Effects of Water and Substratum Nutrient Supplies on Lotic Periphyton Growth: An Integrated Bioassay

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Effects of substratum and water nutrient perturbations on periphyton growth were assessed in a nutrient-poor stream by combining a substratum enrichment technique with a flow-through bioassay system. Periphyton growth (chlorophyll a, total biovolume) responded to combined influences of water and substratum enrichment in an additive manner when both compartments were amended with N and P to yield an optimal ratio (N:P ≃ 16:1). When NO_3-N was added to the substratum and PO_4-P to the water, algal growth response was synergistic. Analysis of the vertical distribution of P fractions in cores taken from nutrient-diffusing substrata indicates that attached microorganisms mediate P release from underlying substrata, acting as a filter or temporary sink. Nutrient-diffusing substrata are useful detectors of limiting nutrients in aquatic systems; however, their function and application differ from water enrichment assays where nutrients are added at a constant rate. Differences are partially attributed to spatial and temporal variability of nutrient release and the strictly localized influence of substratum flora on ambient water chemistry.

On a évalué les incidences de perturbations du substrat et des bioéléments aquatiques sur la croissance du périphyton dans un ruisseau pauvre en bioéléments à l'aide d'une technique d'enrichissement du substrat couplée à un système d'expérimentation à débit continu. La croissance du périphyton (chlorophylle a, biovolume total) a réagi aux influences combinées de l'enrichissement de l'eau et du substrat suite à l'apport de N et de P dans les deux compartiments afin d'obtenir un rapport optimal (N:P ≃ 16:1). Quand du NO_3-N et du PO_4-P ont été ajoutés respectivement au substrat et à l'eau, la réaction des algues en matière de croissance a été synergique. L'analyse de la distribution verticale des fractions de P dans les carottes du substrat producteur de bioéléments indique que les microorganismes présents interviennent dans la libération du P contenu dans les substrats sous-jacents et agissent comme filtres ou trappes temporaires. Les substrats producteurs de bioéléments servent d'indicateurs utiles des bioéléments limitatifs dans les systèmes aquatiques; toutefois, leur fonction et leur application sont différentes des tests d'enrichissement de l'eau où des bioéléments sont ajoutés à un taux constant. Les différences sont partiellement imputées à la variabilité spatiale et temporelle de la libération des bioéléments et à l'influence fortement localisée de la flore du substrat sur la chimie ambiante de l'eau.

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Few studies have addressed how nutrients from different sources interact to determine algal response in aquatic systems. Ambient nutrient levels in natural aquatic environments often do not reflect the contribution of many nutrient-generating sources that contribute to the spatial and temporal variability in nutrient supplies experienced by algal cells. This is especially true in streams where nutrient levels may be high at certain times (e.g. following storms) and where seasonal inputs of decomposing allochthonous materials release nutrients in high initial pulses (Howarth and Fisher 1976; Elwood et al. 1981). The effect of a constant unidirectional current is to homogenize nutrient inputs and decrease spatial nutrient heterogeneity, thereby making examination of individual nutrient effects difficult. Consequently, virtually nothing is known regarding effects of spatial variations in nutrient supplies on algal growth and distribution in streams.

In contrast, effects of nutrient heterogeneity on the distribution of planktonic algae in lakes and oceans have received considerable attention (Moss 1969; Platt 1972; McCarthy and Goldman 1979; Wall and Briand 1980). In lentic systems, individual algal cells experience different rates of resource supply due to depth gradients of nutrients and light, turbulent upwellings and other processes (Tilman et al. 1982). Laboratory experiments have shown that nutrient patches produced by zooplankton are used directly by planktonic algae (Lehman and Scavia 1982). This phenomenon occurs on a more two-dimensional scale and over a sustained period of time when algae are attached to nutrient-generating sources (Pringle and Bowers 1984; Pringle 1985a).

Although some emphasis has been placed on nutritional relationships between epiphytic algal communities and their macrophyte hosts (e.g. Wetzel 1983), nutrients derived from the substratum have received little attention outside of their overall contribution to ambient nutrient levels. In streams, current velocity and diffusion rates from overlying waters have been traditionally emphasized as the nutrient delivery system that maintains the physiological richness of running waters (Ruttner 1926; Whitford 1960; McIntire 1966).

This study quantitatively assesses effects of substratum–water nutrient perturbations on lotic periphyton growth. Artificial substrata that release nutrients in an exponentially decaying fashion were used to stimulate natural nutrient-diffusing

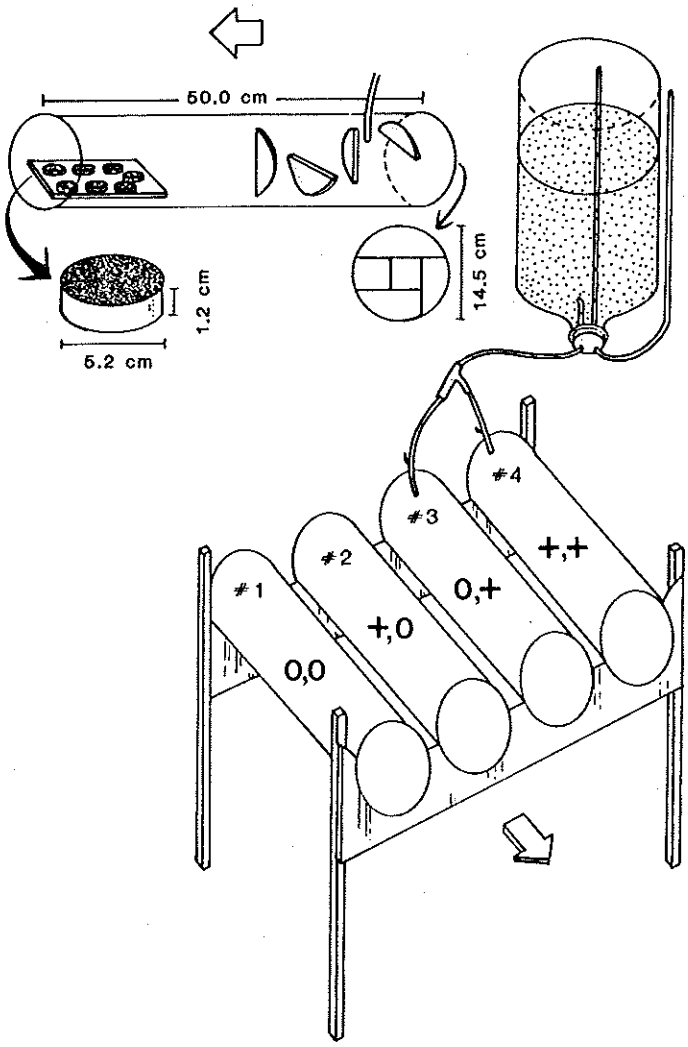

FIG. 1. Schematic diagram of periphyton bioassay system. Unshaded arrows denote current direction. Upper left: single Plexiglas cylinder showing placement of nutrient-diffusing substrata, baffles, and nutrient feeder line. Enlargement of substratum and cross-section of Plexiglas tube (illustrating baffle arrangement) are shown directly below cyclinder. Lower: Bank of Plexiglas cylinders in wooden frame. Nutrients are added to upstream ends of tubes 3 and 4 from a 20-L carboy. Notation on cylinders refers to treatment combination where the first character refers to presence (+) or absence (0) of substratum enrichment and the second character refers to presence or absence of water enrichment.

substrata. An integrated bioassay technique was designed that combines nutrient-diffusing substrata (Pringle and Bowers 1984) with a modified flow-through bioassay system (Peterson et al. 1983) to (1) experimentally separate effects of nutrients derived from substratum versus water on periphyton growth, (2) examine dynamics of phosphorus distribution in nutrient-diffusing substrata, and (3) compare the effectiveness of flow-through systems and nutrient-diffusing substrata as in situ bioassay methods.

Materials and Methods

Study Area

Research was conducted in Carp Creek, located on the University of Michigan Biological Station tract, Cheboygan County, Michigan, (45°33′N, 84°40′W). This second-order stream is fed by numerous springs believed to originate from Douglas Lake, which is located 0.5 km north of the stream's source. It exhibits a stable supply of cold groundwater and has a low gradient, typical of trout streams throughout northern Michigan. The streambed is composed of coarse sand with occasional backwater areas of flocculent detritus.

Bioassay Technique and Experimental Design

The bioassay apparatus (Fig. 1) consists of a bank of four 50-cm-long sections of clear Plexiglas cylinder that are held parallel to the water flow in a wooden frame. The frame was oriented so that cylinders were half filled with flowing water and was secured 0.35 m above the stream bottom by driving four wooden stakes (located at corners of frame) into bottom sediments. Cylinders were installed in current velocities ranging from 18 to 20 $cm \cdot s^{-1}$. Current velocity was measured with a Pygmy Gurley meter every 3 d, at upstream and downstream ends of each cylinder, throughout the duration of each experiment. Water level fluctuations were recorded daily. Four Plexiglas baffles were glued into upstream ends of all cylinders to insure turbulent mixing (Fig. 1). Nutrient-diffusing substrata were secured onto wood boards in downstream ends of cylinders and placed so that substratum surfaces were parallel to the water flow. Nutrients were introduced into two of the cylinders by siphoning concentrated solutions into upstream ends from a 20-L carboy that was installed at the stream's edge. Stopcocks allowed adjustment of rate of nutrient addition to the water.

Rates of nutrient addition to cylinders were monitored and calibrated every day, and nutrient solutions in the reservoir were renewed every 3 d with analytic grade chemicals. The reservoir was covered with a thick layer of black plastic to prevent growth of photosynthetic organisms and/or light degradation of organic compounds. Dye additions at the upstream end of each cylinder indicated thorough mixing by baffle systems. Water flow in tubes appeared to be between 75 and 90% that of adjacent stream waters.

Fertilized and unfertilized sand–agar substrata were exposed to both enriched and unenriched waters in a fully crossed $(2)^2$ factorial design (Table 1). A first set of experiments examined effects of water and substratum enrichment when the same elements were added to each compartment in approximately the same ratio. Experiment I examined periphyton response to both inorganic phosphorus (KH_2PO_4) and inorganic nitrogen ($NaNO_3$), while Experiment II examined effects of organic phosphorus (β-glycerophosphate, $C_3H_7O_6PNa_2 \cdot 5H_2O$) in combination with $NaNO_3$ (Tables 1a, 1b). In both of these experiments, ambient PO_4-P levels were increased fivefold and NO_3-N levels doubled to yield a relatively optimal N:P ratio of ≈14:1. Previous studies have indicated that periphyton growth in Carp Creek is primarily limited by P (Pringle and Bowers 1984). PO_4-P levels were increased only slightly so that periphyton capacity for P uptake would not be near saturation, thereby masking potential interactions between water and substratum. In Experiment II, frequent monitoring of TP and PO_4-P in reservoir batches of concentrated β-glycerophosphate solution and water outflow from Plexiglas cylinders revealed that no degradation of organic P occurred before its addition to stream water.

Experiment III assessed the impact of substratum-derived N on periphyton growth in N-limiting conditions. Periphyton growth response was examined when different elements ($NaNO_3$ and KH_2PO_4) were provided from each source. To

TABLE 1. Experimental design and corresponding nutrient regimes for treatments in Experiments (a) IA, (b) II, and (c) III. Values are expressed in $\mu mol \cdot L^{-1}$. PO_4-P = KH_2PO_4 and P = $C_3H_7O_6PNa_2 \cdot 5H_2O$. (See Table 2 for nutrient chemistry data associated with Experiment IB.)

(a) Experiment IA

Treatment (substratum, water)	Substratum			Water		
	NO_3-N	PO_4-P	N:P	NO_3-N	PO_4-P	N:P
(0, 0)	0.00	0.00	—	2.95	≤0.08	≥37:1
(N + P, 0)	8.00×10^6	0.50×10^6	≃16:1	2.95	≤0.08	≥37:1
(0, N + P)	0.00	0.00	—	5.71	0.40	≃14:1
(N + P, N + P)	8.00×10^6	0.50×10^6	≃16:1	5.71	0.40	≃14:1

(b) Experiment II

Treatment (substratum, water)	Substratum			Water		
	NO_3-N	P	N:P	NO_3-N	P	N:P
(0, 0)	0.00	0.00	—	3.65	≤0.08	≥46:1
(N + P, 0)	1.5×10^7	0.90×10^6	≃16:1	3.65	≤0.08	≥46:1
(0, N + P)	0.00	0.00	—	5.71	0.40	≃14:1
(N + P, N + P)	1.5×10^7	0.90×10^6	≃16:1	5.71	0.40	≃14:1

(c) Experiment III

Treatment (substratum, water)	Substratum			Water		
	NO_3-N	PO_4-P	N:P	NO_3-N	PO_4-P	N:P
(0, 0)	0.00	0.00	—	3.25	≤0.08	≥41:1
(N, 0)	8.00×10^6	0.00	—	3.25	≤0.08	≥41:1
(0, P)	0.00	0.00	—	3.25	1.21	≃2:1
(N, P)	8.00×10^6	0.00	—	3.25	1.21	≃2:1

create potentially N-limiting conditions, water PO_4-P levels were increased by a factor of ≃15, resulting in an N:P ratio of ≃2:1 (Table 1c).

Artificial substrata within cylinders were checked one or two times daily to remove grazers and case-building organisms that can affect periphyton standing crop and/or species composition. Three replicate substrata per treatment were retrieved randomly from cylinders at approximately weekly intervals over a 3-wk period. Experiment IB was concluded at 19 d so that dense periphyton mats could be sampled before sloughing occurred. Ambient water chemistry was monitored every 3 d throughout experiments. Si, Cl, NO_3-N, PO_4-P, and TP were analyzed via standard methods on a Technicon II autoanalyzer (APHA 1980).

Periphyton Analyses

In Experiments IA and II, two cores were taken from each artificial substratum with a cork borer (1.2 cm diameter × 1.2 cm length) after incubation in the stream. Cores were temporarily refrigerated for later nutrient analysis. In all experiments, the top 3 mm of substratum in petri plates was removed with a razor blade. Microscopic inspection indicated that few algal cells were located below 2 mm. The layer was placed in a 200-mL beaker, diluted to 100 mL with distilled water, mixed for 10 min with a magnetic stirrer, and then sonicated for 5 min. This procedure (Pringle and Bowers 1984) resulted in almost complete periphyton separation from sand grains as evidenced by microscopic scanning of residual grains.

Chlorophyll *a* samples (2.0 mL) were taken from the homogenate and filtered (0.45-μm Millipore filters). Samples were extracted in 90% buffered acetone and $MgCO_3$ and stored frozen in dark bottles for later analysis with a Turner III fluorometer. Chlorophyll *a* values were corrected for phaeopigments (Holm-Hansen et al. 1965) and expressed as milligrams per square metre of substratum surface. Three subsamples (0.1–0.2 mL) were also taken from the homogenate and placed upon individual glass coverslips to air dry.

Permanent slide mounts were subsequently prepared with Hyrax® for Experiments IA and II. Diatom taxa comprised at least 95% of the periphyton biovolume in all samples and counts were restricted to diatom taxa. A compound microscope (1000×) was used to enumerate "live" cells containing protoplasm. At least 500 diatoms per sample were enumerated, with a resulting count of ≃1500 frustules per treatment on each sampling date. Mean total diatom biovolume per square metre was determined by estimating cell volumes of specific taxa. Diatom measurements were entered into geometric equations that describe the three-dimensional shape of the frustule (Gruendling 1971). A minimum of 25 cells per diatom taxon were used to determine mean taxon dimensions.

A Student–Newman–Keuls test compared treatment means for chlorophyll *a* and total diatom biovolume between treatments on given retrieval dates. In some instances, multiple comparisons of regression coefficients describing these parameters through time were made using a simultaneous test procedure (Sokal and Rohlf 1981).

Nutrient Analysis of Substratum Cores

In order to assess overall effects of attached biota in mediating nutrient release, a separate experiment compared the ver-

TABLE 2. Selected chemical and physical variables measured ($\bar{x}$ ± SD) in Carp Creek during bioassay Experiments IA (31 Aug. – 22 Sept. 1983), IB (24 July – 15 Aug. 1984), II (5–24 July 1984), and III (10 June – 1 July 1984). Number of observations is indicated in parentheses.

Variable	Experiment IA	Experiment IB	Experiment II	Experiment III
SRP (PO_4-P) (μmol)	0.08 ± 0.02 (n = 5)	0.10 ± 0.02 (n = 4)	0.08 ± 0.04 (n = 5)	0.08 ± 0.01 (n = 6)
TP (μmol)	0.26 ± 0.08 (n = 4)	0.35 ± 0.15 (n = 3)	0.39 ± 0.08 (n = 4)	0.36 ± 0.12 (n = 6)
(NO_2 + NO_3)-N (μmol)	2.95 ± 0.22 (n = 5)	3.93 ± 1.80 (n = 5)	3.65 ± 0.98 (n = 6)	3.25 ± 0.57 (n = 6)
NH_4-N (μmol)	4.11 ± 0.61 (n = 5)	5.01 ± 1.10 (n = 5)	4.62 ± 1.63 (n = 3)	4.43 ± 0.89 (n = 5)
N:P	≥36.88 (n = 5)	39.30 (n = 4)	≥45.62 (n = 5)	≥40.62 (n = 6)
Temperature (°C)	10.10 ± 0.80 (n = 21)	10.20 ± 0.90 (n = 18)	11.50 ± 1.20 (n = 18)	10.30 ± 1.00 (n = 20)

tical distribution of P in field- versus laboratory-incubated substrata. Twelve sand–agar substrata were enriched with 0.50 mol KH_2PO_4 and 12 controls were made with unenriched agar. Six substrata from each treatment were incubated in the laboratory, while remaining substrata were incubated in situ. Two replicate substrata per treatment were cored after 7, 16, and 22 d for both the laboratory and field. In order to determine the presence or absence of a P gradient, cores in this experiment were separated into three 4-mm sections for P analysis. Field-incubated substrata were glued to pine boards which were anchored with wire to the streambed and positioned so that substratum plates were parallel with current flow and ≃15 cm below the water surface. Boards were installed in a median range of current velocities (18–23 $cm \cdot s^{-1}$). For laboratory incubation, 12 wide-mouth 4-L glass jars were acid rinsed and filled with 3 L of distilled water. Individual substrata were placed on elevated plastic platforms in separate jars. Jars were then placed on magnetic stirring units that were installed in an incubator in dark conditions at 10°C. Mixing of water overlying substrata was achieved by magnetic stir bars rotating under platforms. Water in jars was replaced with fresh distilled water every 3 d. Previous laboratory studies (Pringle and Bowers 1984) overestimated nutrient levels remaining in artificial substrata after incubation because water within incubation chambers was not frequently replaced. P levels in overlying water presumably exerted a regulatory influence on diffusion rates from substrata.

In Experiments IA and II, total reactive phosphate (PO_4-P) and total phosphorus (TP) were determined in both the top periphyton/substratum layer and the underlying substratum on selected retrieval dates. In two cores per substratum, the top periphyton/substratum layer (2 mm) was separated from the lower core segment (10 mm) with a razor blade. Wet weight of core sections was determined to three decimal places; sections were then stored frozen in dark conditions until analyzed. Mean P levels, measured in upper and lower segments of substratum cores, were corrected for weight (micrograms per gram) and the latter value was subtracted from the former to yield an approximate value of P associated with attached biota.

Mean background levels of P contributed by agar were determined for each of two batches of agar used to construct artificial substrata, where Batch 1 = Experiments IA and IB and Batch 2 = Experiments II and III. For each batch of agar, PO_4-P and TP were measured in three replicate cores taken from fresh control substrata. Mean P levels in three replicate cores of raw sand were subtracted from levels in sand–agar to determine amounts of P contributed by each batch of agar.

PO_4-P in core sections was analyzed colorimetrically on a Technicon II autoanalyzer employing the molybdenum blue technique (APHA 1980). Frozen substratum cores were placed in acid-cleaned glass tubes, allowed to thaw, mixed into a slurry, and finally diluted to 15 mL with distilled water. Samples were then placed in a hot water bath for 1 h (80°C) to dissolve agar and extract PO_4-P from microorganisms when present (Fitzgerald and Nelson 1966). After this, 5 mL was removed for PO_4-P analysis. Control tubes containing known quantities of β-glycerophosphate were also placed in the hot water bath and analyzed for PO_4-P. This revealed that the heating procedure was not effective in breaking the monophosphate ester bond.

Total particulate phosphorus was also measured as PO_4-P on a Technicon II autoanalyzer following digestion via the 5% persulfate procedure (APHA 1980). Organic and colloidally bound phosphate in substratum core sections was converted by digesting with sulfuric acid and potassium persulfate. The 10.0-mL sample remaining after PO_4-P analysis was brought up to 15 mL with a sulfuric acid and persulfate solution and then autoclaved at 120°C for 1 h. Total reactive phosphorus (PO_4-P) was subtracted from total particulate phosphorous (TP) to determine amounts of organic and colloidally bound P. Analytic methods did not distinguish between P organically bound within attached biota and that which was colloidally bound within the agar–sand matrix.

Results

Mean current velocities affecting substratum plates in downstream ends of cylinders ranged between 18 and 20 $cm \cdot s^{-1}$ ($\bar{x}$ = 19.0 ± 0.8, n = 20). No significant differences in current velocities existed between cylinders (Student t-test, P = 0.05). Water level was always within 0.5 cm of the mean gauge reading with the exception of 12 August 1984 (18 d, Experiment IB), when a storm resulted in water level fluctuations of 8 cm over a 14-h period. Selected chemical and physical variables measured in Carp Creek during bioassay experiments are given in Table 2. Water temperatures typically remained low ($\bar{x}$ = 10.2°C); however, temperatures in the latter half of Experiment II climbed to an unusual 12.7°C. Carp Creek ex-

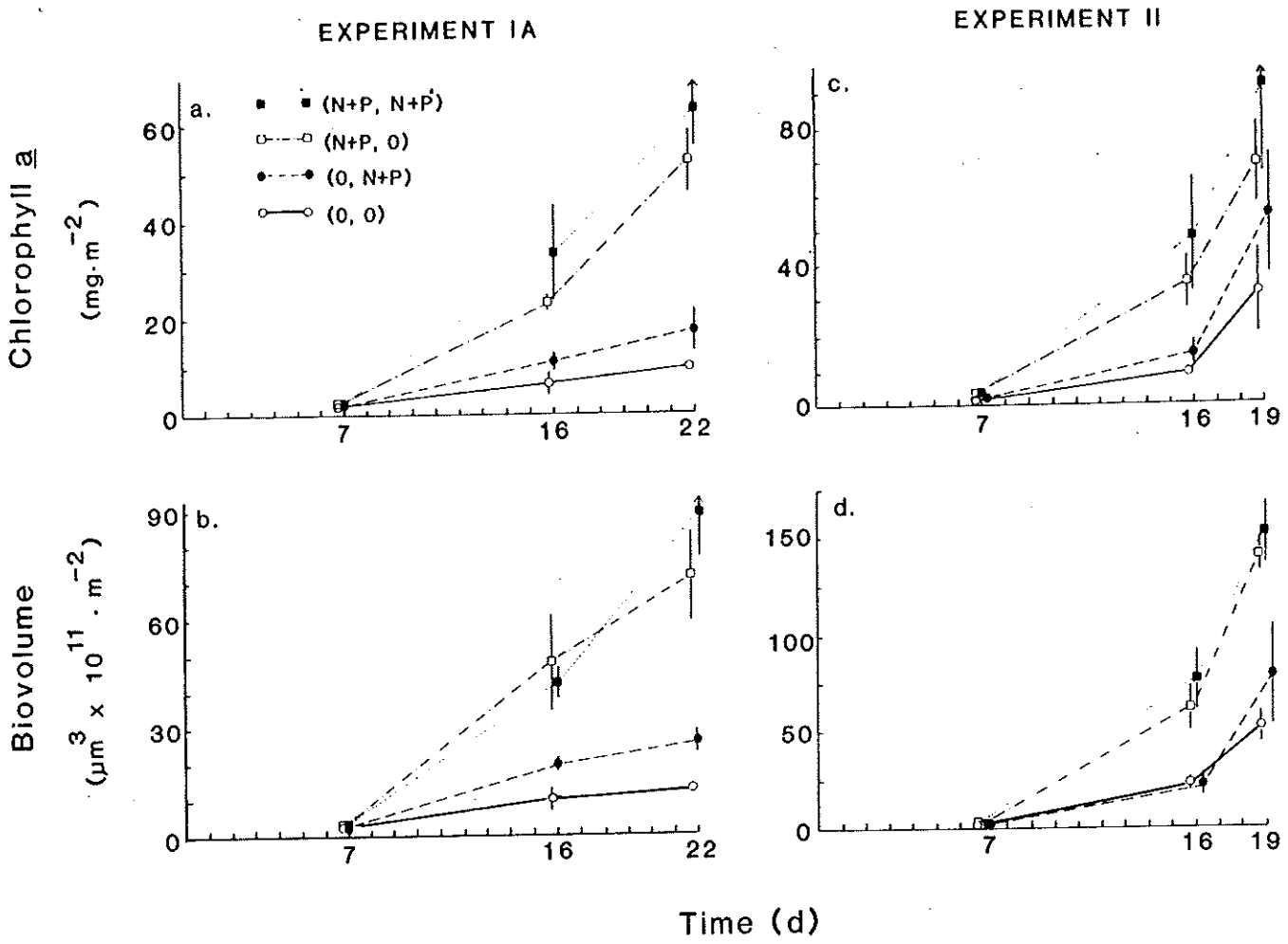

FIG. 2. Mean (±SD) chlorophyll *a* accrual and diatom biovolume for (a and b) Experiment IA and (c and d) Experiment II in response to treatments after in situ exposure in Carp Creek for various time periods. Treatment notation in parentheses refers to treatment combination where the first set of characters refers to the general nature of substratum enrichment and the second to water enrichment. *P = KH_2PO_4 for Experiment IA and P = $C_3H_7O_6PNa_2 \cdot 5H_2O$ for Experiment II.

hibited low levels of soluble reactive phosphorus (PO_4-P) throughout all experiments, with a mean summer concentration of 0.07 μmol.

Periphyton Growth

In Experiment I, when inorganic N and P were added in combination to both substratum and water, algal growth (chlorophyll *a*, diatom biovolume) was about equal to the sum of these two separate treatments relative to controls after 16 and 22 d (Fig. 2a, 2b). A Student–Newman–Keuls test indicated that cylinders representing different treatments supported significantly different standing crops ($P < 0.01$) after 16 and 22 d. A simultaneous test procedure showed that all treatments were significantly greater than the control ($P < 0.01$), although the two enriched substratum treatments were not significantly different from each other ($P = 0.05$). Mean ratios of treatments to controls exhibited similar trends through time for chlorophyll *a* and total biovolume, indicating that these two parameters are in fairly close agreement as estimates of total biomass. At 7 d, ratios of chlorophyll *a* to total biovolume were 1.6–2.3 times higher on all nutrient treatments relative to the control. After 22 d, however, ratios were fairly similar for all treatments (Table 3a).

When Experiment I was repeated (Experiment IB), effects of substratum and water enrichment on chlorophyll *a* accrual again acted in an additive fashion during the first 14 d (Fig. 3). After 17 d of substratum colonization, however, high stream discharge resulted in sloughing of thick periphyton mats on (N + P, N + P) and (N + P, 0) treatments. This is reflected by decreases in chlorophyll *a* at 21 d for both treatments.

In Experiment II, both chlorophyll *a* and total diatom biovolume exhibit an additive trend (Fig. 2c, 2d), with the (0, N + P), (N + P, 0), and (N + P, N + P) treatments, respectively, supporting 1.6, 3.8, and 5.3 times the amount of chlorophyll *a* relative to the control after 16 d (Table 3b). Treatment differences were markedly lower after 19 d. Substratum enriched treatments are generally significantly greater than unenriched treatments with respect to standing crop (total diatom biovolume, chlorophyll *a*) after 16 and 19 d (Student–Newman–Keuls test, $P < 0.05$). There is no significant difference, however, between the (N + P, 0) and (0, N + P) treatments after 19 d. Treatment differences were markedly lower at 19 d, probably due to high water temperatures in the latter half of this experiment which resulted in increased periphyton growth. Comparisons of treatment means for chlorophyll *a* after 16 d indicate significant differences (Student–Newman–Keuls test, $P < 0.05$) between the control (0, 0) and (0, N + P) treatments; however, these two treatments are not significantly different with respect to total diatom biovolume

TABLE 3. Mean ratio of chlorophyll *a* and total diatom biovolume to controls and the ratio of chlorophyll *a* to biovolume for (a) Experiment IA and (b) Experiment II. See Table 1 for treatment explanation code.

Treatment	No. of days colonized	Mean ratio of treatments to controls		Chl *a*:biovolume ($\mu g \cdot \mu m^{-3} \times 10^{-8}$)
		Chl *a*	Biovolume	
		(a) Experiment IA		
(0, 0)	7	1.00	1.00	0.53
	16	1.00	1.00	0.61
	22	1.00	1.00	0.76
(0, N + P)	7	1.59	0.71	1.19
	16	1.69	1.89	0.54
	22	1.82	2.10	0.66
(N + P, 0)	7	1.68	1.09	0.82
	16	3.61	4.55	0.48
	22	5.47	5.74	0.73
(N + P, N + P)	7	1.65	1.06	0.82
	16	5.21	3.98	0.80
	22	6.64	7.12	0.71
		(b) Experiment II		
(0, 0)	7	1.00	1.00	0.66
	16	1.00	1.00	0.42
	19	1.00	1.00	0.62
(0, N + P)	7	1.38	1.31	0.70
	16	1.57	1.04	0.64
	19	1.68	1.52	0.68
(N + P, 0)	7	2.25	1.62	0.92
	16	3.80	2.82	0.57
	19	2.12	2.65	0.49
(N + P, N + P)	7	2.12	1.43	0.98
	16	5.28	3.48	0.65
	19	2.82	2.87	0.61

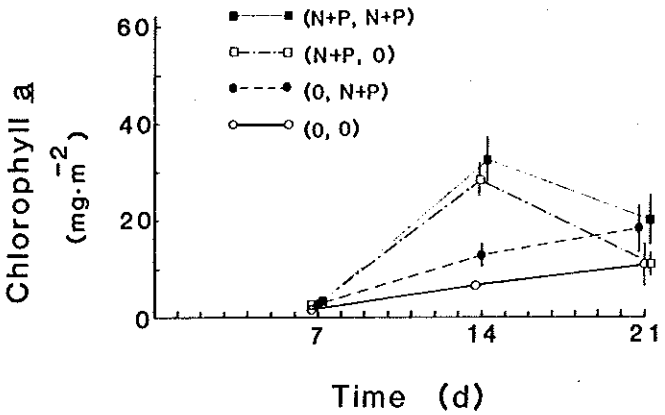

FIG. 3. Mean (±SD) chlorophyll *a* accrual for Experiment IB in response to treatments after in situ exposure in Carp Creek for various time periods. See Fig. 2 for explanation of treatment code.

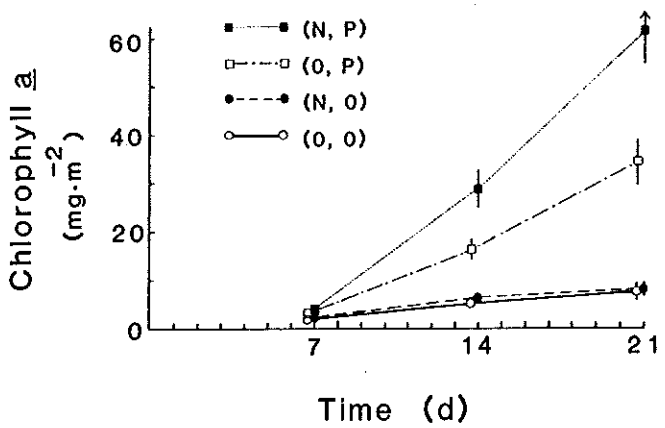

FIG. 4. Mean (±SD) chlorophyll *a* accrual for Experiment III in response to treatments after in situ exposure in Carp Creek for various time periods. See Fig. 2 for explanation of treatment code.

on this sampling date. Mean ratios of chlorophyll *a* to total biovolume were initially higher on substratum enriched treatments relative to unenriched substrata (Table 3b). Ratios decreased through time on enriched substrata and were similar to unenriched substratum treatments on the final sampling date.

In Experiment III, where N and P were added separately to substratum and water, respectively, chlorophyll *a* accrual was greater than the sum of the two individual treatments, indicating a synergistic interaction between the two compartments (Fig. 4). A simultaneous test procedure showed that the (N, P) and (0, P) treatments were significantly different from each other and significantly higher than the control and (N, 0) treatments after 14 and 19 d ($P < 0.05$). No significant difference ($P = 0.05$) was found between regression coefficients describing chlorophyll *a* accrual through time for control or (N, 0) treatments.

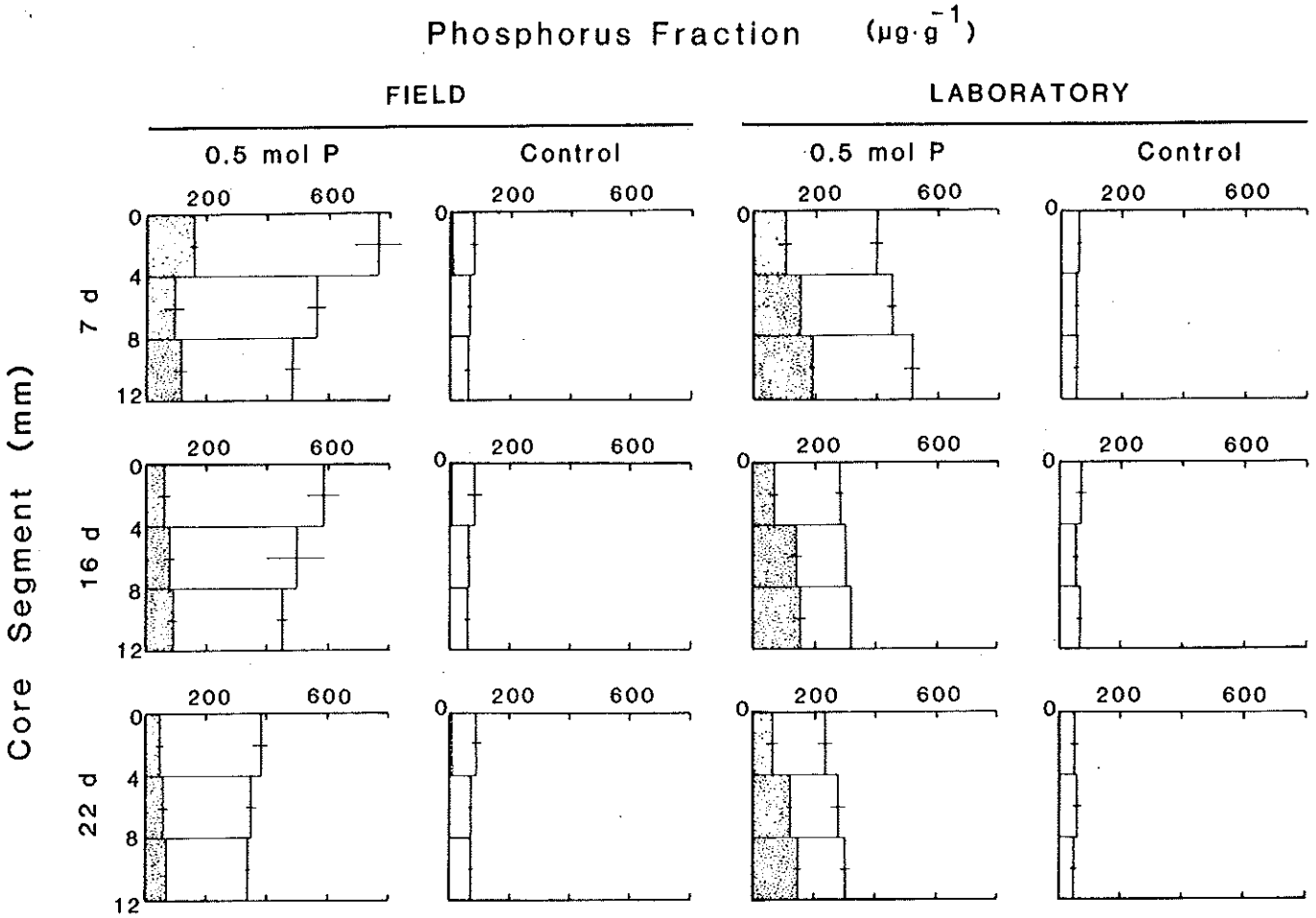

FIG. 5. Vertical distribution ($\bar{x}$ ± SD) of PO_4-P (shaded) and organic and colloidally bound P (unshaded) in field- and laboratory-incubated substrata after 7, 16, and 22 d.

Corollary evidence indicates that periphyton response in different cylinders reflects treatment effects and that potential dissimilarities between cylinders resulting from location effects are minimal. Firstly, Experiment I was repeated two times, and although a high discharge event after 16 d resulted in sloughing of periphyton mats, the same additive trend in chlorophyll *a* accrual was observed between treatments after 16 d (Fig. 2a, 3). Secondly, periphyton growth on glass slides placed within replicate enriched and unenriched cylinders in Experiments I and II was not significantly different ($P = 0.05$) between replicates (Pringle 1985b).

Nutrient Analysis of Substratum Cores

A diffusion gradient of TP and PO_4-P was observed within enriched substrata (0.5 mol PO_4-P) incubated in the laboratory throughout 22 d. In the laboratory, highest levels of TP and PO_4-P existed in deepest substratum layers, decreasing progressively in upper layers (Fig. 5). In the field, however, highest levels of TP occurred in the top periphyton/substratum layer. Field-incubated substrata contained significantly greater amounts of TP than laboratory-incubated substrata on all sampling dates (Student–Newman–Keuls test, $P < 0.05$). Ratios of TP contained in field- to laboratory-incubated substrata and ratios of TP remaining after incubation are presented in Table 4.

Water enrichment with inorganic P did not significantly affect the TP content of the upper substratum layer after 22 d of field incubation in Experiment IA (Fig. 6a). Substratum enriched treatments ((N + P, N + P) and (N + P, 0)) became increasingly similar through time. At 22 d there was no significant difference between them or the (0, 0) and (0, N + P) treatments ($P = 0.05$). In Experiment II, water enrichment with organic P had a positive effect on the TP content of the upper substratum layer. Enriched substratum treatments ((N + P, N + P) and (N + P, 0)) became increasingly different through time. After 19 d, they were significantly different from each other ($P < 0.05$), as were unenriched substratum treatments ((0, 0) and (0, N + P)).

Mean background levels of P ($\bar{x}$ ± SD) in sand used to construct artificial substrata were 1.23 ± 0.92 μg PO_4-P·g^{-1} and 6.38 ± 0.17 μg TP·g^{-1}. Background levels contributed by agar in Experiments IA and IB were 1.26 ± 0.18 μg PO_4-P·g^{-1} and 46.43 ± 0.89 μg TP·g substratum^{-1}, while background levels in Experiments II and III were 0.40 ± 0.14 μg PO_4-P·g^{-1} and 16.00 ± 1.7 μg TP·g substratum^{-1}.

Discussion

As pointed out by Wetzel (1983), "The general assumption that the chemistry of overlying waters has a direct controlling effect on epipelic algae has not been verified experimentally . . . While it is intuitively apparent that changing events in the overlying water can influence epipelic algae, studies of these interactions are essentially nonexistent." This study experimentally quantifies processes that occur in natural aquatic systems where algal communities obtain the same or different

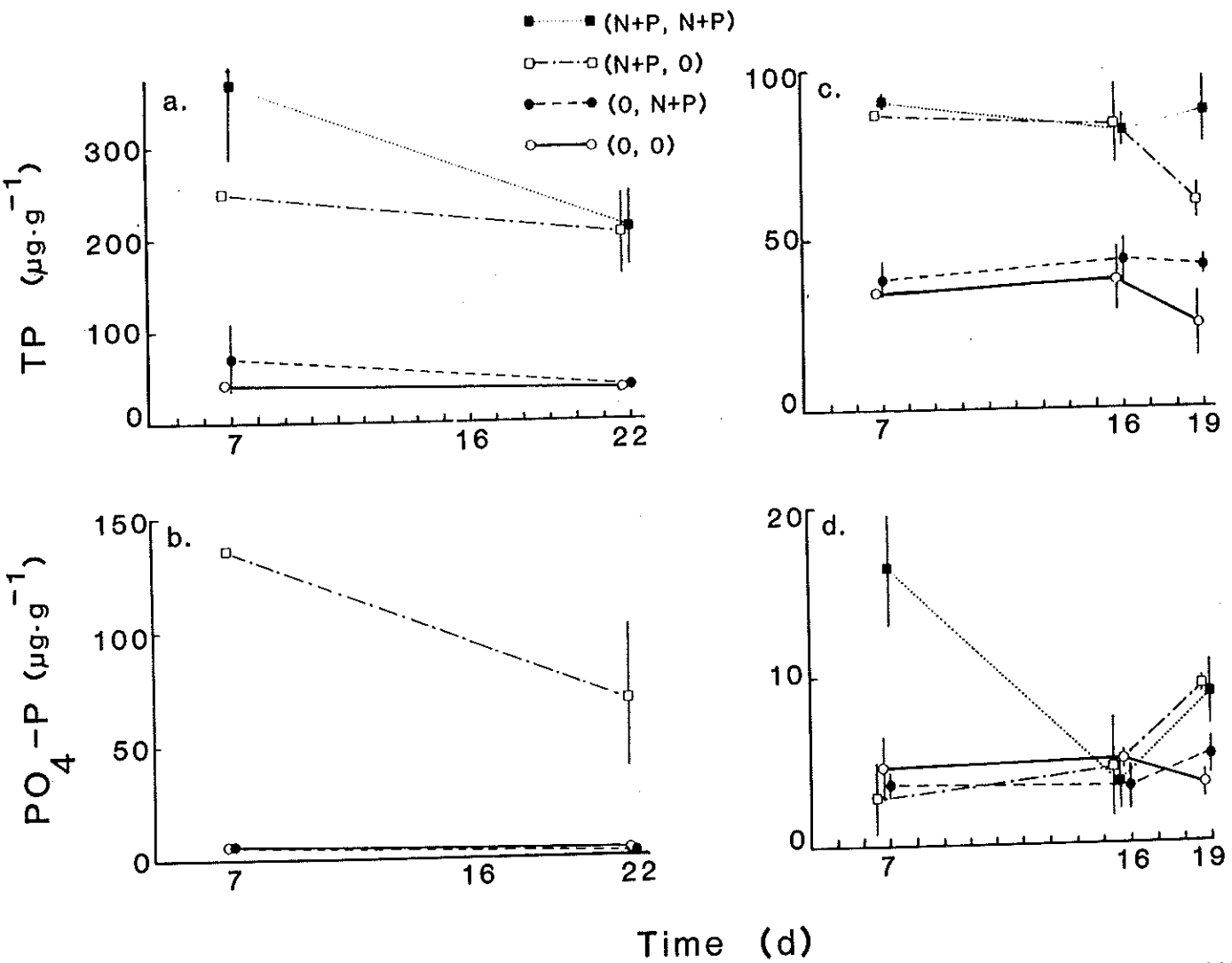

FIG. 6. Amounts (±SD) of total phosphorus (TP) and phosphate (PO_4-P) associated with attached biota on artificial substrata for (a and b) Experiment I and (c and d) Experiment II after in situ exposure in Carp Creek for various time periods. See Fig. 2 for explanation of treatment code.

TABLE 4. Mean ratio of total phosphorus (TP) in field-incubated substrata to laboratory-incubated substrata and mean ratio of TP in field- and laboratory-incubated substrata to amount originally present in fresh substrata.

		Ratio TP remaining in substrata	
Incubation period (d)	TP field:laboratory	Field	Laboratory
7	1.32	0.28	0.21
16	1.70	0.22	0.14
22	1.30	0.16	0.13

nutrients from both water and substratum. Experiments demonstrate both additive and synergistic effects of substratum and water nutrient sources, with the nature of the interaction determined by types and amounts of nutrients provided by each source. Inhibition of growth may also be a potential consequence of water–substratum effects; however, experiments were not designed to assess this interaction.

Nutrient Effects on Periphyton Growth

In Experiments IA and II, the overriding influence of substratum-derived nutrients on algal growth, relative to increases in ambient nutrient levels (Fig. 2), is probably due to overall concentration differences between the two sources (Table 1a, 1b) and/or diffusion limitation of growth in water enriched treatments. After ≈3 wk, total diatom biovolume on artifically enriched substrata was similar to that on natural nutrient-rich substrata in the study stream (Pringle 1985a).

In general terms, Carp Creek would be expected to be a P-limited system, based on low ambient PO_4-P levels and high N:P ratios (Table 2; Pringle and Bowers 1984). This explains the lack of periphyton response to the N-enriched substratum treatment alone (Experiment III) and the significant response to P enrichment of overlying waters. Periphyton subsequently become N limited on the latter treatment, explaining the synergistic response to both treatments in combination (Fig. 4). Results obtained in this mesocosm study reflect processes in the natural environment. Jorgensen (1957) showed how com-

petition for silicate between planktonic and epiphytic diatoms resulted in silicate depletion in the water and a dependence of epiphytes on silicate released from host stems of *Phragmites*. It has also been observed that epipelic diatoms obtain silica from the sediment and interstitial water under conditions of low silica concentration in overlying waters (Werner 1977; Happey-Wood and Priddle 1984).

Effects of substratum-derived nutrients may partially explain natural patchy distributions of attached algae that are so commonly observed in aquatic systems (Patrick 1977). The biomass of algae growing on sediment has been reported to increase as the C and P content of sediments increase (Porcella et al. 1970; Skorik et al. 1972). The larval retreats of tube-building Chironomidae constitute spatially fixed nutrient micropatches (Pringle 1985a); algal communities attached to retreats can exploit chironomid excreta over sustained periods of time. Local nutrient sources can cumulatively represent a significant large-scale phenomenon because of their ubiquitous nature. For instance, given that invertebrate excretion is estimated to account for most of the P released from aerobic sediments in Lake Michigan (Gardner et al. 1981), it is safe to assume that localized effects of benthic invertebrate excretion alone cumulatively can exert a system-wide influence on the growth and distribution of epipelic algae.

Although measurements of ambient nutrient levels may imply limitation by one or more nutrients, such information does not reveal tight nutrient cycling that can occur within periphyton–substratum complexes. Ambient nutrient levels alone are clearly not a reliable indicator of the trophic status of periphyton populations (Elwood et al. 1983).

Periphyton as a Biotic Filter

Attached biota in top layers of fertilized substrata acted as a filter or temporary sink for P, as indicated by the inverse diffusion gradient in field-incubated substrata (Fig. 5). Results imply that the epipelic community was most effective in impeding P loss during the middle of the study period while P was still being released from bottom substratum layers (Table 4) and while algae was still in the exponential growth phase. Algae are not the only component of the epipelic community, however. Fungi and bacteria also play a potentially important role in mediating P release from substrata. Interactions occurring in algal–bacterial systems have become increasingly well-documented in laboratory studies (e.g. Currie and Kalff 1984; Chirac et al. 1985) and warrant further investigation in the field.

Algal communities on enriched substrata exhibit general decreases in ratios of chlorophyll *a* to biovolume through time, suggesting differences in the nutritional state of periphyton communities at different successional stages. Furthermore, the similarity of ratios between enriched and unenriched substratum treatments after 19–22 d (Table 3) may be evidence for an increasing similarity in the nutritional state of periphyton populations, as nutrients in the substratum become depleted. TP associated with the top periphyton/substratum layer of enriched substrata also decreased or remained the same between the first and last sampling dates (Fig. 6a, 6c), while chlorophyll *a* and biovolume increased significantly by up to 30-fold (Fig. 2). While further studies are required to quantify the role of bacterial, fungal, and algal components in mediating P release, given the high relative biomass of attached algae and its demonstrated ability to store P beyond immediate physiological needs (e.g. Rhee 1972), data suggest luxury consumption of P by algae during early colonization. This would account for high rates of algal growth after nutrient levels decrease in the lower substratum.

In Experiment I, where 0.5 mol P substrata were employed (e.g. see Fig. 5), lower layers of enriched substrata may continue to release P, supporting growth of attached algal populations throughout the experiment. In Experiment II (0.1 mol P enriched substrata), however, TP and PO_4-P in lower substratum layers did not decrease significantly between 16 and 19 d, implying that P was not actively being released in measureable quantities. There is also a significant and concomitant decrease ($P < 0.05$) in TP associated with the top epipelon/substratum layer on enriched substrata not exposed to water enrichment during this time interval (Fig. 6c), further suggesting that positive treatment effects were due to luxury consumption or recycling of nutrients within the microbial community.

The availability of organic P in particulate or dissolved forms in natural systems is not well understood (Porcella et al. 1970; Wetzel 1981). Algal ability to use organic P is dependent on the activity of alkaline phosphatase enzymes that are localized at the cell surface and extracellularly released in many taxa (Healey 1973). Due to the influence of current in streams, one might expect extracellular release of phosphatase enzymes to be an ineffective means of uptake in flowing water. In Experiment II, differences in TP between treatments (Fig. 6c) became significant as the epipelic mat became more developed (Fig. 2c, 2d). Data suggest that mat formation in late succession may enhance nutrient recycling by providing a matrix for uptake (Lock 1981), yet it is unclear whether algae are utilizing organic P directly (from the water and/or the substratum surface where it may become adsorbed) or indirectly as inorganic P, made available via bacterial degradation of adsorbed organic P.

Nutrient-Diffusing Substrata as Assays to Evaluate Nutrient Limitation

Major considerations in using nutrient-diffusing substrata include the direction of nutrient transport from the substratum to the attached algal assemblage and the variability in rate of nutrient release. Both factors may result in algal growth responses that are not reflective of responses to constant nutrient additions to the water. Results suggest that early colonizers are able to exploit initial pulses of P released from the substratum through luxury consumption. Recycling and utilization of stored organic P cannot persist idefinitely: continuous inputs of P to the system are needed to support high sustained growth. Thus, if early colonizers are heavily grazed or sloughed off early in colonization before sampling (e.g. Experiment IB, Fig. 3), nutrient effects may not be measured.

As with other assay techniques, long incubation periods may allow an original source of nutrient to degrade to a different and/or more available form (Fitzgerald 1970). While organic P that was directly added to the water did not degrade to PO_4-P before passing over artificial substrata, at least some of the organic P within enriched substrata, was transformed to PO_4-P, possibly via microbial action or ultraviolet radiation (Fig. 6d).

An additional concern is that the nature of the substratum itself may interfere with treatment effects. Agar is a strongly gelling seaweed hydrocolloid that is extracted primarily from *Gelidium* and *Gracilaria* species of the Rhodophyta (Guiseley

1968). It is derived from cell wall polysaccharides and is primarily composed of galactose. The composition of this polysaccharide varies according to the nutritional state of the marine alga from which it was derived, along with different manufacturing and processing techniques. Agar may thus contain background levels of nutrients that interfere with treatment effects. For instance, Lewin (1955) attributed the growth stimulation of *Nitzschia linearis* by agar extract (Patrick and Wallace 1955) to the silica content of agar. Results presented here indicate that the two lots of agar used in substratum construction contributed significantly different amounts of TP and PO_4-P. This P appears to be largely unavailable and colloidally bound within the substratum (e.g. Fig. 5), and growth on controls was similar between experiments that employed different lots of agar (e.g. see Fig. 3 and 4). Nonetheless, results indicate the desirability of using the same lot of a refined brand of commercial agar that contains low background levels of major and micronutrients.

Since nutrient-diffusing substrata constitute localized sources of a finite amount of nutrient, this can also result in algal responses that are different from responses to nutrient additions to the whole system or larger mesocosms. The localized response of attached algae to nutrients in underlying substrata may have little effect in depressing concentrations of other nutrients in the large volume of water in contact with the algal community. Constantly renewable nutrient sources in streams may consequently prevent secondary nutrient limitation. Secondary effects of P enrichment in lakes often include silica and nitrate depletion, causing shifts from diatom-dominated to blue-green-dominated assemblages (Kilham 1971). Assays conducted in closed systems (e.g. Schelske and Stoermer 1971) have created similar conditions. Classic stream assay techniques that involve continuous enrichment of the whole system (e.g. Warren et al. 1964) or of water diverted into long troughs or channels (e.g. Elwood et al. 1981) may also allow periphyton to depress ambient nutrient levels to the extent that secondary nutrient limitation can occur. The localized effect of nutrient-diffusing substrata may partially explain the absence of N-fixing blue-green algae on P-enriched substrata incubated in Carp Creek, while bioassay studies employing P-diffusing clay pots in nearby Douglas Lake (presumably the source of Carp Creek water) revealed an abundance of N-fixing algae (Fairchild and Lowe 1984). The absence of strong unidirectional current in the latter study may have been instrumental in allowing periphyton populations to locally depress N levels, thereby providing an advantage to N-fixers.

In conclusion, this study provides experimental evidence of the following. (1) Nutrients in both the water and substratum interact to determine periphyton growth response; periphyton can respond to combined influences of water and substratum enrichment in an additive or synergistic fashion, depending on amounts and types of nutrients added from each source. (2) Epipelic communities can be an important component of local nutrient cycling in streams, acting as a sink for P released from nutrient-rich substrata. (3) Nutrient-diffusing substrata are useful detectors of limiting nutrients; however, factors that merit consideration before implementing this technique include (a) temporal variability of nutrient release, (b) direction of nutrient transport from the substratum to the attached algal assemblage, (c) the finite source of nutrient and the feasibility of maintaining a specific algal population through time on a given substratum, (d) potential degradation of nutrients within substrata to a more available form during in situ incubation, (e) the presence of growth promoting and/or inhibitory substances in materials used to construct substrata, and (f) the localized effect of the substratum flora on ambient nutrient chemistry.

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