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Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems

ROBERT W. HOWARTH* *and*
STUART G. FISHER
Department of Biology, Amherst College, Amherst, Mass. 01002, U.S.A.

Summary

We investigated the effects of long-term enrichment with nitrate, phosphate, and nitrate + phosphate on the first 5 weeks of leaf detritus processing in laboratory stream microecosystems. Enrichment with nitrate + phosphate accelerated leaf weight loss and increased rates of respiration associated with the leaves. However, whole-system respiration was little changed from that observed in the control stream since respiration in the water was greatly reduced. Enrichment with phosphate alone had little effect except to lower respiration associated with leaf discs. Enrichment with nitrate alone also decreased leaf-disc respiration but resulted in a greatly increased rate of respiration in the water. Net leaching and fragmentation of carbon from the leaves was also increased by nitrate enrichment.

Nitrogen and phosphorus levels in leaf material were little affected by enrichment with nitrate or phosphorus alone. Leaves in those streams and in the control stream released nitrogen and phosphorus to the water. In contrast, percent nitrogen and phosphorus increased greatly in the leaves in the stream enriched with both nitrate and phosphate. The leaves in this system immobilized both nitrogen and phosphorus from the water.

We also studied the importance of nitrogen fixation as a vector for nitrogen incorporation associated with leaf decomposition in streams. Somewhat surprisingly, fixation by microbes associated with the leaves and by microbes suspended in the water occurred under all three experimental enrichment treatments as well as in the control, casting doubt on the effectiveness of nitrate in inhibiting nitrogenase synthesis in nature. However, N_2-fixation is only a minor source of nitrogen for leaves decaying under the conditions studied.

*Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, U.S.A.

Introduction

Stream ecosystems are generally heterotrophic systems where respiration in excess of gross primary production is supported by allochthonous organic matter (Fisher & Likens, 1973; Kaushik & Hynes, 1968, 1971; Minshall, 1967). Since much of the allochthonous detritus in streams enters as leaf litter (Fisher & Likens, 1972; Krumholz, 1972), an understanding of leaf decomposition or 'processing' is requisite to understanding stream ecosystem function.

In both terrestrial and aquatic ecosystems, the nutrient content of leaf litter affects its rate of decay. Leaves high in nitrogen, for example, decay more rapidly than nitrogen-poor leaf species (Bobcock, 1964; Witkamp, 1966; King & Heath, 1967; Kaushik & Hynes, 1971). Since organic carbon is lost during decomposition, the C to N ratio (C/N) of the decomposing litter complex decreases with time. Microbial uptake of dissolved nitrogen from forest throughfall (Gosz, Likens & Bormann, 1973) or from the aqueous medium of aquatic systems (Iversen, 1973) decreases C/N still further, and in some instances may result in an absolute increase of nitrogen in the decomposing litter complex (Witkamp, 1966). Nitrogen fixation as a vector responsible for observed increases of nitrogen in the leaf complex has apparently not been investigated.

Kaushik & Hynes (1971) found that the addition of nitrate or ammonia increased the rate of leaf decay in aquatic laboratory microcosms. When phosphorus was also added (as phosphate), decay rates increased still further. The influence of phosphate alone, however, was not determined in those experiments. Nutrient enrichment may also

accelerate leaf processing at the macroconsumer level since insects are major consumers of leaf detritus in streams (Minshall, 1967; Coffman, Cummins & Wuycheck, 1971) and feed preferentially on leaf material in an advanced state of decay with large microbial populations and low C/N (Cummins *et al.*, 1973; Kaushik & Hynes, 1968).

The present study experimentally determines rates of leaf-detrital processing in small laboratory streams enriched with phosphate and nitrate, alone and in combination. The dynamics of carbon, nitrogen and phosphorus were followed in the detritus complex and in the aqueous medium for the initial 5-week period of decay. Respiration and nitrogen fixation rates by microbes associated with the detritus complex and by microbes in suspension in the water were measured under each of the experimental conditions and in an unenriched control system.

Methods and materials

Two-centimetre diameter leaf discs of sugar maple (*Acer saccharum*) were allowed to decay in recirculating laboratory streams, each consisting of a plexiglass tube 91·5 cm long and 9·7 cm in diameter with a removable cover and sealed ends. Stream water was pumped continuously from one end of the stream to the other at a rate of 200 ml/s, and since each system contained 4 l of water, current velocity was approximately 5 cm/s. Nylon net partitions at intervals of approximately 12 cm retained the leaf discs in 'leaf packs.' Water re-entering the stream from the pump was sprayed through the gas phase of the system to maintain dissolved gases near equilibrium, and compressed air was bubbled constantly through the water to maintain oxygen near 100% saturation. Water temperature was maintained at $18{\cdot}5 \pm 0{\cdot}9$ °C. The streams were covered throughout the experiment to prevent photosynthesis.

Four systems were used. Each contained 4 l of water from a local stream and 370 leaf discs preleached for 24 h in running tap water. One stream was enriched with 10 mg/l NO_3–N, another with 10 mg/l PO_4–P, and a third with 10 mg/l NO_3–N and 10 mg/l PO_4–P. Nitrate and phosphate were added as KNO_3 and K_2HPO_4. The fourth system contained unenriched stream water (0·79 mg/l total N, 0·162 mg/l total P) and served as a control.

The leaf discs remained in the laboratory streams for 5 weeks. At approximately weekly intervals, fifty discs and 320 ml of water were removed for analysis. Water removed in sampling was replaced with an equivalent amount of unenriched stream water.

At weekly intervals, dry weights of the leaf discs were determined after drying at 105 °C for at least 24 h; ash weights were measured after combustion at 550 °C for at least 1·5 h. Carbon content of leaf discs was calculated by assuming organic matter (AFDW) is 47% carbon. Dissolved and fine-particulate organic matter in the water were determined collectively on a weekly basis with a Beckman model 915 total organic carbon analyzer.

Respiration by microbes associated with the leaf discs was estimated weekly by placing samples (six leaf discs with 5 ml of water) in a 5-ml serum bottle and monitoring oxygen changes using a Clark oxygen electrode with a stirrer.

The ammonia content of the water in each of the streams was determined weekly by ammonia electrode (Orion model 95–10). The nitrate content of the water was determined weekly with a colorimetric technique after reduction with hydrazine sulphate.

A modified micro-Kjeldahl digestion technique was used to measure the total nitrogen content of the leaf discs weekly, and total phosphorus at the beginning and end of the 5-week experimental period. The ammonia content of the digests was measured using the Orion ammonia electrode. Phosphorus in the digests was determined spectrophotometrically with the molybdate-antimony technique using an ascorbic acid catalyst.

Nitrogen fixation by microbes associated with leaf discs and fixation by microbes suspended in the water were estimated weekly using the acetylene-reduction assay (Stewart, Fitzgerald & Burris, 1967). For estimating fixation by bacteria in the leaf discs, ten discs and 2·5 ml of water were placed in 5-ml serum bottles. The bottles were sealed with serum stoppers and 0·5 ml of acetylene was added with a 1-ml gas-tight syringe. After a 2-h incubation in the dark, 0·25 ml of a saturated $HgCl_2$ solution was added to each bottle to stop biological activity. One-ml samples of gas were then withdrawn and assayed for ethylene on a gas chromatograph which employed a $2\text{ m} \times 0{\cdot}32$ mm Poropak R (80–100 mesh) column at 45 °C and a carrier gas flow rate of 47·5 ml/min to separate ethylene from acetylene. For estimating fixation by microbes in suspension,

Table 1. Parameters of carbon dynamics in lab-stream microcosms under various conditions of nutrient enrichment. Data indicate changes which occurred over a 5-week incubation period

Parameter	System			
	Control	N	P	N+P
(1) Original disc wt (mg C)	8·61	8·61	8·61	8·61
(2) Total wt loss (mg C)	2·55	3·27	2·28	3·63
% of original wt	30%	38%	26%	42%
(3) Disc respiration (mg C)	1·24	0·91	0·71	2·36
% of original wt	14%	11%	8%	27%
% of total wt loss	49%	28%	31%	65%
% of total respiration	62%	36%	45%	93%
(4) Net leaching & Frag. (mg C) (2–3)	1·31	2·36	1·57	1·27
% of original wt	15%	27%	18%	15%
% of total wt loss	51%	72%	69%	35%
(5) Leachate unutilized (mg C)	0·56	0·74	0·72	1·08
% of original wt	7%	9%	8%	13%
% of total wt loss	22%	23%	32%	30%
% of total leaching	43%	31%	46%	85%
(6) Leachate respired in water (mg C) (4–5)	0·75	1·62	0·85	0·19
% of original wt	9%	19%	10%	2%
% of total wt loss	29%	50%	37%	5%
% of total leaching	57%	69%	54%	15%
% of total respiration	38%	64%	55%	7%
(7) Total respiration (mg C) (3+6)	1·99	2·53	1·56	2·55
% of original wt	23%	29%	18%	30%
% of total wt loss	78%	77%	68%	70%

15-ml water samples were placed in 20-ml serum bottles. The bottles were sealed with serum stoppers, and 1·0 ml of acetylene was added to each. These samples were also incubated in the dark for 2 h and stopped by adding 0·5 ml of $HgCl_2$ solution. One-ml samples of gas were removed and assayed for ethylene by gas chromatography. The rate at which acetylene is reduced to ethylene is directly proportional to the rate of nitrogen fixation, but following the suggestion of Brezonik (1973), our data are reported as ethylene produced.

Results and discussion

Organic carbon dynamics

Weight loss (as organic carbon) of the leaf discs in each experimental system for the 5-week period is reported in Table 1. Disc weight loss was most rapid. in the nitrate and phosphate enriched system (N+P), followed by the nitrate (+N), control, and phosphate enriched (+P) streams. Leaves in the N+P and +N streams lost significantly more weight than those in the +P stream ($P<0·05$). Leaves in the N+P stream lost significantly more weight than those in the control stream at $0·05<P<0·10$. Other differences were not significant.

Weight loss is assumed to be the combined result of aerobic degradation by microorganisms associated directly with the leaves (pathway 1 of Fig. 1) and of leaching and fragmentation of the leaves. Leaching and fragmentation may be either abiotic (pathway 3, Fig. 1) or biotic (resulting from the action of leaf microbes (pathway 4 of Fig. 1). In either case, organic carbon is released to the water. This organic carbon, much of which is dissolved, supports respiration of both suspended microorganisms (pathway 5, Fig. 1) and microbes associated directly with the leaf (pathway 2, Fig. 1). Leaf disc respiration

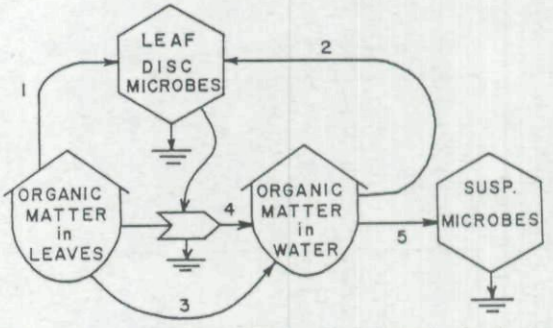

Fig. 1. Diagrammatic model of carbon dynamics in simple lab-stream microcosms. See text for explanation of terms and pathways.

results from the use of both leaf material (pathway 1) and organic carbon in the water (pathway 2) by microbes associated with the leaf disc.

Rates of respiration by microorganisms associated with the leaf discs are two to three times greater in the N+P stream than in the other three streams (Fig. 2). Respiration rates for leaf discs in the +N, +P and control streams are similar to each other (roughly 0·1–0·2 mg O_2/disc/day) and are in the range reported for silver maple leaves in an experimental stream, roughly 0·01–0·02 mg O_2/mg leaf/day (Cummins *et al.*, 1972) and for sugar maple leaves in natural stream ecosystems during the summer, 0·006–0·012 mg O_2/mg leaf/day (Triska, 1970).

The net amount of carbon leached or fragmented from the leaves (Table 1) is the difference between total loss and disc respiration loss. Total carbon respired is calculated by integrating disc respiration curves (Fig. 2) and assuming a respiratory quotient of 0·9. Calculated rates of leaching and fragmentation are net rates only since material respired via pathway 2 (Fig. 1) is not included in the leaching and fragmentation estimate.

Net leaching accounts for only 35% of total weight loss in the N+P system as compared to 51% in the control and nearly 70% in the +N and +P streams. Eighty-five percent of the leached fraction remains unused during the 5-week study period in the N+P stream, while in the other three systems, from 54% to 69% of this material is used in the aqueous phase (Table 1). Since the absolute rate of net leaching (per unit original weight) is no higher in the N+P system than in the other streams, these data may indicate that discs in the N+P system release more refractory organic compounds to the water. Alternatively, gross leaching rates in the N+P stream may be as high as or higher than those in the other systems but the more labile substrates may be quickly metabolized by leaf disc microbes (pathway 2 of Fig. 1). In any event, when both nitrogen and phosphorus are available in excess (the N+P stream), there is a marked tendency for disc organic carbon to be used in the disc complex. While N+P disc respiration rates are two to three times higher than the other systems, N+P total respiration is only 28% higher than the control and is equivalent to the +N system. Thus, enrichment with both nitrogen and phosphorus significantly alters the locus of oxygen consumption in these systems but only slightly affects the total (Table 1).

Enrichment with nitrate alone resulted in an only slightly higher total respiration value than observed in the control system. However, in the +N system, 64% of the total respiration occurred in the aqueous phase as compared to 38% in the control and only 7% in the N+P system (Table 1). Enrichment with phosphate alone resulted in low disc, water, and total respiration values. Fifty-five percent of total respiration occurred in the water in that system.

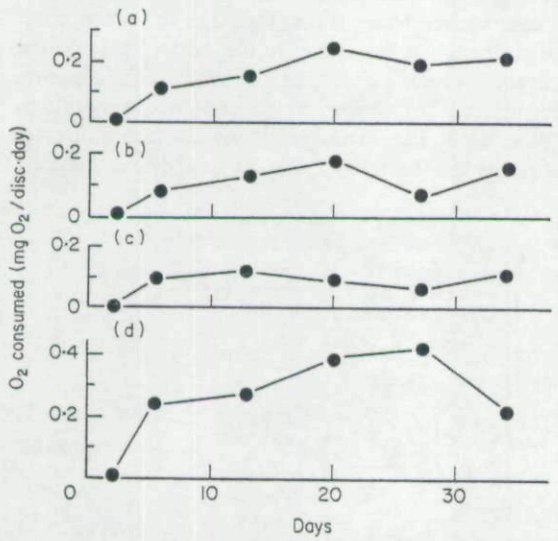

Fig. 2. Rates of respiration of microbes associated with leaf discs in lab-stream microcosms under various conditions of nutrient enrichment. (a) Control; (b) nitrate; (c) phosphate (d) nitrate + phosphate.

Nitrogen dynamics

Percent total nitrogen of leaf material showed small but insignificant ($P \geq 0{\cdot}05$) increases over the 5-week period in the +P, +N and control streams (Fig. 3). By contrast, leaf disc nitrogen increased almost three-fold in the N+P stream from 0·90% (SE = 0·016) at the beginning of the experiment to 2·53% (SE = 0·05) at 5 weeks.

Fig. 4 shows total nitrogen per leaf disc (i.e., nitrogen on an absolute rather than a percent basis) calculated by multiplying nitrogen content values by the average dry weight of the remaining discs. Leaf discs in the N+P system showed an absolute increase in nitrogen content over the 5-week period, indicating a net uptake of nitrogen from other sources. This uptake was mirrored by a precipitous decline in the concentration of nitrate + nitrite in the water of that system (Fig. 4) and a decrease in total nitrogen in the water over 5 weeks (Table 2).

Leaf discs in the other three streams showed

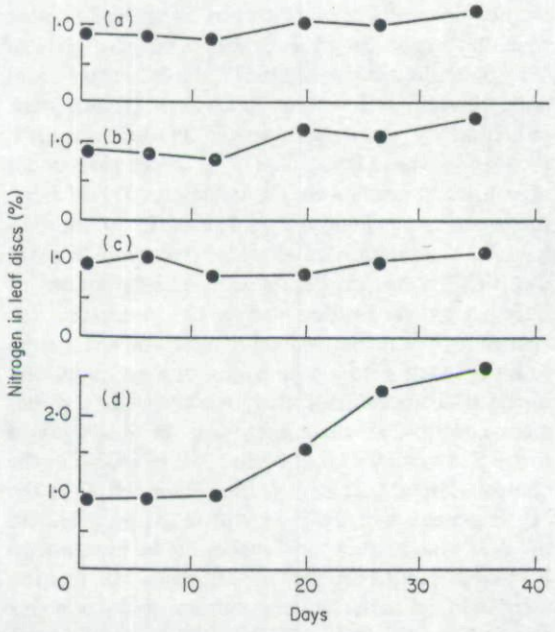

Fig. 3. Percent nitrogen in leaf discs in stream microcosms under various conditions of nutrient enrichment. (a) Control; (b) nitrate; (c) phosphate; (d) nitrate + phosphate.

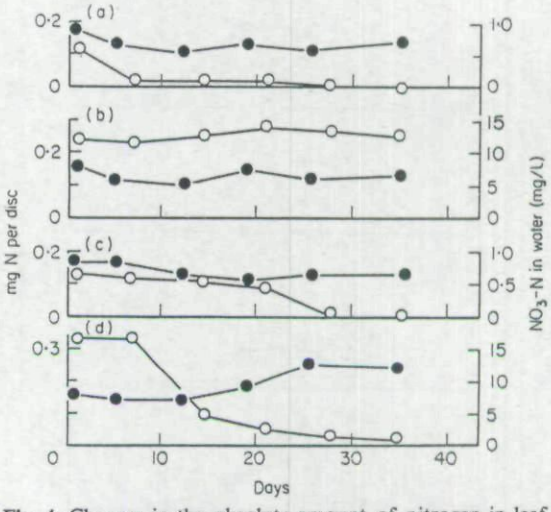

Fig. 4. Changes in the absolute amount of nitrogen in leaf discs (●) and nitrate-nitrogen in solution (○) during the 5-week study period. (a) Control; (b) nitrate; (c) phosphate; (d) nitrate + phosphate.

slight but insignificant decreases ($P \geq 0{\cdot}05$) in total nitrogen per leaf disc over 5 weeks. Rather than immobilizing nitrogen from the aqueous phase, as did the leaf discs in the N+P system, the leaf discs in the +N, +P and control systems released nitrogen to the water. Total nitrogen in the water increased during the experiment in these three streams (Table 2). The concentration of nitrate+nitrite remained high and underwent little change in the nitrate enriched system (Fig. 4). The concentration of nitrate+nitrite in the phosphate enriched and control systems remained low and steady until falling to zero by day 28.

From days 12–34, the absolute nitrogen content (Fig. 4) and the respiration rate of leaf discs (Fig. 2) rose concurrently in all four streams, suggesting that much of the nitrogen present was microbial. This does not preclude a rapid turnover of the nitrogen with the aqueous phase, however. The lack of correlation between nitrogen content and respiration of discs prior to day 12 suggests that leaching is still the dominant factor controlling nitrogen content during this period. That is, microbial biomass was not sufficiently great to retain much nitrogen, and leaf nitrogen was lost by leaching. Ammonia levels remained low in all streams for the entire 5 weeks (Table 2), probably due to rapid turnover.

Phosphorus dynamics

Based on data for day zero and for 5 weeks only, changes in the phosphorus content of the leaf discs appear to parallel the observed changes in nitrogen. The concentration of phosphorus per gram of leaf increased greatly in the N+P stream, increased slightly in the +P stream, and underwent no significant changes in the +N and control streams (Table 2). However, the absolute amount of phosphorus per leaf disc increased only in the +N+P stream. In the other three streams, the leaf discs functioned as sources of phosphorus for the water, not sinks.

Only in the +N stream was there a net increase in the total phosphorus concentration in the water over 5 weeks. A net increase was not seen in the +P or control streams because of sampling removal. The decrease was greater in the N+P stream than in the others due to net uptake of phosphorus by the leaf-disc complex of that system.

Nitrogen fixation

Rates of nitrogen fixation (in terms of acetylene reduced) are reported for microbes associated with the leaf discs and for microbes suspended in the water (Fig. 5). We believe these to be the first reported results indicating nitrogen fixation associated with leaf detritus and leaf leachate in aquatic

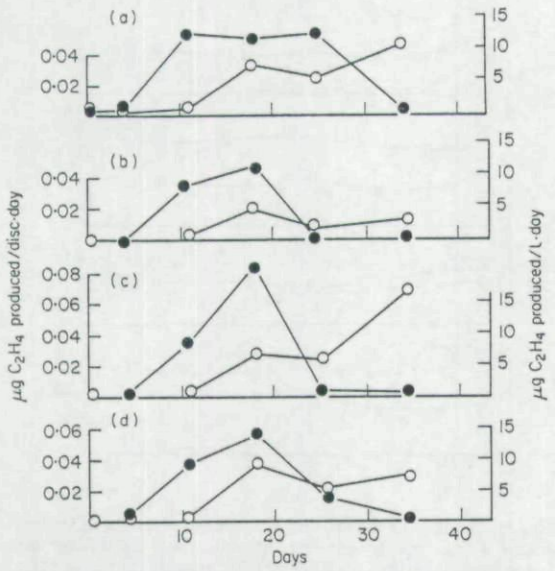

Fig. 5. Acetylene reduction rates associated with the leaf-disc complex (○) and with the aqueous phase (●) of lab-stream microcosms under various conditions of nutrient enrichment. (a) Control; (b) nitrate; (c) phosphate; (d) nitrate + phosphate.

systems. No acetylene reduction by microbes associated with leaf discs was observed in any of the streams until day 18, while reduction by suspended microbes in the water was observed as early as day 10. Acetylene reduction by these suspended microorganisms peaked in all of the systems by 19 or 25 days, and fell to zero by 25 or 35 days, probably due to substrate exhaustion or a build-up of inhibitory metabolites. No such decrease was observed for microbes associated with leaf discs.

We can roughly estimate the rates of nitrogen fixation in terms of μg nitrogen fixed by assuming that the theoretical molar ratio of C_2H_4 : NH_3 of 1·5 holds (Stewart *et al.*, 1967; Hardy, *et al.*, 1968) and correcting for the inhibition of acetylene reduction by elemental nitrogen in our unflushed incubation vials. Brezonik (1973) states that under experimental conditions similar to those used here, the presence of nitrogen would result in an approximate 30% inhibition of acetylene reduction. We will use this figure to estimate the contribution of nitrogen by the fixation vector. On integrating the curves for acetylene reduction and converting the resulting values to μg nitrogen, we estimate that nitrogen fixation associated directly with the leaf discs contributed 0·248 μg N/disc (SE = 0·045) in the +P system, 0·239 μg N/disc (SE = 0·034) in the control system, 0·220 μg N/disc (SE = 0·034) in the N+P system, and 0·095 μg N/disc (SE = 0·032) in the +N system over the 5 weeks of the experiment. Five-week contributions of nitrogen via fixation associated with the aqueous phase of the systems were 123 μg N/l (SE = 22·3) in the control stream, 90·0 μg N/l (SE = 21·8) in the +P stream, 82·6 μg N/l (SE+16·3) in the N+P stream, and 63·3 μg N/l (SE = 16·3) in the +N stream. When compared with values for total nitrogen content of the leaf discs (Fig. 4) or for concentrations of total nitrogen in the water of the systems (Table 2), nitrogen fixation is an insignificant source of nitrogen for the streams. Nonetheless, the observed rates of fixation on the discs are 50–100% higher per unit weight than those observed for *Spartina* detritus in a salt marsh (R. W. Howarth and D. E. Whitney, unpublished data). This may be due to the greater surface area per unit weight of the leaf discs.

It is also interesting that nitrogen fixation occurs in

Table 2. Changes in selected components of nitrogen and phosphorus over 5 weeks in lab-stream microcosms under various conditions of nutrient enrichment

	Control		+N		+P		N+P	
	T_0	T_5	T_0	T_5	T_0	T_5	T_0	T_5
mg P/leaf disc	9·34 (0·73)	6·74 (0·87)	9·34 (0·73)	5·14 (0·52)	9·34 (0·73)	8·00 (0·35)	9·34 (0·73)	26·3 (2·01)
mg P/g of leaf	0·511 (0·017)	0·543 (0·037)	0·511 (0·017)	0·494 (0·024)	0·511 (0·017)	0·635 (0·004)	0·511 (0·017)	2·77 (0·030)
Total P in water (mg/l P)	0·162 (0·002)	0·136 (0·001)	0·162 (0·002)	0·252 (0·080)	10·2	9·53 (0·33)	10·2	8·44 (0·52)
Total N in water (mg/l N)	0·79 (0·05)	1·68 (0·07)	12·7 (0·15)	14·4 (0·44)	0·79 (0·05)	1·63 (0·06)	12·7 (0·15)	5·38 (0·32)
Organic N in water (mg/l N)	0·11 (0·10)	1·63 (0·07)	0·68 (0·25)	2·26 (0·67)	0·01 (0·07)	1·59 (0·07)	0·41 (0·30)	4·91 (0·46)
NH_3 in water (mg/l N)	0·128 (0·0006)	0·025 (0·0039)	0·117 (0·0005)	0·035 (0·0021)	0·110 (0·0007)	0·034 (0·0030)	0·131 (0·0006)	0·038 (0·0050)

all four streams despite the constant presence of fixed forms of nitrogen. The high levels of nitrate present in the +N stream apparently suppressed fixation slightly, yet significant rates still occurred. Other workers have recently demonstrated that nitrogen fixation occurs in natural ecosystems even in the presence of fixed forms of nitrogen (Stewart, 1969; Brooks *et al.*, 1971; Brezonik, 1973), but Stewart (1969) suggests that this is so only because the nitrogenase enzyme complex (the active site of nitrogen fixation) is synthesized at some time (or place) when levels of fixed nitrogen are low and persists for a while in the presence of the fixed nitrogen. In the present study, however, it seems likely that nitrogenase was synthesized in the presence of high levels of nitrate (at least in the +N stream) since no detectable fixation occurred until day 18 while nitrate levels remained high over 5 weeks.

Conclusions

Leaf decomposition in nature, and especially under the experimental conditions employed here, is a heterotrophic process. Our data clearly show that leaf 'processing' is both quantitatively and qualitatively affected by inorganic nutrient levels in the ambient environment. Enrichment of stream microcosms with inorganic fixed nitrogen, either alone or with concurrent phosphate enrichment, increases slightly the rate at which leaves lose weight. Total system respiration (oxygen demand) is also increased slightly by enrichment with nitrogen alone or with phosphorus. Enrichment with phosphorus alone has little effect on leaf processing rates, in fact it slightly depresses both leaf weight loss and total system respiration.

The major effects of nutrient enrichment are, however, qualitative. Enrichment with nitrate alone produces an oxygen demand in the water which is more than twice that observed in the control system, while enrichment with nitrogen and phosphorus together nearly doubles oxygen demand associated with leaf discs in the system. It appears that the control system is primarily limited by available nitrogen. When this limitation is alleviated (the +N system), respiration of suspended microorganisms increases while respiration of leaf-disc microbes is unaffected. When nitrate and phosphate are added together (the N+P system), respiratory activity is shifted to the leaf-disc complex and the oxygen demand associated with the water decreases markedly. This suggests that in the presence of ample nitrogen, activity at the leaf-disc locus is limited by available phosphorus. When leaf-disc activity is high, labile organic compounds are probably used rapidly in the disc complex and only more refractory compounds are released to the water. If this is true, it would explain both the low rate of respiration by suspended microbes and the large fraction of unused leachate in the N+P system (Table 1).

Data on nutrient dynamics support the nutrient limitation hypothesis. Only in the N+P system is there a net immobilization of both nitrogen and phosphorus from the aqueous phase to the leaf disc compartment (Table 2). Thus when nutrient levels are ample, the locus of heterotrophic activity and nutrient accumulation is the original source of energy—the leaf disc. When one or more nutrients are in short supply, energy (organic carbon) is released and used elsewhere.

Extrapolation from simple laboratory systems to nature is always hazardous, but the principles elucidated here are of potential significance to stream ecosystem metabolism and clearly warrant testing in the field. Nutrient levels in natural streams may be influenced by leaf litter accumulations which may release nutrients under some conditions and immobilize them under others. Immobilization and storage is of course temporary—nutrients are later released, yet may be released in quite different chemical and physical combinations, e.g., nitrate may be immobilized by particulate organic material and later released as dissolved or particulate organic nitrogen or ammonia. These transformations should be of particular interest to watershed ecologists who take stream-water chemistry to be equivalent to nutrient outputs from terrestrial ecosystems.

Finally, the shifting locus of heterotrophy under different nutrient conditions has potential implications for the study of organic matter processing strategies in streams. While macroconsumers were deliberately excluded from our systems, their role in nature may be appreciable. We suspect that in hard-water nutrient-rich streams, the role of macroinvertebrates in leaf litter processing may be considerably more significant than in soft-water, nutrient-poor streams where the locus of consumption is shifted to the aqueous phase and thereby to microconsumers.

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