

In situ nutrient assays of periphyton growth in a lowland Costa Rican stream

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Abstract

Nutrient limitation of primary production was experimentally assessed using an *in situ* bioassay technique in the Quebrada Salto, a third-order tropical stream draining the northern foothills of the Cordillera Central in Costa Rica. Bioassays employed artificial substrata enriched with nutrients that slowly diffuse through an agar-sand matrix (Pringle & Bowers, 1984). Multiple comparisons of regression coefficients, describing chlorophyll-*a* accrual through time for different nutrient treatments, revealed positive micronutrient effect(s). Micronutrient treatment combinations (Fe, B, Mn, Zn, Co, Mo, EDTA), supplemented with and without nitrate and phosphate, exhibited significantly greater chlorophyll-*a* accrual over all other treatments ($P < 0.05$), supporting over three times that of the control after 14-d of substratum colonization. Neither of the major nutrients (N or P) produced a significant stimulation, although the N treatment displayed $\approx 50\%$ more chlorophyll-*a* than the control after 14-d. Similarly, Si, EDTA, and Si + N + P treatments did not exhibit chlorophyll-*a* response curves that were significantly different from the control. During the experiment, mean $\text{NH}_4\text{-N}$ and $(\text{NO}_2 + \text{NO}_3)\text{-N}$ concentrations in the Salto were $2.0 \mu\text{M}$ ($28.6 \mu\text{g}\cdot\text{l}^{-1}$) and $7.2 \mu\text{M}$ ($100.2 \mu\text{g}\cdot\text{l}^{-1}$), respectively. High concentrations of $\text{PO}_4\text{-P}$ ($\bar{x} = 2.0 \mu\text{M}$; $60.9 \mu\text{g}\cdot\text{l}^{-1}$) and TP ($\bar{x} = 3.0 \mu\text{M}$; $94.0 \mu\text{g}\cdot\text{l}^{-1}$) were also found, and consequently low molar N:P ratios ($\bar{x} = 4.7$). Despite the potential for N limitation in the system, both N and P appear to be at growth saturating levels. This may be due to micronutrient limitation and/or light limitation of periphyton growth in densely shaded upstream portions of the stream.

Introduction

Nutrient limiting factors have been studied in a variety of temperate and arctic streams and regional information has been compiled. Many streams in desert/semi-desert areas of the southwestern US (Cole & Batchelder, 1969; Sommerfeld *et al.*, 1974; Grimm *et al.*, 1981) and the Pacific Northwest (Gregory, 1980) are suspected or have been found to be nitrogen limited, exhibiting low N:P ratios. In

contrast, streams of the eastern US, draining predominantly forested watersheds, have been found to exhibit relatively high molar N:P ratios of 68:1, suggesting phosphorus limitation (Omernik, 1977). Likewise, periphyton populations in pristine streams in the midwestern US (Pringle & Bowers, 1984), the southern US (Elwood *et al.*, 1981), northern Canada (Stockner & Shortreed, 1978) and arctic Alaska (Peterson *et al.*, 1983), have been stimulated by *in situ* additions of phosphorus, exhibiting little or no response to nitrogen additions (also indicating phosphorus limitation).

Nutrient limitation of periphyton abundance is poorly understood in tropical lotic systems relative

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to the amount of information that exists for temperate streams. This is unfortunate given the rapid degradation of tropical watersheds and the abundant evidence that deforestation can severely alter nutrient input from the watershed (e.g. Bormann & Likens, 1979). An increasing amount of work has been done in the Amazon (Sioli, 1975; Tundisi & Curi, 1984; Setaro & Melack, 1984), however, other areas have remained unexplored. Specifically, the nutrient chemistry of Costa Rica's Atlantic and Pacific slope streams is little known and, to our knowledge, no *in situ* manipulations have been attempted. Many of these watersheds are underlain by volcanic basalt and are generally characterized by young soils that contain more phosphorus relative to older soils of the Amazon (Vitousek, 1984). Virtually nothing is known regarding how this geochemistry is reflected by stream nutrient chemistry and primary production.

In this study we report preliminary analyses of nutrient chemistry for a stream in Costa Rica which flows through a remnant of primary rainforest. Using an *in situ* substratum enrichment technique (Pringle & Bowers, 1984), we evaluated nutrient limitation of periphyton growth.

Description of study site

The study site was located in the lower watershed of the Quebrada Salto, a river that drains the Atlantic slope foothills of Costa Rica's Cordillera Central, dropping from 300 m to ≈ 36 m above sea level where it empties into the Río Puerto Viejo. The lower watershed of the Salto is located in the La Selva Biological Reserve ($10^{\circ}26'N$, $84^{\circ}00'W$), owned and operated by the Organization for Tropical Studies. The upper two thirds of the river is contained within 'Zona Protectora La Selva', a corridor of land that comprises part of the last altitudinal transect of tropical rainforest remaining in Central America. According to topographic and land-use maps (Pringle *et al.*, 1984), less than 5% of the Salto's watershed is estimated to be in pasture and secondary forest. In general, the Salto is heavily shaded by many overhanging trees. At the study site, where La Selva's Central Trail crosses the river via a bridge, the canopy is relatively open allowing direct sunlight penetration for $\approx 3-4$ h \cdot d $^{-1}$. The Salto is a third-order stream at

this point and river bed sediments range from fine gravel to large boulders.

The Salto watershed lies in an area of basaltic bedrock overlain by residual volcanic soils derived from this parent material (Bourgeois *et al.*, 1972). Though soils are highly weathered, they are classified as inceptisols and entisols, which are more fertile relative to older soils characteristic of many tropical areas.

Materials and methods

In constructing nutrient-diffusing substrata, quartz sand (medium particle size) was washed and autoclaved to kill attached microorganisms, then consolidated into plastic disposable petri dishes (5.2 cm diameter; 1.2 cm deep) with 2.0% agar solutions enriched with various nutrients according to the method of Pringle & Bowers (1984). Each bioassay plate consisted of 10 ml of agar and ≈ 40 g of sand. Treatments included the following: (a) *control* (unenriched agar), (2) *nitrate* (0.50 M NaNO₃), (3) *phosphate* (0.50 M KH₂PO₄), (4) *micronutrient combination* (0.03 M FeC₆H₅O₇, 0.01 M H₃BO₃, 0.02 M MnCl₂ · 4H₂O, 3.01 × 10⁻⁴ M ZnCl₂, 3.79 × 10⁻⁵ M CoCl₂ · 6H₂O, 3.06 × 10⁻⁴ M Na₂MoO₄ · 2H₂O, and 4.46 × 10⁻³ M EDTA), (5) *micronutrient combination* (see #4), *nitrate and phosphate* (1.50 M NaNO₃, and 0.09 M KH₂PO₄; N:P = 16:1), (6) *silicate* (0.10 M Na₂SiO₃ · 5H₂O), (7) *silicate, nitrate and phosphate* (0.10 M Na₂SiO₃ · 5H₂O, 1.50 M NaNO₃, and 0.09 M KH₂PO₄; N:P = 16:1), (8) EDTA (4.46 × 10⁻³ M EDTA). The EDTA treatment assessed bioavailability of metals in the water (Goldman & Horne, 1983). Micronutrient ratios followed those used by Moss (1972) for lentic systems and were scaled to one tenth of the KH₂PO₄ concentration used in treatment 5. Treatment combinations were chosen to help identify potential limiting factors that might not be apparent if only single nutrient spikes were used (Schelske, 1984).

Nutrient enriched substrata were glued onto wooden boards in a non-random design which clumped replicates within treatments to minimize lateral and downstream nutrient effects between treatments. Boards were subsequently installed within the Quebrada Salto during the rainy season

in September, 1984. Care was taken to insure that differences between substratum treatments resulting from location effects were minimal by locating substrata in a narrow range of current velocities and uniform light. Boards were placed ≈ 10 cm below the water surface, ≈ 60 cm above the stream bottom, and were oriented so that substratum surfaces were parallel to the current flow. Boards were secured in this position by tying them securely to wooden projections that extended outward from a wooden bridge that spans the stream at the study site. Artificial substrata were checked 1–2 times daily throughout the experiment and cleared of any accumulated debris. Three replicate substrata per treatment were retrieved at 5, 10, and 14 d intervals for analysis of chlorophyll-*a* accumulation. Previous studies in the Quebrada Salto (Paaby & Phillips, unpubl. data), indicated that substantial periphyton populations developed on glass slides after a 14 d period.

Upon retrieval, the upper 3 mm of agar and sand were carefully removed with a razor blade. The layer was placed in a 200 ml beaker, diluted with 100 ml of distilled water and then mixed for 10 min with a magnetic stirrer. This procedure (Pringle & Bowers, 1984) resulted in almost complete periphyton separation from sand grains, as evidenced by microscopic scanning of residual grains. Samples (2.0 ml) taken from the suspended homogenate were filtered (0.45 μm Millipore filters) and then placed in 5 ml of buffered, 90% acetone and stored frozen in dark bottles for 2 wks until analyzed. The fluorescence of extracted chlorophyll-*a* was subsequently determined on a Turner III fluorometer according to standard methods (APHA, 1980). Values were corrected for phaeophytin and expressed in $\text{mg chlorophyll-}a \cdot \text{m}^{-2}$ substratum surface. Multiple comparisons of regression coefficients, describing chlorophyll-*a* accrual through time for different treatments, were made via a simultaneous test procedure (Sokal & Rohlf, 1981).

Ambient water chemistry was monitored throughout the duration of the experiment every 1–2 d (and twice a day for a period of 4 d). Two replicate water samples were collected on each sampling date and filtered through pre-rinsed, Whatman (GFC) glass-fiber filters; two replicate unfiltered samples were also collected. Water samples were stored frozen in polyethylene bottles and shipped frozen back to the University of Califor-

nia, Davis, for chemical analyses. Soluble reactive phosphorus ($\text{PO}_4\text{-P}$) and total phosphorus (TP) were measured with the molybdenum blue technique (preceded by acid hydrolysis in the case of TP analyses; APHA, 1980). Filtered samples were analyzed for ($\text{NO}_2 + \text{NO}_3$)-N using the hydrazine reduction method (Kamphake *et al.*, 1967). Ammonia, iron and silica analyses were carried out on unfiltered samples using the indophenol method (Solorzano, 1969), the ferrozine technique (Stokey, 1970), and the molybdosilicate complex method (APHA, 1980), respectively. Cations (Zn, Ca, Mg, Na, K) were measured with atomic absorption spectrometry. Temperature and oxygen were monitored daily using a combined oxygen-temperature meter (YSI model). Alkalinity was determined potentiometrically and pH was measured with an Orion probe. Suspended solids concentrations were determined by filtering known quantities of water through pre-weighed Whatman (GFC) glass-fiber filters, drying at 103°C and reweighing. Water level fluctuations were recorded daily and current velocities of waters flowing over artificial substrata were measured at the beginning of the experiment by timing the distance traveled by a small plastic float (Hynes, 1970).

Results

Analysis of substratum plates after 14 d indicated that chlorophyll-*a* accumulations on the micronutrient treatment ($26.7 \pm 2.8 \text{ mg} \cdot \text{m}^{-2}$) and the micronutrient + N + P treatment ($30.7 \pm 1.8 \text{ mg} \cdot \text{m}^{-2}$) were over three times those of control substrata ($8.6 \pm 0.5 \text{ mg} \cdot \text{m}^{-2}$). Chlorophyll-*a* levels, $\approx 50\%$ greater than those of the control, were displayed by the N treatment ($13.1 \pm 1.1 \text{ mg} \cdot \text{m}^{-2}$) and the Si + N + P treatment ($13.7 \pm 1.1 \text{ mg} \cdot \text{m}^{-2}$; Fig. 1, Table 1).

A one-way ANOVA indicated that some treatment effects were highly significant ($P < 0.01$). A simultaneous test procedure showed that the micronutrient and the micronutrient + N + P treatments were significantly higher than the control and all other treatments, though not significantly different from each other ($P < 0.05$). No significant differences ($P < 0.05$) were found between regression coefficients describing chlorophyll-*a* accrual through time for control,

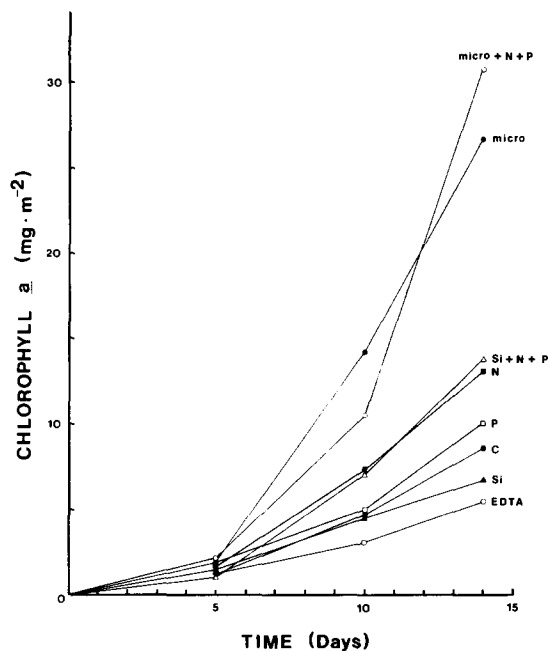


Fig. 1. Mean chlorophyll-*a* accretion on bioassay substrata after 5, 10 and 14 d of *in situ* exposure in the Quebrada Salto; Where: *micro* = micronutrient combination; *N* = nitrate; *P* = phosphate; *Si* = silicate; and *C* = control (see Table 1 for standard error associated with plotted means).

Table 1. Mean chlorophyll-*a* accretion \pm standard error upon bioassay substrata during the experiment. Substratum retrieval dates were September 15, 20, and 24, 1984. (See Fig. 1 for treatment explanation code.)

Treatment	Substratum exposure period		
	5-d	10-d	14-d
C	1.3 \pm 0.1	4.8 \pm 0.5	8.6 \pm 0.5
N	1.7 \pm 0.3	7.4 \pm 0.7	13.1 \pm 1.1
P	1.9 \pm 0.3	5.0 \pm 0.9	10.1 \pm 0.4
Micro	1.8 \pm 0.2	14.2 \pm 1.5	26.7 \pm 2.8
Micro + N + P	2.2 \pm 0.3	10.5 \pm 2.4	30.7 \pm 1.8
Si	1.5 \pm 0.2	4.5 \pm 0.3	6.7 \pm 1.0
Si + N + P	1.0 \pm 0.4	7.0 \pm 0.5	13.7 \pm 1.1
EDTA	1.2 \pm 0.1	3.1 \pm 0.2	5.5 \pm 1.1

EDTA, Si, P, N, and Si + N + P treatments.

Chemical and physical conditions of the Quebrada Salto are summarized in Table 2. Rates of water flow over artificial substratum plates ranged from 32.0–40.0 cm \cdot s⁻¹ and water levels were usually within 3 cm of the mean gauge reading (total range = 19 cm). Macroinvertebrate grazer populations were very low at the study site and virtually no grazers colonized artificial substrata.

Table 2. Ranges, means and standard deviations for measurements of physical and chemical variables in the Quebrada Salto during the bioassay experiment.

Variable	Unit	No. of observations	Min.	Max.	Mean	Standard deviation
SRP (PO ₄ -P)	μ M	24	1.2	2.4	2.0	0.3
Total P	μ M	24	2.5	3.6	3.0	0.3
(NO ₂ + NO ₃)-N	μ M	24	1.4	11.4	7.2	3.2
NH ₄ -N	μ M	21	1.4	2.7	2.0	0.3
N:P ¹	μ M	20	2.1	6.1	4.7	–
Total Fe	μ M	23	5.7	24.6	9.3	4.7
Si	μ M	26	391.5	569.4	495.4	14.3
Zn	–	4		below detection		–
Ca	μ M	4	49.6	155.8	122.2	47.4
Mg	μ M	4	62.9	266.3	209.9	98.8
Na	μ M	4	95.3	364.7	276.8	122.7
K	μ M	4	34.8	98.5	76.3	28.3
Temperature	°C	12	23.4	24.9	23.9	0.4
Oxygen	mg \cdot l ⁻¹	12	7.2	8.3	7.6	0.3
Total alkalinity	mg CaCO ₃ \cdot l ⁻¹	12	27.3	52.5	42.8	7.4
pH	–	12	6.4	6.9	6.7	0.2
Suspended solids	mg \cdot l ⁻¹	15	3.3	7.9	6.8	4.3

¹ Where N:P = (NO₂ + NO₃)-N + NH₄-N:PO₄-P.

Discussion

Bioassay results reflect high ambient N and P levels, indicating that periphyton standing crop was not primarily limited by either of these macronutrients. Despite the potential for N limitation in the system [given high $\text{PO}_4\text{-P}$ and TP concentrations, with consequently low N:P ratios ($\bar{x} = 4.7$), relative to Redfield's ratio of 16:1 (Redfield *et al.*, 1963)], ambient N and P levels both appear to be at growth saturating conditions. The lack of periphyton response to P, coupled with high ambient P levels ($\bar{x} = 2.0 \mu\text{M PO}_4\text{-P}$) is in accordance with studies of Elwood *et al.* (1981) which reveal that periphyton capacity for P uptake in a temperate stream was saturated at $2.0 \mu\text{M PO}_4\text{-P}$.

Several other investigations have observed a lack of periphyton response to both N and P in lotic systems (Patrick, 1966; Kilkus *et al.*, 1975; Moore, 1977). The streams studied, however, were disturbed nutrient-rich systems draining agricultural watersheds. The study of Wuhrmann & Eichenberger (1975) is very similar to ours. They added N and P to pristine ground water and detected no stimulation of periphyton growth, whereas trace metal mixtures and dilute sewage did elicit a response. In this latter study it is possible that initially high N and P levels in the ground water had not yet been depressed through metabolic uptake by photosynthetic organisms; water diverted through unshaded stream channels for a significant distance might have become P limited (Peterson *et al.*, 1983). In a similar fashion, light limitation of periphyton populations in densely shaded upstream portions of our study stream may partially explain high ambient N and P concentrations. Thus, though micronutrient(s) appear to limit periphyton abundance at our study site and at the site of Wuhrmann & Eichenberger (1975), the possibility that other factors (e.g. light) may be primarily limiting in other parts of the stream should not be overlooked.

The highly significant periphyton response to micronutrient treatments indicates the desirability of distinguishing among individual micronutrient effects within the combination employed in our preliminary experiment. Although several studies have assessed effects of individual and/or combined trace metal additions upon phytoplankton populations in lakes (e.g. Goldman, 1964; Henry &

Tundisi, 1982), comparatively few have dealt with periphyton response in lotic systems (Patrick *et al.*, 1969; Wuhrmann & Eichenberger, 1975). Interpretation of results of such studies is often confounded by difficulties in measuring the biological availability of trace metals with conventional analytical methods (Schelske, 1984).

Possibly one or more trace metals are in short supply within the Salto watershed because of geological and/or biological phenomena. For instance, Goldman (1966) has shown that some ions (e.g. molybdate) are strongly absorbed to hydrated oxides of iron and manganese, which effectively remove them from aquatic environments that favor formation of these colloids. The Salto watershed provides such an environment. When basalt weathers, minerals are eventually converted to clay, aluminum and iron oxides (note high Fe levels in Table 2), with bases released into solution (Greenland, 1981).

Major questions arising from our study that we are currently pursuing include: (1) Which micronutrients limit periphyton growth, (2) Why are $\text{PO}_4\text{-P}$ and TP levels so high in the Salto relative to many undisturbed systems in temperate areas, and (3) What effects do such high levels have on nutrient cycling processes? Data for other rivers that also flow through primary forest within different watersheds on the La Selva Reserve, indicate similarly high P levels in both rainy and/or dry seasons (Luvall & Jordan, 1984; Glover & Balthelt, unpubl. data; Pringle *et al.*, unpubl. data). Factors governing bulk nutrient concentrations in freshwater systems include soil chemistry and exchange capacity, anthropogenic inputs into the watershed, precipitation and vegetation cover. Since terrestrial systems at La Selva are characterized by tropical andisols, which have a high phosphorus binding capacity (Parfitt, 1980), we speculate that high $\text{PO}_4\text{-P}$ and TP concentrations in the Salto arise from soil input and desorption processes. During our study, suspended sediment loads in the Salto were visibly high during periods of rainfall. The presence of high concentrations of iron, which is not soluble in oxic conditions, also indicates significant sediment inputs. Furthermore, the presence of ferric hydroxides implies removal of soluble phosphorus present in the system via adsorption (Nürnberg, 1984). This is reflected by high TP levels in unfiltered samples. Grobbelaar (1983) also

reports high amounts of P absorbed onto suspended sediments in Amazon rivers, suggesting that adsorbed nutrients could substantially drain tropical ecosystems of phosphorus through sedimentation and/or discharge processes.

In conclusion, three lines of evidence indicate that periphyton growth was not primarily limited by either N or P at our study site during the experiment: (1) relatively high ambient N and P concentrations, (2) lack of periphyton response to *in situ* N and P additions, and (3) significant periphyton response to addition of micronutrients. As cited above, other studies have also recorded unusually high ambient P levels in La Selva streams. Our study, combined with those observations, indicates that nutrient regulation of periphyton growth and nutrient cycling processes in lowland Costa Rican streams warrant further investigation.

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