid uptake of DOC (Dahm 1981) by either heterotrophs or abiotic means could have masked pulses of DOC release by autotrophs. Because bacterial numbers declined over

the experiment, it is likely that whatever DOC was leaked through cell degradation after light was eliminated was insufficient to make up for the lost DOC that had been excreted from actively photosynthesizing cells when light was still present.

Declines in areal carbon fixation after 13 wk of darkness were attributable partly, but not exclusively, to declines in autotrophic biomass. Areal carbon fixation declined between 78.4% (LG, OT) and 83.7% (LG, REC), whereas chlorophyll a values only declined between 37.3% (LG, OT) and 59.8% (LG, REC). Because rates of chlorophyll-specific carbon fixation also declined, physiological stress associated with the extended period of darkness may have contributed to the lower rates of carbon fixation. Handa (1969) suggested that observed drops in chlorophyll a, carbohydrate, protein, and lipid in a marine diatom after it was placed in the dark explained its decreased photosynthetic activity after it was reilluminated. No data were obtained on the chemical composition of the periphyton assemblages in the present study, so we cannot evaluate the importance of these changes to photosynthesis (cf. Steinman et al. 1988), nor did we evaluate the possibility of heterotrophy by algae in these communities. Dehning and Tilzer (1989) reported that reduced metabolic rates, and not heterotrophy, was the main factor that allowed *Scenedesmus* cells to survive 3 months of darkness. Other factors that may influence the dark survival of autotrophic algae include species composition and temperature (Smayda and Mitchell-Innes 1974).

In planktonic systems, protozoa play a critical role in nutrient cycling by remineralizing nutrients during consumption of bacteria (Azam et al. 1983 Goldman et al. 1987, Bloem et al. 1989). Very little information is available on the role of benthic protozoa in lotic ecosystems, however. Bott and Kaplan (1989) reported that microflagellates appeared to be more important consumers of bacteria than ciliates, but did not address their respective roles in nutrient regeneration. In the present study, protozoa were observed (particularly amoebae) with diatoms in their guts, suggesting that protozoans can remineralize nutrients from consumption of either bacteria or algae. It is unlikely that rates of remineralization by protozoans were large enough to account for the net decrease in upstream-downstream differences in SRP and NO_3-N concentrations over time in the present study because protozoan densities were approximately 3 to 4 orders of magnitude lower than those reported in Baldock et al. (1983) and Bott and Kaplan (1989). Thus, the net decline between the upstream and downstream ends of the channels was probably due to decreased autotrophic demand in the dark.

In studies where black plastic has been placed over natural streams, the distribution of aquatic invertebrates that feed on algae has shifted away from beneath the shaded area to lighted reaches (Towns 1981, Fuller et al. 1986). In the present study, snails could have moved to the lighted upstream section of each stream, but daily (non-quantitative) observations suggested that this was not the case. Rather, occasional observations beneath the black plastic revealed the snails moved very little, with the snails either completely withdrawn into their shells or passively attached to cylinders with their mouthparts retracted. Lamberti et al. (1989) reported similar behavior by the snail *Juga,* but in that study snails were inactive because of satiation. Thus, the absence of a significant decline in *Elimia* fresh weight may have been due in part to a behavioral response to darkness; decreased vagility could result in less metabolic costs. If the snails ingested just enough food to maintain their basal rates of metabolism, snail biomass would remain relatively constant. Reduced grazing in the dark also explains why algal biomass did not decline faster in streams with *Elimia* compared with those where *Elimia* was absent.

Although algal species diversity and number declined in all streams after light elimination, the declines were greater in HG than LG streams, where species diversity and numbers were lower to begin with. These data are in agreement with the notion that species diversity is positively associated with system stability (Margalef 1963). The high SIMI values between pre- and post-light elimination communities were somewhat surprising given the large declines in diversity and number. However, SIMI gives greatest weight to the dominant taxa (McIntire and Moore 1977), indicating that the dominant species did not change after light elimination, and those that were lost must have been very rare. Thus, it is not surprising that even though species number declined dramatically in HG streams, AFDM was much less affected, because the algal species that were lost comprised a small part of the total periphyton community.

Relatively high resistance of one property to light elimination did not necessarily indicate that other properties would have high resistance. For example, algal taxonomic structure was quite resistant, but biomass was less resistant, in contrasts between LG and HG streams. This finding suggests that high resistance of community structure does not necessarily translate into high resistance of other system attributes (see also McNaughton 1977, Pimm 1982). Indeed, Boesch and Rosenberg (1981) point out that although individuals or populations may be effective at resisting stress, the high cost of adaptability by complex communities presumably precludes resistance at the community levels in benthic marine systems. In addition, Van Voris et al. (1980) noted that static indices of system complexity, such as diversity or richness, may be inadequate descriptors because "stability is a property of the dynamic response of a system to perturbation". They found that ecosystems with high functional complexity also had higher stability.

In conclusion, neither grazing nor nutrient level had an overwhelming influence on ecosystem resistance to a light elimination event. Most variables measured in-

dicated that the grazing and nutrient treatments imposed before the light-elimination event had resulted in significantly distinct stream systems. This suggests that the general failure of treatments to significantly affect resistance was not because initial differences among streams were too small. The results suggested instead that high grazing pressure and a combination of low grazing and high nutrient regime may confer resistance to a stream in terms of periphyton biomass, but this resistance does not necessarily extend to other structural and functional properties. Thus, the type and duration of the disturbance may have more importance to system behavior than relatively fundamental properties such as nutrient level or plant-animal interactions. The implication for natural systems is that ecosystem resistance may be more dependent on the qualities of the disturbance than upon the attributes of the system. If this is true, generalizations about how ecosystems resist disturbance will largely be disturbance-dependent. Future research needs to examine the effects of other disturbances on system resistance, with particular attention being paid to disturbance magnitude, duration, and frequency.

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References

Anon. 1983. U.S. Environmental Protection Agency (USEPA). Methods for chemical analysis of water and wastes, EPA-600/4–79–020 (350.1–1 to 350.1–6). – Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Anon. 1985. American Public Health Association. Standard methods for the examination of water and waste water. 12th edition. – American Public Health Association, Washington, D.C.

Azam, F., Fenchel, T., Fields, J. G., Gray, J. S., Meyer-Reil, L.-A., and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. – Mar. Ecol. Prog. Ser. 10: 257–263.

Baldock, B. M., Baker, J. H. and Sleigh, M. R 1983. Abundance and productivity of protozoa in chalk streams. – Holarct. Ecol. 6: 238–246.

Bloem, J., Albert, C., Bar-Gilissen, M. B., Berman, T. and Cappenberg, T. E. 1989. Nutrient cycling through phytoplankton, bacteria, and protozoa, in selectively filtered Lake Vechten water. – J. Plankton Res. 11: 119–131.

Boesch, D. F. and Rosenberg, R. 1981. Response to stress in marine benthic communities. – In: Barrett, G. W. and Rosenberg, R. (eds), Stress effects on natural ecosystems. Wiley, New York, pp. 179–200.

Bothwell, M. L. and Jasper, S. 1983. A light and dark trough methodology for measuring rates of lotic periphyton settlement and net growth. – In: Wetzel, R. G. (ed.), Periphyton of freshwater ecosystems. Junk Publishers, The Hague, pp. 253–265.

– , Suzuki, K. E., Bolin, M. K. and Hardy, F. J. 1989. Evidence of dark avoidance by phototrophic periphytic diatoms in lotic systems. – J. Phycol. 25: 85–94.

Bott, T. L. and Kaplan, L. R. 1989. Densities of benthic protozoa and nematodes in a Piedmont stream. – J. N. Am. Benthol. Soc. 8: 187–196.

Brock, T. D. and Clyne, J. 1984. Significance of algal excretory products for growth of epilimnetic bacteria. – Appl. Environ. Microbiol. 47: 731–734.

Burris, J. A., Bamford, M. S., and Stewart, A. J. 1990. Behavioral responses of marked snails as indicators of water quality. – Environ. Toxicol. Chem. 9: 69–76.

Cairns, J., Jr. and Dickson, K. L. 1977. Recovery of streams from spills of hazardous materials. – In: Cairns, J. Jr., Dickson, K. L. and Hendricks, E. E. (eds), Recovery and restoration of damaged ecosystems. Univ. Virginia Press, Blacksburg, Virginia, pp. 24–42.

Cole, J. J., Likens, G. E., and Strayer, D. L. 1982. Photosynthetically produced dissolved organic carbon: An important carbon source for planktonic bacteria. – Limnol. Oceanogr. 27: 1080–1090.

Dahm, C. N. 1981. Pathways and mechanisms for removal of dissolved organic carbon from leaf leachate in streams. – Can. J. Fish. Aquat. Sci. 38: 68–76.

DeAngelis, D. L., Mulholland, P. J., Palumbo, A. V., Steinman, A. D., Huston, M. A. and Elwood, J. W. 1989a. Nutrient dynamics and food web stability. – Ann. Rev. Ecol. Syst. 20: 71–95.

– , Bartell, S. M. and Brenkert, A. L. 1989b. Effects of nutrient recycling and food- chain length on resilience. – Am. Nat. 134: 778–805.

– , Mulholland, P. J., Elwood, J. W. Palumbo, A. V. and Steinman, A. D. Biogeochemical cycling constraints on stream ecosystem recovery. – Environ. Manage. *in press.*

Dehning, I. and Tilzer, M. M. 1989. Survival of *Scenedesmus acuminatus* (Chlorophyceae) in darkness. – J. Phycol. 25: 509–515.

Findlay, S. E. G., Meyer, J. L. and Edwards, R. T. 1984. Measuring bacterial production via rate of incorporation of [^{3}H] thymidine into DNA. – J. Microbiol. Methods 2: 57–72.

Fisher, S. G. 1983. Succession in streams. – In: Barnes, J. R. and Minshall, G. W. (eds), Stream ecology: application and testing of general ecological theory. Plenum, New York, pp. 7–27.

Fuller, R. L., Roelofs, J. L., and Fry, T. J. 1986. The importance of algae to stream invertebrates. – J. N. Am. Benthol. Soc. 5: 290–296.

Goldman, J. G., Caron, D. A. and Dennett, M. R. 1987. Nutrient cycling in a microflagellate food chain: IV. Phytoplankton-microflagellate interactions. – Mar. Ecol. Prog. Ser. 38: 75–87.

Grime, J. P. 1979. Plant strategies and vegetation processes. – Wiley, New York.

Grimm, N. B. and Fisher, S. G. 1986. Nitrogen limitation in a Sonoran Desert stream. – J. N. Am. Benthol. Soc. 5: 2–15.

Handa, N. 1969. Carbohydrate metabolism in the marine diatom *Skeletonema costatum.* – Mar. Biol. 4: 208–214.

Harrison, G. W. and Fekete, S. 1980. Resistance of nutrient cycling systems to perturbations of the flow rates. – Ecol. Modelling 10: 227–241.

Hobbie, J. E., Daley, R. J. and Jasper, S. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. – Appl. Environ. Microbiol. 33: 1225–1228.

Lamberti, G. A., Ashkenas, L. R., Gregory, S. V., Steinman, A. D. and McIntire, C. D. 1989. Productive capacity of periphyton as a determinant of plant-herbivore interactions in streams. – Ecology 70: 1840–1856.

Lee, J. J., Hutner, S. H., and Bovee, E. C. (eds) 1985. Illustrated guide to the Protozoa. – Allen Press, Lawrence, KS.

Lowe, R. L. and Hunter, R. D. 1988. Effects of grazing by *Physa integra* on periphyton community structure. – J. N. Am. Benthol. Soc. 7: 29–36.

Margalef, R. 1963. On certain unifying principles in ecology. – Am. Nat. 97: 357–374.

McIntire, C. D. and Moore, W. W. 1977. Marine littoral diatoms: ecological considerations. – In: Werner, D. (ed.), The biology of diatoms. Univ. of California Press, Berkeley, pp. 333–371.

McNaughton, S. J. 1977. Diversity and stability of ecological communities: a comment on the role of empiricism in ecology. – Am. Nat. 111: 515–525.

Mulholland, P. J., Steinman, A. D., Palumbo, A. V., Elwood, J. W., Kirschtel, D. and DeAngelis, D. L. Ecosystem response to nutrient constraint in low-grazed and high-grazed streams. – Ecology *submitted*.

O'Neill, R. V. 1976. Ecosystem persistence and heterotrophic regulation. – Ecology 57: 1244–1253.

– , Harris, W. F., Ausmus, B. S. and Reichle., D. E. 1975. A theoretical basis for ecosystem analysis with particular reference to element cycling. – In: Howell, F. G., Gentry, J. B. and Smith, M. H. (eds), Mineral cycling in southeastern ecosystems. U.S. Energy Research and Development Administration. Washington, D.C., pp. 28–40.

Palumbo, A. V., Mulholland, P. J. and Elwood, J. W. 1987. Extraction with DMSO to simultaneously measure periphyton photosynthesis, chlorophyll, and ATP. – Limnol. Oceanogr. 32: 464–471.

– , Mulholland, P. J. and Elwood, J. W. 1989. Epilithic microbial populations and leaf decomposition in acid-stressed streams. – In: Rao, S. S. (ed.), Microbial interactions in acid stressed aquatic ecosystems. CRC Press, Boca Raton, FL, pp. 69–90.

Pimm, S. L. 1982. Food webs. – Chapman and Hall, London.

Power, M. E. and Stewart, A. J. 1987. Disturbance and recovery of an algal assemblage following flooding in an Oklahoma stream. – Am. Midl. Nat. 117: 333–345.

SAS. 1988. SAS/STAT user's guide. Release 6.03 edition. – SAS Institute, Cary, NC.

Shannon, C. E. and Weaver, W. 1949. The mathematical theory of communication. – Univ. Illinois Press, Urbana, IL.

Shoaf, W. T. and Lium, B. W. 1976. Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. – Limnol. Oceanogr. 21: 926–928.

Smayda T. J. and Mitchell-Innes B. 1974. Dark survival of autotrophic planktonic marine diatoms. – Mar. Biol. 25: 195–202.

Steinman, A. D. and McIntire, C. D. 1986. Effects of current velocity and light energy on the structure of periphyton assemblages in laboratory streams. – J. Phycol. 22: 352–361.

– , McIntire, C. D., Gregory, S. V., Lamberti, G. A. and Ashkenas, L. 1987. Effect of herbivore type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. – J. N. Am. Benthol. Soc. 6: 175–188.

– , McIntire, C. D. and Lowry, R. R. 1988. Effects of irradiance and age on chemical constituents of algal assemblages in laboratory streams. – Arch. Hydrobiol. 114: 45–61.

Towns, D. R. 1981. Effects of artificial shading on periphyton and invertebrates in a New Zealand stream. – N. Z. J. Mar. Freshw. Res. 15: 185–192.

Van Voris, P., O'Neill, R. V., Emanuel, W. R. and Shugart, H. H. Jr. 1980. Functional complexity and ecosystem stability. – Ecology 61: 1352–1360.

Vitousek, P. M., Reiners, W. A., Melillo, J. M., Grier, C. C. and Gosz, J. R. 1981. Nitrogen cycling and loss following forest perturbation: the components of response. – In: Barrett, G. W. and Rosenberg, R. (eds), Stress effects on natural ecosystems. Wiley, New York, pp. 115–128.

Webster, J. R., Waide, J. B., and Patten, B. C. 1975. Nutrient recycling and stability of ecosystems. – In: Howell, F. G., Gentry, J. B. and Smith, M. H. (eds), Mineral cycling in southeastern ecosystems. – U.S. Energy Research and Development Administration, Washington, D.C., pp. 1–27.

– , Gurtz, M. E., Hains, J. J., Meyer, J. L., Swank, W. T. and Wallace, J. B. 1983. Stability of stream ecosystems. – In: Barnes, J. R. and Minshall, G. W. (eds), Stream ecology: application and testing of general ecological theory. – Plenum New York, pp. 355–395.