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Source: *Ecology*, Vol. 62, No. 1 (Feb., 1981), pp. 146-158

Published by: Ecological Society of America

Stable URL: <http://www.jstor.org/stable/1936678>

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THE LIMITING ROLE OF PHOSPHORUS IN A WOODLAND STREAM ECOSYSTEM: EFFECTS OF P ENRICHMENT ON LEAF DECOMPOSITION AND PRIMARY PRODUCERS¹

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Abstract. The limiting role of phosphorus on leaf decomposition and primary producers was investigated in a second-order woodland stream in Tennessee by experimentally enriching, for 95 d, adjacent reaches with an average of 60 and 450 $\mu\text{g PO}_4\text{-P/L}$, respectively, over upstream control levels of $\approx 4 \mu\text{g/L}$. Red oak (*Quercus rubra*) leaf packs in the enriched sections lost mass 24% faster than control packs ($P < .05$). Nitrogen content of the enriched packs increased 60% more, and P content increased 83% more than the respective increases in the control packs ($P < .05$). Differences in mass loss and N and P levels between the low and high enrichments were not significant ($P > .05$). Respiration rates of subsampled leaf discs were significantly higher than control rates only at the high level of enrichment. The increased respiration rates in the low and high enrichments accounted for 10 and 34% of the increased mass loss in the respective enriched sections, suggesting that the enrichment also produced increases in mechanical breakdown through faster microbial conditioning, increases in macroinvertebrate feeding, or both. Effects of the enrichment on aufwuchs initially consisted of increased chlorophyll *a* levels, followed by increased aufwuchs biomass levels. Dense growth of filamentous algae, including some *Oscillatoria*, which may be a nitrogen fixer, developed immediately downstream of P inputs. In addition, *Nostoc*, a known nitrogen-fixing blue-green alga, sampled after the enrichment, was significantly more abundant in the enriched sections than the control ($P < .05$). Densities of the snail, *Goniobasis clavaeformis*, a grazer-shredder sampled after the enrichment, also were significantly greater in the enriched reaches, suggesting that the lack of a sustained response of chlorophyll *a* to the enrichment may have been a result of increased grazing on algal biomass. These findings indicate that nutrient limitation of detrital processing is a significant factor in natural streams. The apparent increases in densities of benthic macroinvertebrates in the enriched sections, along with reported relationships between detrital food richness and macroinvertebrate growth and survivorship, suggest that nutrient limitation in streams also has ramifications on higher trophic levels.

Key words: aufwuchs; chlorophyll *a*; enrichment; *Goniobasis clavaeformis*; invertebrate density; leaf decomposition; nitrogen content; *Nostoc*; nutrient limitation; phosphorus; respiration; stream.

INTRODUCTION

While much work has been published on the limiting role of nutrients in primary production and detrital decomposition of lake and marine systems, their similar role in streams has received far less attention. Despite the fact that detrital decomposition and algal production are known to be influenced by both nitrogen and phosphorus (e.g., Hynes and Kaushik 1969, Rhee 1978, Smith 1979), very little is known about nutrient limitation in natural streams.

One can argue that because of the continuous flow of water, the mechanism of nutrient limitation in streams should be different from that in lakes or marine systems, where biomass can increase to the point of exhaustion of some nutrient. In contrast, lotic ecosystems receive a continual supply of nutrients from upstream so that one would not expect nutrients to exert a primary limitation on algal or microbial (bac-

teria, fungi) biomass. Concentrations of phosphorus and nitrogen typical of streams draining forested watersheds, however, may be sufficiently low that rates of microbial growth and algal photosynthesis are limited though a concentration limitation on nutrient uptake kinetics. Studies on the effects of current on phosphorus uptake by epilithic bacteria and algae (Whitford and Schumacher 1961, Schumacher and Whitford 1965, Lock and John 1979) suggest that at low concentrations, nutrient uptake may be limited by diffusion through either a laminar boundary layer around the cells (Whitford 1960), a film of microorganisms and the polysaccharide-like matrix they produce which surrounds the cells (Gessey et al. 1977), or both. Under a diffusion limitation, rates of algal production and microbial decomposition in streams effectively would be limited by the concentration of nutrients in stream water, even though the nutrient supply from upstream is continuous. Algal and microbial biomass thus would be limited secondarily by nutrients with other factors (e.g., grazing, sloughing) exerting the primary limitation.

In this paper, we describe the results of an experi-

¹ Manuscript received 26 September 1979; revised 5 April 1980; accepted 1 May 1980.

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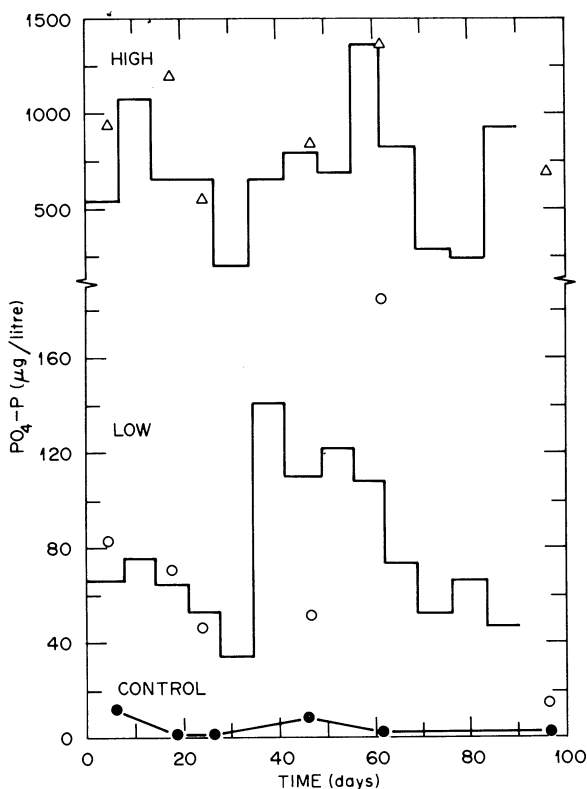


FIG. 1. Phosphorus ($\text{PO}_4\text{-P}$) concentrations in water in the control, low-level, and high-level P-enrichment sections vs. time (days) after the enrichment was started (Day 0 = 10 February). Solid lines are average weekly concentrations computed from P input and streamflow records.

ment to examine the effects of phosphorus enrichment on leaf decomposition and primary producer biomass in Walker Branch, a second-order woodland stream in eastern Tennessee, USA. Concomitant effects on macroinvertebrate populations in leaf packs and on the stream bottom also were examined. We refer to a nutrient as being limiting if an increase in the supply of that nutrient affects the parameter in question. Thus, a parameter whose value is limited by a given nutrient also may respond to increases of another (simultaneously limiting) nutrient, or to changes in rate-controlling factors such as temperature.

We tested for phosphorus limitation because Walker Branch has low levels of soluble reactive phosphorus throughout the year with a mean annual flow-weighted concentration of $3.0 \mu\text{g/L}$, and a molar ratio of inorganic N ($=\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) to P of 31:1, a ratio at which a response of algae to P enrichment would be expected (Rhee 1978, Smith 1979). In addition, aufwuchs and fine particulate organic matter (POM) in the sediments of Walker Branch contain high levels of alkaline phosphatase (Saylor et al. 1979), an inducible enzyme formed under phosphorus limitation by some algae (e.g., Fitzgerald and Nelson 1966) and bacteria

(Torriani 1960). Phosphatase level has been shown to be a good indicator of phosphorus deficiency (e.g., Rhee 1973, Healey and Hendzel 1979).

STUDY SITE

The study site was a 340-m reach of Walker Branch, a second-order woodland stream in Walker Branch Watershed located in eastern Tennessee. The study reach lies immediately downstream from the confluence of two small, spring-fed streams collectively draining 97.5 ha. Annual combined discharge, measured by 120° V-notch weirs, ranges from a fall-winter maximum daily average of 340 L/s to a summer average minimum of 4 L/s (Elwood and Cushman 1975). The mean discharge in the study reach during the 95-d study period (8 February–16 May 1978) was 24 L/s.

The flow through the reach is augmented 10–20% by springs and groundwater inputs. Otherwise the reach is uniform with respect to gradient, substrate, and canopy cover. Water temperatures increased from 7°C (mean daily average) initially to 13.5°C in mid-April (≈ 60 d after leaf packs were introduced) and then declined to 12°C , apparently corresponding to development of the canopy cover. There were no measurable temperature gradients within the reach.

The average annual concentrations of phosphorus, as soluble reactive phosphorus (SRP), $\text{NO}_3\text{-N}$, and $\text{NH}_3\text{-N}$, are ≈ 3 , 13, and $22 \mu\text{g/L}$, respectively, from past chemical records. Bicarbonate alkalinity in the stream is $\approx 170 \text{ mg/L}$ (Elwood and Nelson 1972).

MATERIALS AND METHODS

The study reach was divided into three sections: a 70-m upstream control section in which $\text{PO}_4\text{-P}$ concentrations remained below $10 \mu\text{g/L}$, a 150-m section which was continuously enriched to a design level of $100 \mu\text{g/L}$ $\text{PO}_4\text{-P}$, and a 120-m downstream section enriched to a design level of $1000 \mu\text{g/L}$ $\text{PO}_4\text{-P}$. Enrichment solutions were prepared weekly in two 55-gal (200-L) drums with phosphoric acid and stream water. Phosphoric acid concentrations in the drums were based on flow measurements in the stream at the time of mixing. The solutions were siphoned through surgical tubing at $\approx 20 \text{ mL/min}$ to the head of each stream section and introduced at points where rapid mixing occurred, as verified by Rhodamine-B dye observations.

Water samples were taken twice weekly immediately downstream of the inputs in 250-mL, acid-washed polyethylene bottles which were pre-equilibrated with spring water. Levels of orthophosphate were determined by Chamberlain and Shapiro's (1969) 6-s method modified by the addition of potassium antimony tartrate to preserve color (Murphy and Riley 1962). Because of fluctuating streamflows and occasional malfunctioning of the siphon, actual enrichment levels fluctuated (Fig. 1), averaging $\approx 60 \mu\text{g/L}$ and $450 \mu\text{g/L}$ in the middle and downstream sections, respectively.

Water samples also were collected for P analysis at other locations downstream of the inputs several times during the enrichment. There were no detectable downstream gradients in phosphorus concentrations of water in either of the enriched sections. Ammonia-N and NO₃-N were determined on a Technicon Auto Analyzer II using the colorimetric phenate method and the cadmium reduction method, respectively (Environmental Protection Agency 1974).

To measure decomposition of detritus, leaf packs were prepared with red oak (*Quercus rubra*) leaves which were hand picked, preleached for 4 d, and air-dried for 1 wk. Red oak leaves were used because they are a major component of the standing crop of detritus in Walker Branch during winter. Six to 7 g of air-dried leaves were placed in each of 120 3-mm octagonal mesh, nylon net bags, tethered to bricks and placed in the stream on 10 February 1978. Forty packs were placed in each section and spread over a distance of ≈30 m; those in the enriched sections were located from 30 to 60 m downstream of the points of phosphorus input. The mean water depths over the packs, measured at average flow conditions, were 12, 8, and 12 cm in the control, low-level, and high-level sections, respectively. The current, measured just upstream of the individual leaf packs, averaged 0.11, 0.12, and 0.13 m/s in the respective sections; these were not significantly different ($P > .25$). Randomly chosen packs were removed from each section on days 18 ($n = 4$ packs per section), 32 ($n = 4$), 46 ($n = 6$), 60 ($n = 6$), 90 ($n = 10$), and 95 ($n = 10$). Contents of the packs were gently rinsed in distilled water to remove stream sediments. Macroinvertebrates were picked from the leaf packs and enumerated. Subsamples of leaves were removed from the center of the pack with a cork borer and analyzed for microbial activity on a 20-unit Gilson differential respirometer at ambient stream temperature. Packs and subsamples were oven-dried overnight at 100°C and mass was determined to the nearest 0.01 g. All previous air-dried masses were converted to oven-dried masses by an average air-dry to oven-dry difference of 4.5%. Dried leaves were then ground in a Wiley mill and analyzed for nitrogen content on a Perkin-Elmer model 240 elemental analyzer.

To determine phosphorus content of leaves, we ashed ≈0.5 g of ground material overnight at 450°C and dissolved the residue in 4 mL of hot 3N HCl. Deionized water was added to the dissolved ash solution to attain a volume of 10 mL. This solution was analyzed for total phosphorus concentration on a Technicon Auto Analyzer II using the ascorbic acid reduction method (EPA 1974).

To estimate mass loss rates of leaf packs, we used the model $Y_t = Y_0 \exp[-k(t - t_0)]$, in which Y_t is the mass remaining (as percent) after t days in the stream, Y_0 is an estimate of the mass remaining at t_0 , when exponential decay begins, and k is the instantaneous

decay rates (in days⁻¹). In order to test for differences in the rate of mass loss among the three treatments, we used a linearized form of the model which allowed for simultaneous estimation of the slopes:

$$\ln Y_t = \ln Y_0 - \left(\sum_{i=1}^3 k_i X_i \right) (t - t_0),$$

in which $X_i = 1$ if Y_t is from treatment i , otherwise $X_i = 0$, and k_i is the decay rate for treatment i . The lag period t_0 was estimated from the data subjectively. The other parameters were estimated by linear regression of the log-transformed Y values. The hypotheses, H₁: $k_C \neq k_L \neq k_H$; H₂: $k_C = k_L \neq k_H$; and H₃: $k_C \neq k_L = k_H$, where k_C , k_L , and k_H are the decay coefficients for the control, low-level, and high-level enrichments, respectively, were tested against the hypothesis (H₀: $k_C = k_L = k_H = k$) of no treatment effect on decay rate using the extra sums of squares principle (Draper and Smith 1966).

To determine the response of primary producers to phosphorus enrichment, aufwuchs biomass and chlorophyll a levels on artificial substrates were measured over an 85-d period during the enrichment. Glass slides taped to nails were placed on the stream bottom in the vicinity of the leaf packs in each of the three sections on 10 February. Eight slides were randomly selected from each reach for analysis at 14, 28, 42, 56, and 85 d after being placed in the stream. Aufwuchs on the upper surface (19.35-cm² exposed surface) of four of the slides from each section was scraped and analyzed for organic content by determining dry mass loss on combustion at 450°C for 24 h. Chlorophyll a content on the upper surface of the remaining four slides from each section was measured by extracting the chlorophyll in 90% acetone and reading absorbance of the extract at 663, 645, 630, and 750 nm on a Perkin-Elmer model 200 spectrophotometer.

Nitrogen-fixing blue-green alga, *Nostoc*, was sampled on 12 June, 27 d after the P enrichment was terminated. Based on the observation that *Nostoc* was predominantly associated with moss on rocks, we selected five moss-covered rocks at random from each section. The moss was scraped from each rock and the *Nostoc* was then separated from the moss by hand. A ratio of *Nostoc*:moss biomass for each rock was then determined, based on the dry mass loss on combustion at 450°C.

Chlorophyll a data for each treatment were fitted by least squares to the semilogarithmic regression of chlorophyll a density on time, $Y_t = Y_0 e^{kt}$, where Y_t is the concentration of chlorophyll a per unit area at time t , Y_0 is the intercept of the regression, k is the instantaneous rate of increase in chlorophyll a , and t is the time (in days) that substrates had been in the stream. Because of variance of chlorophyll a within a treatment was not homogenous for all observation times, we used a weighted regression analysis where the weights were reciprocals of the sample variance

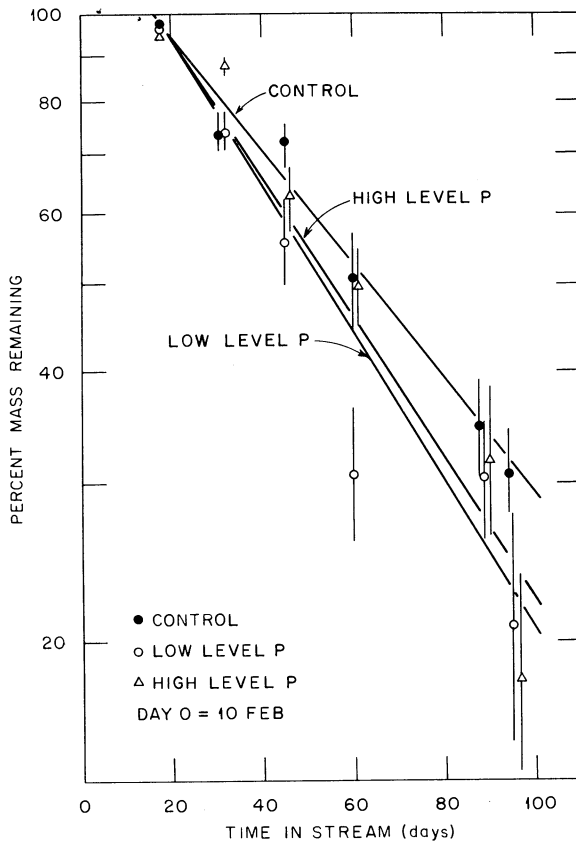


FIG. 2. Mass loss of red oak leaf packs expressed as percent of original mass remaining in P-enriched and control sections of Walker Branch, Tennessee. Geometric means (± 2 SE) of 4–10 leaf packs are plotted. Superimposed are the regression lines, $Y_t = 100e^{-k(t-t_0)}$ where Y_t is percent mass remaining, t is time (days) after leaf packs were placed in the stream, t_0 is when exponential decay of the leaf packs began, and k is the decay rate. Regressions were fitted using all samples ($n = 40$ for each treatment and control).

at each time (Draper and Smith 1966). The test for a treatment effect on the rate of increase in chlorophyll a was based on an analysis of the residual sums of squares for each regression. The two-sided Wilcoxon signed ranks test (Hollander and Wolfe 1973) was used to test for a treatment effect on chlorophyll a and aufwuchs biomass. The hypothesis of a change in the rank distribution of chlorophyll a concentration or aufwuchs biomass in the enriched sections ($\Delta \neq 0$) within each observation time was tested against the null hypothesis of no change in the distribution of ranks of these two variables ($\Delta = 0$). Unless otherwise specified, results are regarded as being statistically significant whenever $P \leq .05$.

RESULTS

Mass loss of leaf packs

Regressions of the log-transformed mass loss data produced decay coefficients $k_C = 0.015$, $k_L = 0.019$,

and $k_H = 0.018 \text{ d}^{-1}$ (Fig. 2). The null hypothesis ($k_C = k_L = k_H$) was rejected for all three alternate hypotheses ($H_1: k_C \neq k_L \neq k_H$; $H_2: k_C = k_L \neq k_H$; $H_3: k_C \neq k_L = k_H$), indicating that the decay rates of leaf packs in both enriched sections were significantly greater than those of the control. However, H_1 could not be rejected in favor of H_3 , indicating that the difference between the two enrichment levels was not significant. The decay rate for the two enrichment levels combined, k_E , was 0.018 d^{-1} , $\approx 24\%$ above the control decay rate.

Respiration

Results of a two-way ANOVA (date, treatment) showed a significant treatment effect on respiration rates of leaf packs (Fig. 3). The time-weighted mean respiration rate for the high-level enrichment ($279 \text{ L O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) was 37% greater than for the control ($203 \text{ L O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). The mean respiration rate for the low enrichment level ($224 \text{ L O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$), however, was not significantly different from either the control or high-level enrichment rates (Duncan's multiple range test, $P > .05$). Analyses of variance of respiration rates on individual dates showed significant treatment effects only on days 60 and 90.

We converted respiration rates to equivalent decay rates, k , assuming a respiratory quotient (CO_2 expired/ O_2 consumed) of 0.9 (Winberg et al. 1971), and using a measured carbon content in red oak leaves of 0.45 g C/g ash-free dry mass. The time-weighted mean rates of mass loss from microbial decomposition alone for the control, low-level, and high-level enrichment were 0.005, 0.006, and 0.007 d^{-1} , respectively. This means that microbial respiration accounted for roughly 33, 31, and 39% of the mass loss in the three sections. The increased respiration rates in the low- and high-level enrichments accounted for only 10 and 34% of the increased rates of mass loss reported in the respective enrichment sections, suggesting that the enrichment also produced increases in mechanical breakdown (through faster microbial conditioning), increases in macroinvertebrate feeding, or both. The smaller role of respiration in mass loss in the low-level enrichment may be related to macroinvertebrate feeding activity (see below).

Nitrogen and phosphorus content of leaf packs

The nitrogen content of the leaf packs (as percent of dry mass) was significantly greater in the phosphorus-enriched sections than in the control section (two-way ANOVA). As with the decay rates there was no significant difference between the two enrichment levels (Duncan's multiple range test). The N content of leaf packs in the enriched sections increased significantly between 0 and 18 d, whereas the control leaf packs showed no increase during this period (Fig. 4). By 32 d the N content of leaf packs in all sections had

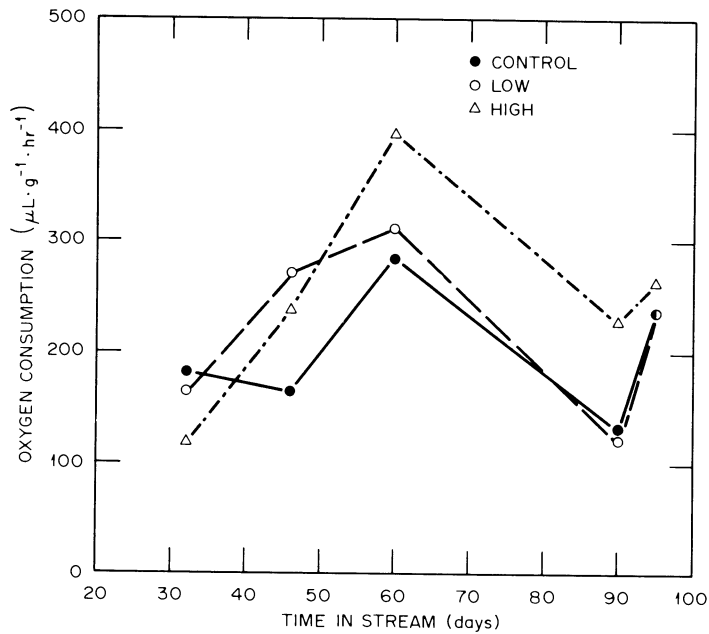


FIG. 3. Mean respiration rates of red oak leaf packs from P-enriched and control sections of Walker Branch, Tennessee.

increased, but the increase in the enriched sections was ≈60% greater than that in the control. The total amount of nitrogen in the leaf packs increased in the enriched sections in the 1st 18 d (a period of very small mass loss), but declined thereafter. The control packs showed a net loss of nitrogen, in absolute terms, throughout the study. Nevertheless, since increases in nitrogen content (as percent) of the control packs exceeded mass losses due to respiration between 18 and 32 d, it appears that N uptake from the water was occurring in all three sections.

Phosphorus content was measured only for leaf

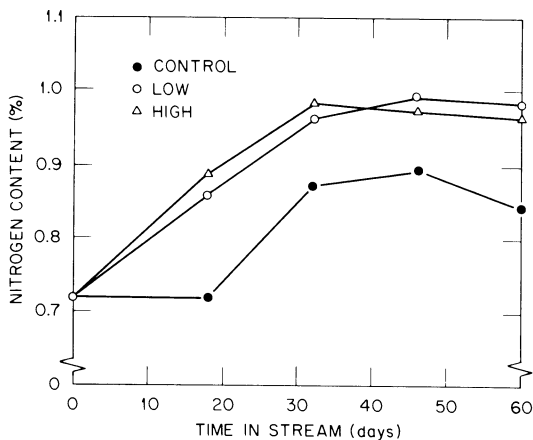


FIG. 4. Nitrogen content of red oak leaf packs in P-enriched and control sections of Walker Branch, Tennessee. Values plotted are means of four to six samples. SE < 0.06% for all estimates.

packs removed after 18 and 32 d. The P content of leaves in both enriched sections was significantly greater than in the controls (Table 1). At 18 d the high-level enrichment showed a significantly higher P content than the low level, but by 32 d differences between the two enrichments were not significant. The increase in P content of leaves between 0 and 32 d was ≈83% greater in the enriched sections than in the control.

Benthic macroinvertebrates

We analyzed the numbers of benthic macroinvertebrates on the leaf packs using two-way (time and treatment) analysis of covariance (Steel and Torrie 1960) with mass of leaf remaining in the pack as the covariate. The effects of all three factors were significant ($P \leq .01$). Fig. 5 shows the mean numbers of invertebrates per pack after adjusting for treatment differences in mean leaf pack mass within each sampling time. This was done using the slope of the regres-

TABLE 1. Median percent phosphorus in leaf packs (range [maximum - minimum] given in parentheses; $n = 4$ packs for 18 d, $n = 6$ packs for 32 d) in P-enriched and control sections of Walker Branch, Tennessee. The initial P content of packs placed in stream was 0.021% (0.003).

Number of days incubation	Control	Low-level P enrichment	High-level P enrichment
18	0.023 (0.004)	0.031 (0.006)	0.041 (0.004)
32	0.033 (0.005)	0.052 (0.008)	0.046 (0.008)

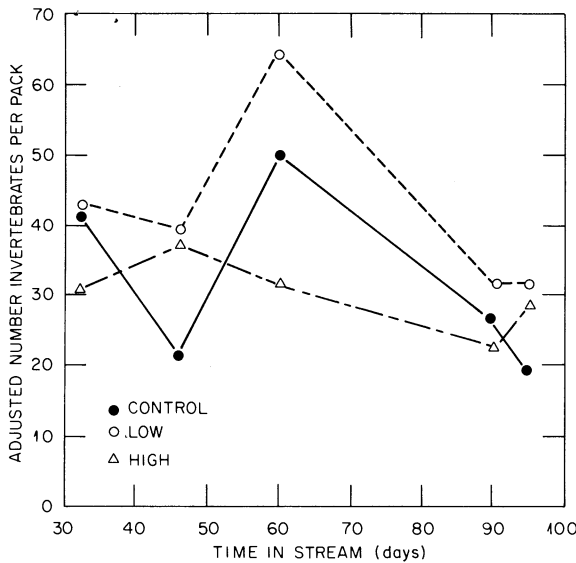


FIG. 5. Adjusted mean numbers of benthic macroinvertebrates per leaf pack from P-enriched and control sections of Walker Branch, Tennessee. The adjusted means, determined from two-way analysis of covariance, are estimates of the expected numbers of invertebrates per leaf pack if the treatment means of leaf pack masses at each time were the same. Adjustments to actual means were all ≤ 8.2 organisms per pack.

sion line for the covariate (leaf pack mass). Adjusted means in the low-level enrichment were consistently greater than those of the control, but the means from packs in the high-level enrichment showed no consistent pattern, with the average (over all times) being nearly identical to that of the control. This absence of an apparent effect in the high-level reach suggests that the effect observed in the low-level reach may be spurious, arising from chance reach-to-reach variations.

A sampling of the benthos on 22 May 1978 (Day 101; Table 2) showed that densities of organisms on the stream bottom were not significantly different among the sections (one-way ANOVA of log-transformed numbers). However, there was a trend of in-

TABLE 2. Geometric mean densities of benthic macroinvertebrates and *Goniobasis clavaeformis* in P-enriched and control sections of Walker Branch, Tennessee, 101 d after the enrichment was initiated. Ten 0.1-m² samples were taken in each section.

	Number/m ²		
	Control	Low-level P enrichment	High-level P enrichment
Total	187	253	334
<i>Goniobasis clavaeformis</i>	119	233	294

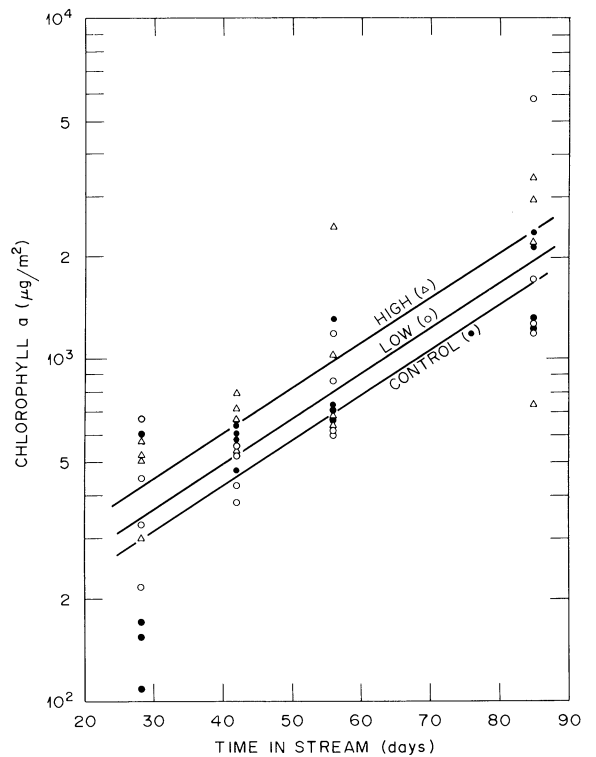


FIG. 6. Chlorophyll *a* per unit area on glass slides in P-enriched and control sections of Walker Branch, Tennessee. Superimposed are the weighted regression lines $Y_t = Y_0 e^{kt}$, where Y_t is the chlorophyll *a*/per square metre at time t (days), k is the instantaneous rate of increase in chlorophyll *a* (in days⁻¹), and Y_0 is the estimated intercept of Y . Regressions were fitted using all samples ($n = 16$ for each treatment and control).

creasing densities with higher levels of enrichment. The snail, *Goniobasis clavaeformis*, a facultative grazer-shredder which constituted $\approx 83\%$ of the total number of macroinvertebrates, and $>95\%$ of the macroinvertebrate biomass, was significantly more abundant in the enriched sections (Table 2). The difference in snail density between the two enriched sections was not significant. We did no benthic sampling prior to the experimental enrichment, so it is possible that these differences were already present. However, since the study reach is uniform with respect to physical characteristics, and the density increases of 2 and 2.5 times above control levels occurred within a distance of 250 m, we tentatively ascribe these differences in snail density to the enrichment effect.

Chlorophyll a and aufwuchs biomass

Chlorophyll *a* levels on glass slides in both the control and enriched sections increased throughout the 85-d period (Fig. 6). For the samples collected on days 28 through 85, chlorophyll *a* levels can be adequately described as semilogarithmic functions of the length

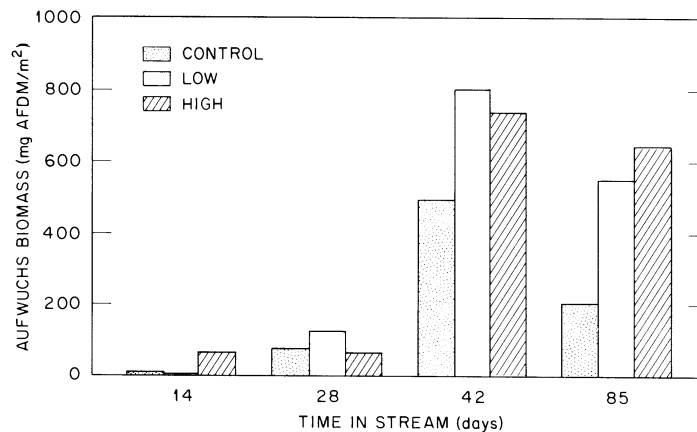


FIG. 7. Median aufwuchs biomass as milligrams ash-free dry mass (AFDM) per unit area on glass slides in P-enriched and control sections of Walker Branch, Tennessee, in relation to the time the substrates had been in the stream.

of time substrates had been in the stream. Levels of chlorophyll *a* on glass slides in all three sections on day 14 had not yet attained measurable quantities. Analysis of the residual sums of squares for the regression coefficients of chlorophyll *a* density on time showed that the slopes of the three regressions were not significantly different ($P > .05$) from each other (i.e., $k_c = k_l = k_H$), indicating that the enrichment had no effect on the rate of chlorophyll *a* accumulation from days 28 through 85. While there was a trend of increasing chlorophyll *a* levels with higher levels of enrichment from days 28 through 85, the difference between the control and enriched sections was significant only on day 28. Differences in chlorophyll *a* between the two enriched sections, however, were not significant at any of the observation times. These results suggest that colonization and growth of algae in the enriched sections was greater than the control from days 0 to 28. The intercept of the regression for the high-level enrichment was also significantly greater than the control, another indication of faster colonization and growth of algae in the high enrichment during the 0- to 28-d period. Thereafter, the estimated rate of increase in chlorophyll *a* in the enriched sections was not significantly different from the control (Fig. 6).

Aufwuchs biomass levels in the enriched sections were significantly greater than the control on days 42 and 85 but not on days 14 and 28. Biomass differences between the two enriched sections were not significant at any of the observation times. Whereas chlorophyll *a* levels in all three sections increased throughout the 85-d period of measurement, aufwuchs biomass on glass slides reached a maximum on day 42 and had declined by day 85 (Fig. 7). Aufwuchs were lost from the 56-d slides collected for biomass measurements after they had been sampled so biomass data for this time were not included in the analysis.

The blue-green alga, *Nostoc*, sampled randomly

from moss habitat, was more abundant in the enriched reaches than in the control, and more abundant in the high enrichment than in the low enrichment ($P \leq .001$, Kruskal-Wallis test, five samples per reach). The mean densities of *Nostoc* were 5.1, 17.0, and 77.7 mg *Nostoc*/g moss in the control, low-, and high-enrichment reaches, respectively, with no overlap of the ranges between treatments. We do not have pre-enrichment *Nostoc* densities, but it is unlikely that differences of this magnitude and consistency could have occurred in the absence of enrichment.

In addition to the greater density of *Nostoc* in the enriched sections, dense growths of predominantly filamentous green algae, but including some of the blue-green alga, *Oscillatoria*, developed in the enriched sections immediately downstream of the phosphorus inputs. These growths, however, were not quantified.

DISCUSSION

The continuous P enrichment of Walker Branch increased rates of detrital processing as measured by decomposition, respiration, and N immobilization. The effect of the enrichment on aufwuchs initially consisted of increased chlorophyll *a* levels, followed by increased aufwuchs biomass levels. In addition, the blue-green alga, *Nostoc*, was much more abundant in the enriched sections than in the control section, and blooms of filamentous algae were observed immediately below the points of enrichment. The grazer-shredder *Goniobasis* was also more abundant in the enriched sections.

Based on laboratory studies of N and N + P enrichment, Hynes and Kaushik (1969) and Howarth and Fisher (1976) reported increases in decay rates of leaves of 26–40% over control rates. In comparison, the combined decay rate of leaves in the P-enriched sections of Walker Branch was 24% greater than the control rate. The decay rates reported here are similar to those reported by Suberkropp et al. (1975) for white

oak leaves in a natural stream with a comparable thermal regime.

The respiration rates of leaves from Walker Branch were within the ranges reported for leaf packs in natural streams (Petersen and Cummins 1974, Triska et al. 1975), and followed a temporal pattern similar to those reported by Triska and Sedell (1976). Both the initial peak and the subsequent decline in respiration rates, however, occurred considerably sooner for the leaf packs in Walker Branch.

In view of the importance of nitrogen in decomposition processes (e.g., see Park 1976), the stimulatory effect of phosphorus on N content of leaf detritus in Walker Branch (Fig. 4) is strong evidence that phosphorus was limiting the decomposition process. Because the variability of N content between replicate samples was low (SD = 0.064%), N content appears to be a more sensitive indicator of P limitation than are mass loss and respiration rates, both of which are subject to large variability.

The N content of the leaf packs in all sections never exceeded 1%, whereas the peak N content of decomposing leaves in natural streams generally lies between 1 and 3% (Mathews and Kowalczewski 1969, Kaushik and Hynes 1971, Iversen 1973, Triska et al. 1975, Suberkropp et al. 1976, Triska and Buckley 1978). Of the studies cited, the only reported peak N contents of leaves <1% (Triska et al. 1975, Triska and Buckley 1978) were from a stream with very low N concentrations in the water (5 $\mu\text{g/L}$), but relatively high P levels (200 $\mu\text{g/L}$) (Sedell et al. 1975). Thus, it appears that N accumulation by microbes on leaves in the control section of Walker Branch was P-limited, while that in the enriched sections was N-limited.

In contrast to the responses of mass loss, respiration, and N content of leaf packs, the increases in P content do not necessarily imply a limiting role for P. Phosphorus may simply have been taken up by microorganisms in excess of requirements, or alternatively it may have been adsorbed onto leaf surfaces. In either case, however, the capacity for P uptake appears to have been saturated at the low-level enrichment of 60 $\mu\text{g/L}$.

While the discrepancy between low- and high-level enrichment densities of the invertebrates in leaf packs remains unexplained, the higher densities in the low-level enrichment (Fig. 5) may have been responsible for the apparently (but not significantly) faster mass loss in that reach. Several workers have explained differences in decomposition rates in terms of macroinvertebrate populations (Hart and Howmiller 1975, Iversen 1975, Sedell et al. 1975), but others have found no relationship (Mathews and Kowalczewski 1969, Kaushik and Hynes 1971, Reice 1977, Meyer 1980).

Although chlorophyll *a* levels on the glass slides responded initially to enrichment, the apparent absence of an effect from 42 through 85 d suggests that either P did not limit algal growth during this period,

or that other factors obscured an effect on chlorophyll levels. Levels of both total inorganic nitrogen (TIN = $\text{NO}_3\text{-N} + \text{NH}_3\text{-N}$) and soluble reactive phosphorus in Walker Branch are low, averaging annually ≈ 35 and 3 $\mu\text{g/L}$, respectively. During the period of enrichment, TIN in the control averaged 41 $\mu\text{g/L}$. Assuming a P concentration of 3 $\mu\text{g/L}$, the N:P ratio for stream water in the control was in the range of reported molar N:P ratios for algae of 16:1 (Redfield 1958) to 30:1 (Rhee 1978) and would include the range of N:P ratios where dual N and P limitation would be expected (Smith 1979). At these low P levels, small changes in concentration of either nutrient, particularly P, will have a significant effect on the N:P ratio. Thus, with the low N and P levels in stream water, algae in Walker Branch may effectively be under a dual N and P limitation as the N:P ratio fluctuates below or above the optimum for algal growth. The initial response of chlorophyll *a* on the glass slides, the response of *Nostoc*, and the growths of filamentous algae, however, all indicate that P limited growth of some algal species during at least part of the enrichment period. Qualitative examination of algae growing on glass slides after 85 d of enrichment showed a predominance of diatoms, with no apparent differences in species composition between the control and enriched sections.

Stockner and Shortreed (1976) observed a twofold increase in chlorophyll *a* and aufwuchs biomass over controls when $\text{PO}_4\text{-P}$ and $\text{NO}_3\text{-N}$ levels were simultaneously doubled in Carnation Creek water which was diverted into channels adjacent to the stream. They suggested that P was probably the more critical nutrient in their stream because of the low concentrations present throughout the year. The N:P ratio in Carnation Creek of ≈ 56 would indicate a P limitation on algal growth in this stream. In a later experiment (Stockner and Shortreed 1978) in which they added N and P singly and in combination, addition of N alone had no appreciable effect on algal growth. However, tripling of the P concentration resulted in an eightfold increase in algal biomass over controls after 35 d. Addition of N + P resulted in an even greater increase in algal biomass. Manuel and Minshall (*in press*) also found significantly higher levels of aufwuchs biomass and chlorophyll in streamside channels enriched with N and P, using stream water with an ambient N:P ratio of 12, a value at which N limitation would be expected. Enrichment of the stream itself, however, resulted in no significant increase in aufwuchs biomass or chlorophyll relative to the control. Manuel and Minshall suggested that the effects of enrichment on aufwuchs biomass may have been obscured by greater grazing and sloughing of biomass in the stream. In contrast to these studies in which stimulatory effects of enrichment on stream aufwuchs were observed, Wuhrmann and Eichenberger (1975) saw no significant effect of P, N, or N + P enrichment on primary producers in ar-

tificial streams receiving groundwater with a $\text{PO}_4\text{-P}$ concentration of $10 \mu\text{g/L}$. The ambient N:P ratio in their channels was 96:1, a value where phosphorus limitation on algal growth clearly would be expected. In a second experiment, however, they observed significant increases in aufwuchs biomass when the water was enriched with a mixture of trace elements. We cannot exclude the possibility that primary producers in Walker Branch were similarly limited by one or more essential micronutrients. Other reported attempts to relate standing crop of algae to P and/or N levels in streams have not been successful (Kilkus et al. 1975, Moore 1977) probably because the streams studied were well lighted, enriched environments draining agricultural watersheds where N and/or P limitation would be less likely to occur.

It is possible that differences in grazing pressure between the control and enriched sections prevented a sustained increase in rates of chlorophyll *a* accumulation in the enriched reaches. As described earlier, the mean density of *Goniobasis clavaeformis*, the predominant grazer in Walker Branch, was significantly greater in the enriched sections (Table 2). Earlier estimates of grazing on aufwuchs in Walker Branch indicated that total grazing rates were comparable to and, during certain periods, exceeded primary production rates (Elwood and Nelson 1972). Results of laboratory-measured grazing rates by *Goniobasis* at different constant temperatures (Elwood and Goldstein 1975) indicate that this species alone could limit primary production rates in Walker Branch by controlling the standing crop of aufwuchs. *Goniobasis* were observed grazing on the glass slides in both the enriched and control sections, but attempts to exclude them from the artificial substrates, in order to eliminate any effect resulting from possible differences in grazing pressure between sections, were unsuccessful.

The response of algae and grazers in Walker Branch to the phosphorus enrichment thus may have been similar to that observed for phytoplankton and zooplankton in Great Central Lake, British Columbia, Canada, after the lake was enriched with both N and P (McAllister et al. 1972, LeBrasseur et al. 1978). Except for a brief period immediately after N and P additions to the lake, the standing crop of phytoplankton remained unchanged. The primary production rate, however, was double that measured in the year prior to enrichment and the standing crop of zooplankton increased by a factor of eight. Thus, despite increased primary production after fertilization, increased grazing pressure was sufficient to maintain a constant biomass of phytoplankton in the lake. Results from several laboratory and field studies have indicated the importance of grazing in limiting algal biomass in streams (Douglas 1958, McIntire 1968, Elwood and Nelson 1972, Kehde and Wilhm 1972, Smrchek et al. 1976, Moore 1977, Eichenberger and Schlatter 1978, Manuel and Minshall, *in press*). However, the quan-

titative effects of grazers on primary production rates in natural streams have not been directly demonstrated. The response of algal biomass to phosphorus enrichment observed by Stockner and Shortreed (1978) occurred under conditions of low grazing pressure.

The significantly greater biomass of aufwuchs in the enriched sections on days 42 and 85, and the lack of a significant difference in chlorophyll levels at these same times, could be a result of several possible factors, including differences in the selectivity of grazing on algal and non-algal biomass between the control and enriched reaches, changes in the chlorophyll *a* content of algal cells, or increased formation of non-algal aufwuchs in the enriched sections. Calow (1973) demonstrated that the selectivity of grazing on certain algal species (primarily diatoms) by *Ancylus fluviatilis*, a pulmonate snail similar to *Goniobasis*, increased as the standing crop of epilithic algae increased. Besides algae, aufwuchs communities in woodland streams contain several other components which are also potential sources of food to grazers. These include bacteria, fungi, and epilithic detritus with its associated microflora. Much of the non-algal biomass in the aufwuchs community of forested streams such as Walker Branch has been shown to consist of this detritus which is predominantly fragments of leaves and other vegetative parts from streamside vegetation (Madsen 1972, Karlstrom 1978). Analysis of four detritus size classes (<0.045, 0.045–0.150, 0.150–0.250, 0.250–1.000 mm) collected from the sediments in the west fork of Walker Branch prior to this experiment showed an increasing trend in the C:N ratio with decreasing particle size, ranging from 18:1 to 30:1 (mass basis). Based on these results, the C:N ratio in the epilithic detritus in Walker Branch would be expected to be greater than the 17:1 ratio generally considered to be maximum for the required protein intake by non-ruminant animals (Russell-Hunter 1970). Since diatoms, which are the predominant algal species in the aufwuchs in Walker Branch, have a lower C:N ratio than this, grazers such as *Goniobasis* may select algae over this allochthonous detritus. A shift in feeding selectivity together with the increase in grazer density thus could have obscured differences in the rate of chlorophyll *a* accumulation between the enriched and control sections. Since grazing pressure on non-algal biomass in the enriched sections theoretically would decrease, this could account for the significantly greater standing crop of aufwuchs in the enriched sections, most of which was apparently non-algal biomass, on days 42 and 85.

It is also possible that the differences in the chlorophyll:aufwuchs biomass ratio between the enriched and control sections resulted from a decrease in the chlorophyll *a* content of algal cells in the enriched sections. Rhee (1978) observed a twofold increase in chlorophyll *a* per cell in *Scenedesmus* cultures when the N:P ratio of the medium was increased from 30:1

to $\approx 50:1$ by adding $\text{NO}_3\text{-N}$ alone. He concluded that N limitation impairs chlorophyll *a* synthesis much more than P limitation. This suggests that the low N:P ratios ($<1:1$) in the stream water in the enriched sections may have reduced the chlorophyll *a* content of algal cells. However, since differential grazing, differences in chlorophyll *a* content, or increased formation rate of non-algal aufwuchs by heterotrophic decomposition of coarse POM to fine POM could explain our observations, we can only suggest that these factors were actually involved.

In general, responses produced at the high-level P enrichment ($450 \mu\text{g/L}$) were not significantly different from those at the low-level enrichment ($60 \mu\text{g/L}$), indicating that phosphorus limitation occurred at a P concentration $\leq 60 \mu\text{g/L}$. The low N:P ratio ($1.5:1$) in the low-level enrichment water suggests that N limitation may have been important once the P limitation was removed. In contrast to the other variables we measured, *Nostoc* abundance was much greater in the high-level reach than in the low-level reach. But since *Nostoc* is able to supply its own nitrogen, this result tends to support the hypothesis that the P enrichment induced nitrogen limitation in other primary producers and the decomposers. The ability of a stream ecosystem to respond to phosphorus enrichment does not, of course, preclude the possibility that nitrogen was simultaneously limiting. However, a nitrogen enrichment of the same reach of stream the following year produced no stimulatory effects, indicating that Walker Branch was not under dual N and P limitation (J. D. Newbold, *personal communication*).

On the basis of these considerations we can suggest that the optimal N:P ratios for decomposition and primary production in Walker Branch lie between $31:1$ (the ratio in the control water) and $1.5:1$. This range includes the $30:1$ ratio reported by Rhee (1974, 1978) as optimal for growth of the alga, *Scenedesmus*, a portion of the optimum range ($29\text{--}46$) for phytoplankton photosynthesis in lakes (Smith 1979), as well as the $16:1$ ratio widely found in marine particulate matter (Redfield 1958, Goldman et al. 1979). A number of laboratory experiments on decomposition rates in closed systems (Kaushik and Hynes 1971, Fenchel and Harrison 1976, Howarth and Fisher 1976) have shown an interdependence of N and P in limiting decomposition rates, but there has been no systematic investigation of optimal ratios. However, studies examining the effect of enrichment with P alone have shown no stimulatory effects (Egglishaw 1972, Howarth and Fisher 1976, Carpenter and Adams 1979), whereas a response to enrichment with N alone was demonstrated in several of the studies cited here. This pattern, however, was established in closed laboratory systems. While it suggests that optimal N:P ratios may be quite high, and that P limitation would not normally be found in unpolluted water, it is quite possible that the absence of response to P fertilization in laboratory

microcosms is an artifact of the closed systems used in these studies. For example, closed systems have been shown to cycle P very rapidly (Barsdate et al. 1974); if this cycling is faster than that of N, closed systems would probably show a bias towards N limitation. Results from closed systems may be further confounded by such factors as nutrient depletion, build-up of extracellular metabolites, and development of a planktonic community.

While nitrogen stimulation in closed systems is frequently observed, Triska and Sedell's (1976) nitrogen enrichment of an open system (artificial outdoor channels) did not produce significant stimulatory effects on leaf decomposition, even though both the N:P ratio ($\approx 4:1$) and absolute nutrient concentrations ($18\text{--}24 \mu\text{g/L}$ P, $37 \mu\text{g/L}$ N) were relatively low. The suggestion that Triska and Sedell's channels were P limited is consistent with our results, and, if true, implies an optimal N:P ratio for leaf decomposition of $4:1$ or less. Of course, limitation by some factor other than N or P provides an equally plausible explanation for their results. In a comparison of two Oregon streams with very low N:P ratios ($0.05:1$ and $0.3:1$, respectively), Sedell et al. (1975) and Triska et al. (1975) found differences in rates of decomposition and nitrogen immobilization that were apparently related to the differing $\text{NO}_3\text{-N}$ levels in the stream water. Nitrogen limitation would be expected at these N:P ratios, which are well below the lower limit for phosphorus limitation ($1.5:1$) that we found for Walker Branch.

The significantly greater abundance of *Nostoc* in the enriched sections, the presence, immediately downstream of the P inputs, of *Oscillatoria*, which may be a strain that can also fix nitrogen (Kenyon et al. 1972), and the fact that nitrogen fixation has been observed in the microbial decomposition of leaves and wood in streams (Howarth and Fisher 1976, Triska and Buckley 1978) all suggest that streams may behave in a manner similar to that suggested by Schindler (1977) for lakes. That is, the capacity for nitrogen fixation may render streams phosphorus limited even when the supply of nitrogen is low. Schindler had hypothesized that by reducing the N:P ratio in fertilizer added to experimental lakes in Canada, blue-green algae, which are capable of fixing nitrogen, would be favored. In every year that fertilizer with an N:P ratio of $5:1$ was added, nitrogen-fixing blue-green algae dominated the lake phytoplankton. Based on Schindler's results, Stockner and Shortreed (1978) suggested that a reduction in the N:P ratio in their stream channel to as low as $5:1$ would have led to a predominance of nitrogen-fixing blue-green algae. The N:P ratio in their P-enriched channel was $13:1$, compared to ratios of $\approx 1.5:1$ and $0.2:1$, respectively, in the low- and high-enrichment sections of Walker Branch. Thus, while the abundance of nitrogen-fixing algae, relative to other algae, in the enriched sections of Walker Branch is unknown, data on *Nostoc* abundance appear to confirm

Stockner and Shortreed's prediction that the importance of nitrogen-fixing blue-green algae would increase when the N:P ratio was reduced to <5:1. The N:P ratio in Rocky Creek, California, during the period when Horne and Carmiggelt (1975) observed maximum growth of *Nostoc*, was $\approx 3:1$, which also tends to support this prediction.

Although our work has focused on nutrient limitation, we do not underrate the importance of other factors such as current, temperature, and invertebrate activity. We have shown, however, that in a natural stream subject to the influence of a large variety of other factors, nutrient limitation can be significant. This conclusion is supported by the work of Durbin et al. (1979), who found increases in decomposition rates in streams following large inputs of phosphorus and nitrogen from spawning alewives. Our observation of increased macroinvertebrate densities, taken together with the reported relationship between food richness and macroinvertebrate growth rates (Iversen 1974, Anderson and Grafius 1975, Ward and Cummins 1979) and survivorship (Kostalos and Seymour 1976), suggest that the nutrient limitation also extends to higher trophic levels.

Information on the spatial and temporal extent of nutrient limitation in streams obviously must await additional experimental work. Omernik (1977) reported that streams draining predominantly ($\geq 90\%$) forested watersheds in the eastern United States had orthophosphate concentrations averaging $6 \mu\text{g/L}$, a level comparable to that in the control section of Walker Branch. Further, the molar ratio of inorganic nitrogen to orthophosphorus in these forested streams was 68:1. Based on our results from Walker Branch, this suggests that phosphorus limitation on detrital decomposition and primary production in woodland streams may be widespread.

ACKNOWLEDGMENTS

A number of people contributed substantially to this work. A. L. Sheldon sorted and identified the macroinvertebrates from the leaf packs; M. N. Ferguson and M. A. N. Silver provided chemical analyses; K. D. Kumar and J. J. Beauchamp assisted with statistical analysis; and J. M. Loar and F. S. Sanders reviewed the manuscript.

This research was supported by the National Science Foundation's Ecosystem Studies Program under Interagency Agreement DEB 78-03012 with the United States Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

This paper is Publication 1517, Environmental Sciences Division, Oak Ridge National Laboratory.

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