

The Effects Of Nutrients, Light and Insect Herbivory on Algal Biomass

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Abstract Algae are an important source of primary productivity in stream ecosystems, but the effect of nutrients on their growth, in relation to light limitation and herbivory is not well known in lotic systems. To better understand these two effects and the links between them, I examined the response of algal growth to light availability, nutrient availability and herbivory, by the use of nutrient diffusing substrates (nitrogen, phosphorus, and nitrogen + phosphorus) within the South Fork Eel River, Mendocino Co., CA. The study was conducted within nine streams with varying degrees of canopy cover. Of these nine streams, four were chosen to examine the effect of herbivorous insects on increased algal growth. Nutrient diffusing substrates were used to assess the effects of nutrients on algal biomass. To see if herbivores respond to the expected increase in algal biomass by increased consumption and presence, treatments allowing for herbivore inclusion were added. Chlorophyll- α (productivity) and ash-free dry mass (AFDM, biomass) data were analyzed via multiple regression. I concluded that nutrients, when alone and not in conjunction with one another, showed to have either an increased (N) or decreased (P) effect on algal productivity and biomass. In a Two-Way ANOVA, herbivores were not shown to reduce organic biomass in streams, nor did the data show them to have a significant preference towards nitrogen treatments. This could mean that either algal abundance does not play a role in grazer nutritional preferences, or that grazing of algae on nutrient treated substrates allowed other algae, not preferred by grazers, to colonize nutrient treated substrates.

Introduction

There are many factors that affect trophic systems in streams and much has been studied regarding the variability of community structures, in combination with differing environmental aspects. Because algae are important in both primary productivity and nutrient cycling within aquatic ecosystems, it is important to understand how the effects of nutrient limitation, light and herbivory on algal growth and how these factors vary within streams. Although much has been studied regarding algal reactions to environmental manipulation in marine and lentic (lakes, ponds) ecosystems, less attention has been paid to effects of nutrients, light and herbivory in lotic ecosystems (streams).

Nutrient and light availability in stream systems has an affect on various stream populations and processes such as algal growth, invertebrates and leaf litter decomposition (Elwood *et. al.* 1981, Peterson *et. al.* 1985, Hill and Knight 1988). It has been suggested that ratios of light to nutrient availability, as well as, the total availability of nutrients within the water column may have an effect on decomposition and invertebrate growth (Elser *et. al.* 2000, Sterner and Elser 1997). Phosphorus, nitrogen, and a phosphorus/nitrogen combination have been shown to limit algal growth in streams (Elwood *et. al.* 1981, Fairchild *et. al.* 1985, Grimm and Fisher 1986, Tate 1990).

Previous studies have shown that as light availability increases, so does algal biomass (Rier and Stevenson, 2002, Hill and Knight 1988). Presumably, as stream size increases, canopy cover will decrease, and hence microbial biomass will increase (Hamilton *et al* 2001). In addition, *in situ* shading experiments, affecting light availability, have shown to decrease chlorophyll- α concentrations of algae (Hill and Knight 1988, Findlay and Howe 1993). It has also been shown that as stream size increase to $\geq 10 \text{ km}^2$, algal production becomes increasingly important to all consumer groups excluding shredders. In the case of streams smaller than $\leq 10 \text{ km}^2$, consumer groups follow the River Continuum Concept of community consumption patterns (Vannote *et. al.* 1980, Finlay 2001). It is suggested that this is due to a decrease in probably due to a decrease in terrestrial allochthonous inputs in relation to stream width and increasing algal production due to light availability (Vannote *et. al.* 1980).

The importance of algae for herbivorous invertebrates has been documented (Hill *et. al.* 1995, Hill and Knight 1998, Lawrence and Gresens 2004), and various studies have examined

the effects of nutrients in combination with herbivory (Hill and Knight 1988, Finlay 2001, Rier and Stevenson 2002 Urabe *et. al.* 2002). This study examines how nutrient limitations and light availability affect algal growth in streams and how this growth is affected by herbivores.

This investigation is, in essence, an extension of previous research performed in the UC Angelo Reserve by Walter Hill and Allen Knight in 1988. The Hill & Knight studies were conducted in two second-order streams and consisted of two parts. The first and most relevant to my study, found that algal biomass and herbivorous insects were greater on substrates with increased nutrient availability in the unshaded stream. Because increased algal biomass was found to be significantly greater in the shaded stream, when light could penetrate the canopy, it is suggested that light is a primary limiting factor in algal growth. The second study found that caddisfly larvae (*Neophylax*) biomass was inversely proportional to the reduction of algal biomass and mayfly nymphs (*Ameletus*) were shown to be selective grazers.

In order to more fully understand how nutrient and light limitation affects algal growth within the stream drainage area of the South Fork Eel River, I investigated how environmental differences and nutrient manipulations affect algal biomass within 9 streams and whether the number of herbivores on nutrient rich substrates is significantly different from control substrates. The experiment concerning herbivores was conducted within 4 of the proposed 9 streams. This makes the study an expanded, *in situ* analysis of the Hill and Knight studies within the South Fork Eel watershed area, allowing characterization of the environmental inputs that affect primary and secondary production.

The hypotheses are that there will be an overall increase in algal growth on pots that are equipped with augmented nutrient and increased light availability, a greater presence of herbivores on pots that allow herbivore access, as well as, a decrease of algal biomass on herbivore inclusion pots. Results complementing these hypotheses will suggest that light and nutrient availability are significant limiting factors in algal biomass. In addition, complementary results will show that herbivores are attracted to resources of greater availability and higher nutrient content. If these results are not seen, such an outcome suggests that nutrients, light, and herbivory are affected by other factors not included within this study.

Methods

The study site is comprised of nine, relatively pristine second order streams along the South Fork Eel River within the UC Angelo Reserve in Mendocino County, California, USA. The

reserve is part of the Nature Conservancy's Northern California Coast Range Preserve (39°45' N, 123°40' W) and has been a reserve since clear-cutting occurred within the area in the 1950's and since has been subject to little human disturbance. For this reason the reserve is a good place to study algal nutrient reactions within streams, there are no point sources for additional nutrient inputs that may offset the study's results.

In each stream, the following combination of four treatment pots was multiplied by five to create a five block set for all nine study sites: nitrogen (N), phosphorous (P), nitrogen + phosphorous (N/P), control (C) (Fig.1a). In four of the nine sites, two additional treatments of the nitrogen (NH) and control (CH) pots were added to each block for the herbivore effect portion of the experiment (Fig.1b).

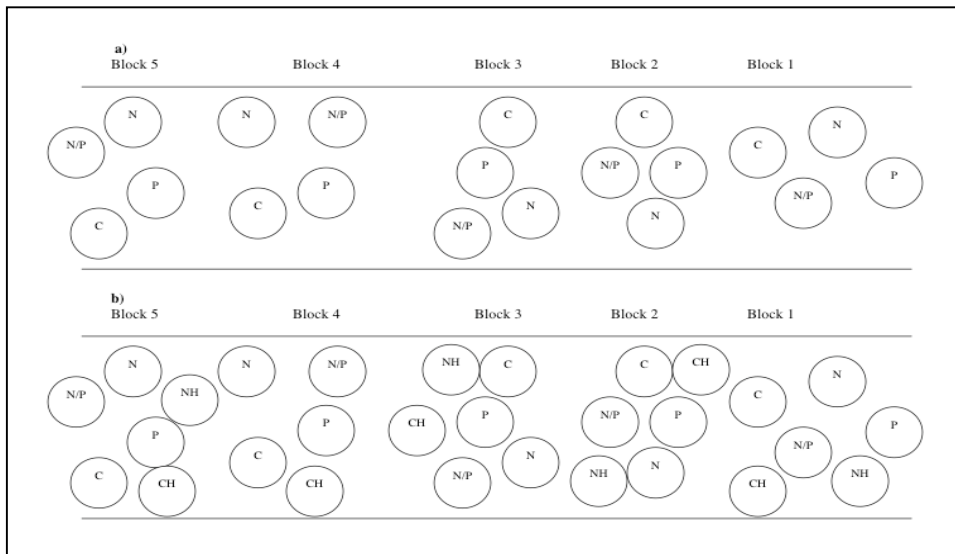


Figure 1. a) Experimental set-up in all nine of the study sites. b) Experimental set-up in four of the nine study sites.

Streams chosen for additional treatment were perceived to have an abundance of herbivores. The placement of the blocks went from downstream to upstream. Within blocks, treatments were placed in random order to avoid positional differences in data.

The treatments were prepared by filling clay flowerpots with a 2% agar solution alone, with 0.5M nitrogen (NaNO_3), 0.1M phosphorous (KH_2PO_4), or a combination of 0.5M nitrogen and 0.1M phosphorous. Pots were first lined with plastic wrap prior to pouring the agar solution to avoid clogging the porous surface. Once the agar had congealed, the plastic wrap was removed to seal in the agar plug with a Petri dish and a silicone adhesive. These were then placed Petri

dish side down on top of a deli container that rose either two inches or four inches high, depending on the depth of the stream they were placed (Fig.2).

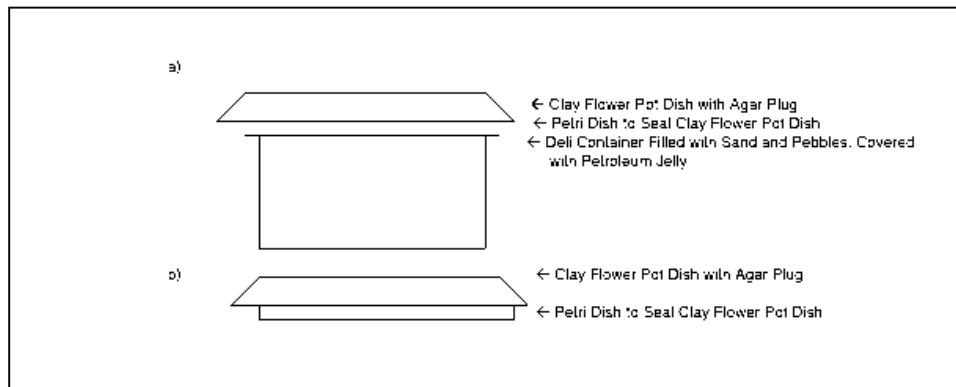


Figure 2. a) Nutrient diffusing pot set-up for herbivore exclusion treatments. b) Nutrient diffusing pot set-up for herbivore inclusion treatments (treatments: NH and CH)

The containers were then covered in petroleum jelly to prevent herbivores from climbing up the sides. We did nothing to prevent drifting herbivores from landing on the pots because the amount of work did not seem feasible in the short amount of time and number of replications. The two additional treatment pots were prepared in the same manner; however, they were not set atop deli containers and were made as accessible as possible to crawling herbivores. In total the streams Elk, Jack of Hearts, McKinley, Misery and Barnwell received five treatment blocks with four treatments in each block, totaling 100 treatment pots (Fig.3). Fox Creek, Jane's Riffle, Upper and Lower Elder received five treatment blocks with six pot treatments in each block, totaling 120 treatment pots. Pots were set out on either July 15th & 16th and left for four weeks until August 12th or 13th.

Because we want to be able to explain as much of the variability in algal growth as possible, the following measurements were either collected on July 29th and 30th or gathered from concurrent studies occurring at the same sites: Water depth (cm), flow (cm/s), percent canopy cover, temperature and watershed size (Table 1).

Algal Sampling and Analysis After twenty-eight days were completed, pots were scrubbed with a small nylon brush and any epilithon (measurement of 1° productivity) was collected into a small Tupperware container. All samples were then placed into 50 ml tubes, one for each pot, and brought to 50ml with stream water. These algal slurries were processed between the timeframe of 6 to 24 hours on ashed (500°C x 1 hour) Whatman GF/F (glass fiber) filters.

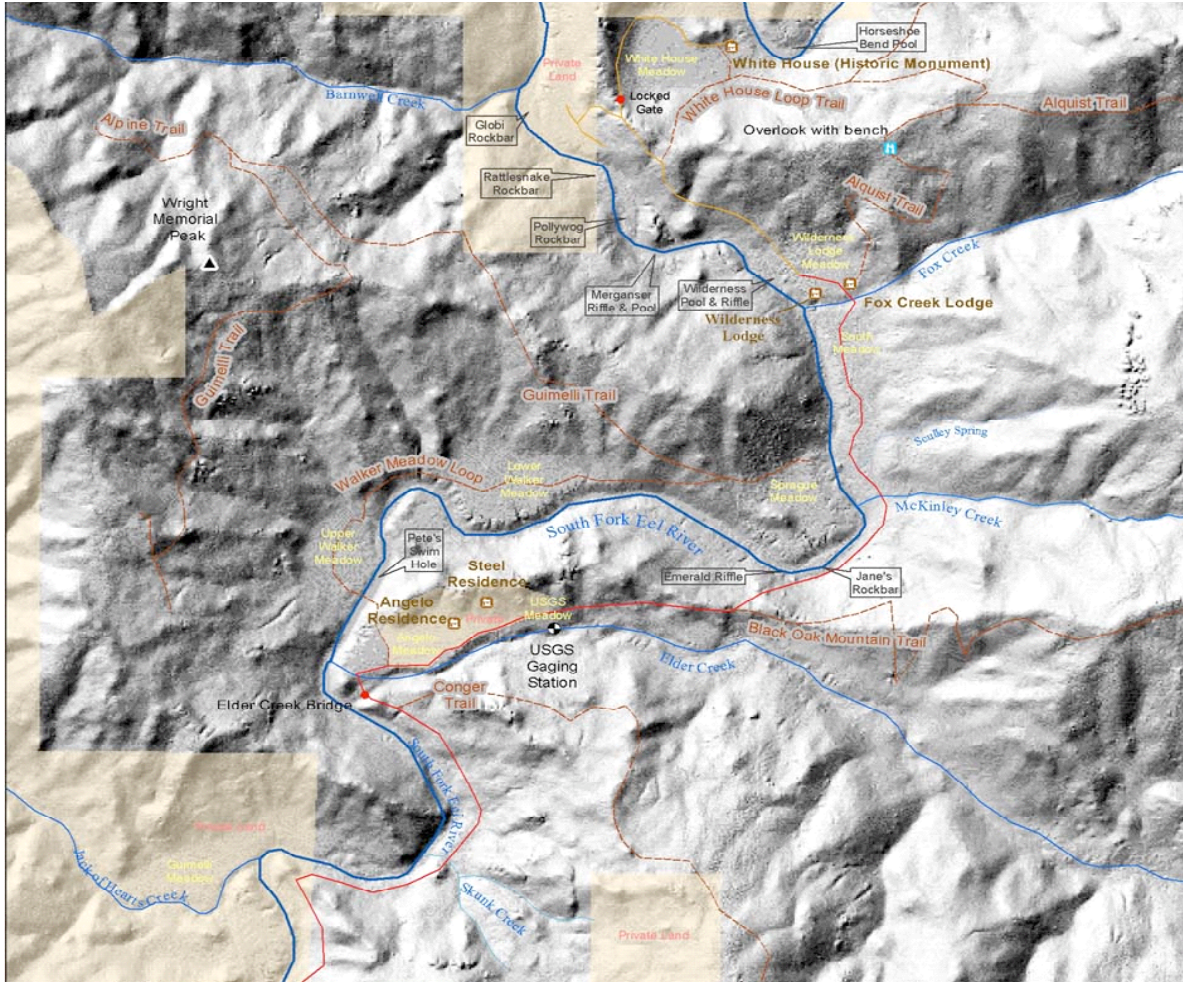


Figure 2: Relief map of the UC Angelo Reserve study site showing stream locations. Misery and Elk not pictured.

Table 1: Additional Measured and Collected Variables

Variable	Method	Reason
Flow (cm/s)	PDFM-IV Portable Doppler Flow Meter (Massena, NY)	water flow shown to be positively correlated w/ nutrient uptake (Whitford and Shumacher, 1964)
Depth (cm)	measurements taken at top of each pot	light and temperature can vary at different depths
Percent Canopy Cover (%)	convex densiometer	measure light availability to stream
Watershed Size (km ²)	gathered from concurrent research being done in the reserve	gathered in direct support of first hypothesis that algal growth increases with stream size

For each sample we placed filtered 1ml of slurry for later AFDM (ash free dry mass) measurements and 1ml of slurry for chlorophyll- α measurements on Whalman GF/Fs. Filtered material for chlorophyll- α samples were placed directly in a freezer for preservation. In the lab,

samples for AFDM were placed in a drying oven at 60° for at least 24 hours. After drying, filters were cooled in a desiccator, weighed and placed in the muffle furnace at 500°C for 1 hour. After ashing was completed, samples were misted with distilled water to replace water lost by minerals and placed in the drying oven for at least 24 hours before being placed in the desiccator and weighed again.

Chlorophyll- α filters were placed in a 10ml tube in 90 percent ethanol for 24 hours to extract chlorophyll. After this period, the extracted liquid would be placed in a TD-700 Laboratory Fluorometer for $\mu\text{g/ml}$ chlorophyll- α readings.

Grazing Insects Grazing insects were counted and identified to family as accurately as possible at the time samples were collected.

Analysis Prior to running multiple regression for AFDM and Chlorophyll- α I log-transformed the data to create a more even distribution for residuals using JMPin software version 4.0.4 (SAS Institute Inc.; Cary, NC 2001). To examine differences between various independent and dependent variables I used ANOVA and I examined whether herbivore treatments had an effect by using Two-Way ANOVA.

Results

AFDM The final multiple regression model for AFDM includes site, site*treatment, site*flow, treatment (N, P, N/P), block, flow (cm/sec) and total number of insects, this yields an R^2 value of 0.94. Independent variables site and canopy do not contribute significantly to the model ($p > 0.05$), however, interactions between site*canopy cover ($p = 0.03$), total insects*canopy cover ($p = 0.005$), site*flow ($p = 0.0006$) are all significant. Treatments revealed that nitrogen ($p < 0.0001$) has a significant positive effect on AFDM, while phosphorus has a significant negative effect ($p < 0.0018$) on AFDM. Interactions between canopy cover and site supports my hypothesis that as canopy cover increases, streams will have increased allochthonous materials. This is seen particularly in the case of McKinley, a small (0.56 km^2), slow moving, dark stream has the highest positive relationship with measured AFDM. The p-values associated with decreased AFDM, when total number exceeded 36, were significant ($p < 0.05$) in all streams but Barnwell, Elk and Misery. However, coefficients were positively associated with the streams Elk, McKinley and Misery although in these cases (not including McKinley which has very high AFDM), these coefficients were very small fractions. Flow was also negatively associated with AFDM and significant interactions occurred in all but one stream (Elk). This finding makes

sense when one considers that as stream flow increases, periphyton may be “washed off” of substrates.

Chlorophyll- α The final multiple regression model for log-transformed Chlorophyll- α includes site, site*block, block, and treatment, yielding an R^2 value of 0.89. Site and block interactions were significant ($p=0.0017$), as well as, site ($p<0.0001$) and block ($p=0.042$) by themselves. These terms have a considerable effect on predicted chlorophyll concentration, however, as was the case for AFDM, the terms represent explanatory power not shown by my own independent variables. Again, treatments N and P had an effect on chlorophyll concentration and N/P treatments did not. The positive value of the nitrogen coefficient suggests that its affect increases chlorophyll concentration. Phosphorus has a negative coefficient and works to decrease chlorophyll concentration. When canopy cover was regressed alone it explained 43% of the variability for chlorophyll, however, when multiple independent variables were added, this effect is “swamped out” by other variables.

Herbivore Treatments ANOVA analysis of herbivores by treatment did not reveal any differences by site. A Two-Way Analysis of herbivore treatments for nitrogen and controls revealed that for chlorophyll- α concentrations and AFDM, although treatment differences did have a small effect, neither the herbivore exclusion vs. inclusion treatments, nor the nitrogen vs. control treatments were significantly different (Fig. 4a, Fig. 4b).

a)

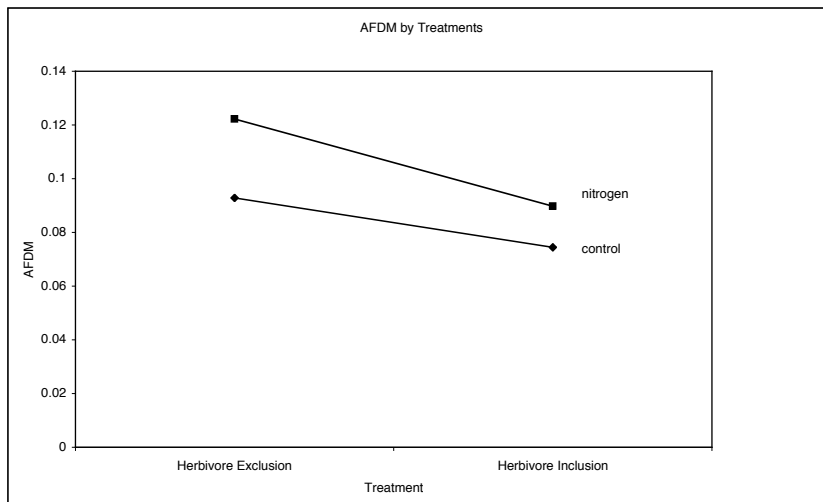


Figure 4. a) Interaction plot showing effects of treatments on chlorophyll- α concentration (Tukey's least squares means, $p=0.19$).

b)

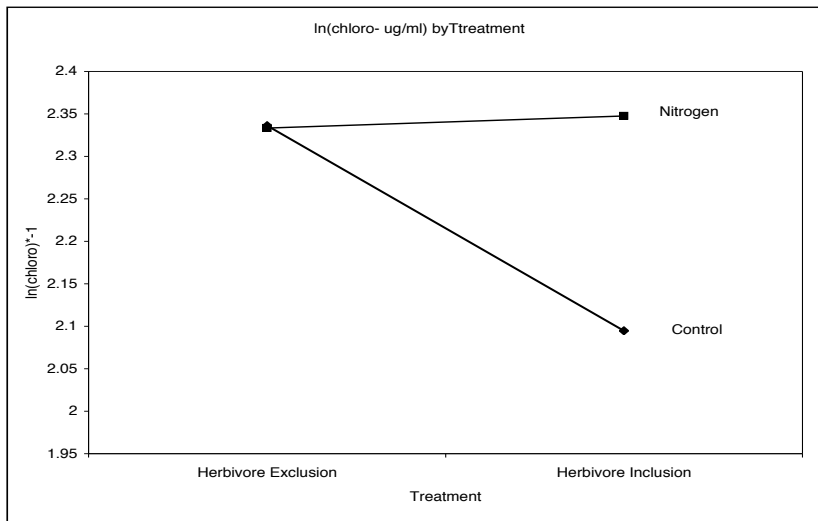


Figure 4. b) Interaction plot showing treatment effects on AFDM, (Tukey's least squares means, $p=0.89$)

Discussion

Although the McKinley stream did seem to have results leaning in the direction of light limitation, overall, canopy cover as a variable does not appear to support the hypothesis that increases in light availability to streams will increase algal biomass. Other studies have shown that through *in situ* shading manipulation or comparison between vastly different settings, light availability does result in a decreased amount of algal biomass (Hill and Knight 1988, Findlay and Howe 1993). Therefore, it may be that our stream sites were largely too similar in characteristics to yield a favorable result.

Nutrient additions did appear to produce results favoring the hypothesis that because algae are nutrient limited, their biomass will be increased by nutrient additions. Phosphorus was not expected to increase algal biomass due to previous results reported by Hill and Knight. For this reason phosphorus treatments were not included in the herbivore inclusion/exclusion portion of this study. Results from my analysis show that phosphorus actually decreases algal biomass. I would like to say that this is a result from phosphorus limited herbivore consumption, however, no differences in herbivore numbers were found between nutrient pot treatments.

Herbivore treatments appear to have a larger effect from inclusion/exclusion experiment when plotted against log-transformed chlorophyll- α . Control pots showed decreased chlorophyll- α concentrations while nitrogen pots showed a very slight increase. The end result

of the analysis shows interaction occurring between the treatments and that there is no significant difference between nutrient treatments (nitrogen, control) for chlorophyll- α concentration. In the case of AFDM, there was no interaction and both treatments showed decreased AFDM. Although AFDM measurements were higher on nitrogen pots overall, the pattern of decrease was not different. These results may be because nothing was done to exclude drifting herbivores from the exclusion treatments. I also believe that better herbivore identification may have affected the outcome differently. The results may also explain, in the case of chlorophyll- α , herbivore grazing may have the effect of increasing algal productivity by reducing algal competition.

Overall, my research concludes that nutrients alone (N or P) and not in combination (N/P) have different effects on algal biomass. Canopy cover alone as a variable did not sufficiently explain increases in algal biomass and that other variables not included in this study may have better explanatory power. Algal counts may have helped this study, in addition to, C:N:P ratios for stream study sites. Although herbivores were associated with decreased AFDM, herbivores exclusion and inclusion treatments did not adequately demonstrate that herbivores are attracted to increased algal growth and hence, nutrient availability.

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