Organic Enrichment with Leaf Leachate in Experimental Lotic Ecosystems

Introduction

In recent years it has become clear that a critical component of running water ecosystem structure and function resides in the organic matter-microbial complex (Hynes 1970, Triska 1970, Fisher 1971, Fisher and Likens 1972, Cummins 1972). A simplified view of stream ecosystem structure (Fig. 1) illustrates the relationships between organic matter (particulate and dissolved), microbial consumers (fungi and bacteria), producers (photosynthesizers), and animal consumers (detritivores, herbivores, carnivores). Any strategy of stream management directed towards maintaining normal structure and function must be concerned with at least these basic interrelated compartments. The model has been useful in formulating experiments dealing with the processing of organic matter, particularly leaf litter and leachate derived from leaves.

The degree to which dissolved organic matter is converted to living biomass and CO_2 in streams, and the rate of these conversions, depends upon the nature and absolute quantity of the material, input rate and the characteristics of the receiving stream system. If biological diversity is to be maintained in the recipient stream, chemical diversity of the organic input is important. Deterioration of a high quality stream system is not necessarily the result of the input of particular classes of resistant organic substances that are difficult to decompose, since such materials probably constitute a significant fraction of natural inputs. Thus, observed stream deterioration may often be due not only to large absolute amounts and high rates of organic inputs but also to the lack of sufficient chemical diversity of the inputs. This experiment was designed to investigate the fate of natural complex dissolved organic matter (leaf leachate) introduced into simulated natural streams as a pulse of sufficient magnitude to be classed as a major perturbation.

Methods

This experiment is part of a general program in stream ecosystem analysis at the Kellogg Biological Station using two large (12 m long, 1.5 m wide, 0.6 m deep), temperature, light and flow controlled, recirculating channels (Cummins 1972) designed to interface simplified laboratory culture experiments with the full complexity of natural streams (Warren and Davis 1971). Water is circulated at the rate of 1,150 ℓ per min (0.7 cfs) in stream channels holding 2,000 to 10,000 ℓ depending upon the volume of sediments and water depth. Large particulate drift is retained by passing the water through 1 mm mesh screens at the downstream end of each channel.

The capability of the stream community to process an approximately tenfold increase in dissolved organic matter (DOM), in the form of natural leaf leachate was investigated. Such an increase constituted carbon enrichment approximating levels reported for grossly polluted systems (e.g. Wuhrmann et al. 1967). Ten months prior to initiation of the experiment (10 November 1971), the channels were filled and natural stream sediments and organisms added from Augusta Creek, a small, hardwater, brown trout stream in southwestern Michigan (Kalamazoo and Barry counties). This allowed for the establishment of a stream community exhibiting organism diversity and densities typical of average Augusta Creek levels. To provide a background of normal autumnal detritus and detritivore densities, additional animals and detritus were introduced 30 and 14 days prior to this date. To insure similar light penetration characteristics and to allow for evaporative loss during the experiment, both channels were initially filled to maximum depth. Two days were allowed for the new water to mix thoroughly with the long-term conditioned channel water prior to the initiation of experimental measurements. Both the initial and additional water supply were from Gull Lake, a deep, hard water trout lake.

After an initial five-day period of measurements (Fig. 2, period prior to leachate introduction), dried and preweighed pignut hickory [*Carya glabra* (Mill.)] and silver maple (*Acer saccharinum L.*) leaves enclosed in two 1 mm mesh nylon screen bags were placed in the upstream reservoir of the treatment stream: a total of 3849 g, 2092 g (54.3%) hickory and 1757 g (45.7%) silver maple. An organic leachate input of 573 g dry weight was estimated and a maximum expected concentration of 61.2 mg/ℓ soluble organic matter was calculated, based on previously determined leaching rates (% dry wt loss - 23.2 for silver maple and 7.9 for hickory) and changes in percent ash (silver maple = 1.4, hickory = 2.4) measured over the 30 hrs that the leaves remained in the treatment channel. Using a mean carbon content of 50.7% (King and Heath 1967, Herring 1967) for the leaves, the calculated input was 31.3 mg/ℓ dissolved organic carbon (DOC) as compared to the 35.6 mg/ℓ DOC actually measured after 30 hrs of leaching (Fig. 2). After removal from the stream the leaves were dried and reweighed. Of the observed weight loss, estimated leaching accounted for 47%, the remaining 53% was attributed to fragmentation. Since the maximum particulate organic matter measured was one-tenth of this value, rapid settling into the sediments and removal by filter feeders probably accounts for this discrepancy.

Dissolved organic carbon and particulate organic carbon (POC >0.5 μm) were measured according to the procedures described by Wetzel (1971). Nitrate, ammonia and dissolved organic nitrogen (U.V. decom-

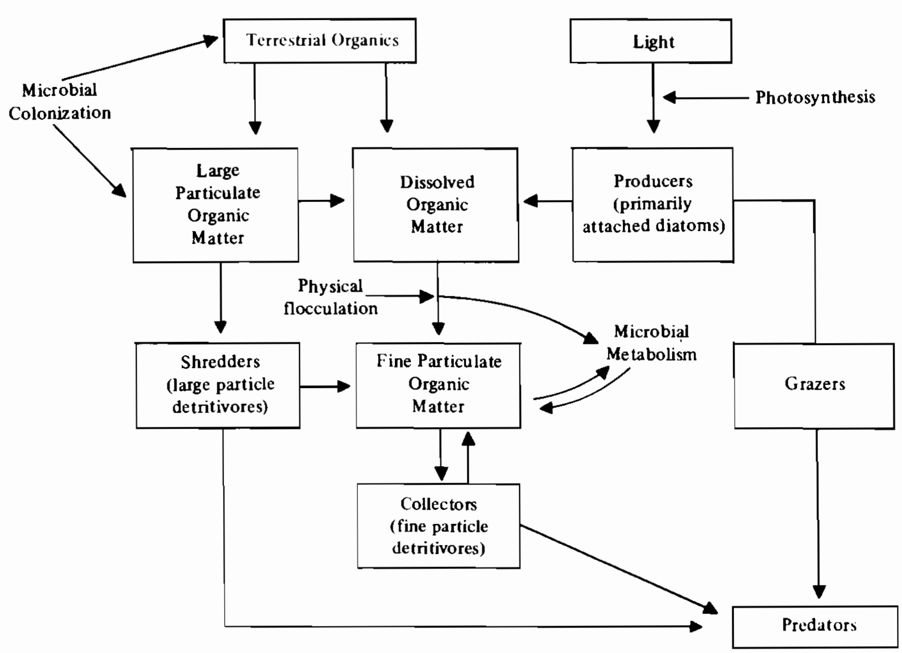

Fig. 1. A simplistic view of stream ecosystem structure and function emphasizing the processing of allochthonous organic matter (Cummins 1972).

position) were determined as reported by Manny et al. (1971). Total particulate matter (PM) in transport was fractionated with nitex screen and membrane filters and measured gravimetrically in two size fractions: >75μm and <75μm>0.45μm. Dissolved oxygen, pH and total alkalinity were measured periodically (APHA 1965).

Fungal populations were examined qualitatively through periodic observations of hickory leaf packs (Cummins 1972) placed in the streams 15 days prior to leachate introduction. Estimates of bacteria in transport (cells/mℓ) were based upon serial dilutions on nutrient agar and incubation in the dark at 16°C. Oxygen consumption in a Gilson differential respirometer and glucose-^{14}C uptake in agitated flasks were determined for the microbial community associated with silver maple leaf discs (2.2 cm diameter) incubated for varying periods in the two streams. Sets of three discs on insect pins were stuck to styrofoam blocks anchored in the channels for the incubation period.

To avoid severe disturbances just prior to and for a critical period after (Fig. 2) leachate introduction, densities of animal benthos both in the sediments and leaf packs were measured 8 days before and 15 days after enrichment. Animal drift was measured by collecting all animals on the downstream retaining screens at 24-hour intervals; these animals were reintroduced at the upstream ends of the channels. Dissolved and particulate organic carbon, dissolved inorganic and organic nitrogen and particulate matter are reported on a per liter basis corrected to the initial volumes in each stream channel at the time of the first measurements, since the treatment and control streams lost 35 and 47 liters per day, respectively, due to splashing, evaporation and sample removal. Stream temperatures of 5 to 15°C during the period resembled fluctuations in Augusta Creek during the same interval.

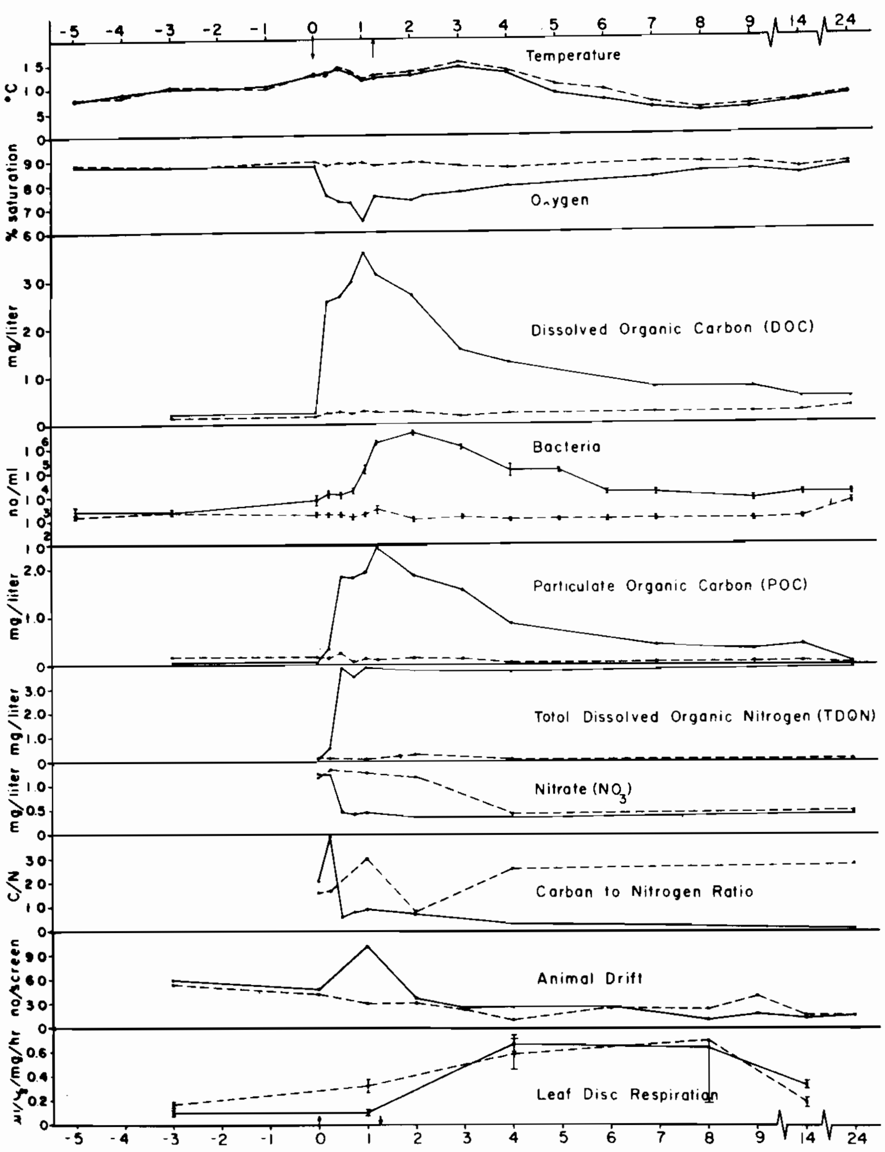

Fig. 2. Changes in physical, chemical and biological parameters prior to, during and following leaf leachate introduction into an artificial stream channel. Period of leachate introduction indicated by arrows (day 0 to 1.25); control channel dashed line, treatment channel solid line. Vertical bars on bacterial and leaf disc respiration = ± 1 SD.

Results and Discussion

Measured parameters in the untreated control stream showed little or no change, while highly significant changes were observed in the treated channel (Fig. 2). Maximum water coloring and minimum oxygen levels (7.7 m/gℓ, 70.5% saturation), together with maximum DOC, POM, DON and animal drift, were reached in the treated stream after 30 hrs. Minimum NH_4, maximum labile DON, high PM and low NO_3 were also observed after 30 hrs. Maximum estimate of bacteria appeared 24 hrs later, 2.25 days after leachate introduction was initiated.

Microbial community oxygen consumption associated with leaf discs was significantly higher ($P<0.01$) in the control than in the treated channel after 3 to 7 days incubation in the streams (Fig. 2). Since all other parameters measured, such as dissolved oxygen, were essentially the same in both channels prior to leachate introduction, initial microbial levels were probably lower in the treatment channel. After 10 and 14 days incubation (4 and 8 days after leachate introduction) oxygen consumption by the disc communities had doubled and leveled off in both streams (the difference between channels no longer being distinguishable within the variation between disc sets). This similarity between streams was observed despite a 1 to 2 order of magnitude greater density of bacteria in transport in the treatment channel. Thus, the oxygen consumption was apparently due to the original leaf disc colonizers, and reduced respiration after 20 days of incubation (14 days after leachate introduction) probably reflects depletion of the readily metabolized leaf substrate. However, at this reduced level, respiration was significantly higher ($P<0.01$) in the treatment channel. This higher rate could have resulted from colonization by certain bacteria from those in suspension, still ten times higher in the treated stream at that time, and/or an increase in metabolic activity by the microbes associated with the leaf discs that had adapted to the leachate. Glucose uptake indicated increased substrate utilization after 21 days incubation as compared to 8 days (15 and 2 days respectively after leachate introduction, Table 1). The pattern of carbon uptake and respiration resembles that of the oxygen consumption associated with leaf discs (Fig. 2), although no tracer data were collected in the middle of the period. Other leaf disc experiments with glucose-^{14}C uptake have shown an approximate 3-hr lag in uptake if the leaves are treated with a surface cleaning agent (benzalkonium chloride). Thus, glucose up-

TABLE 1. Percent[1] distribution of ^{14}C label in 3 hr experiments with glucose-^{14}C uptake by microbial community associate with leaf discs. T = treatment channel, C = control

Days incubation of leaf discs	Days after leachate introduction	Leaf disc incorporation		Leaf disc respiration		Water respiration		Insoluble products[2]		In solution[3]		Total utilization	
		Stream		Stream		Stream		Stream		Stream		Stream	
		T	C	T	C	T	C	T	C	T	C	T	C
8	2	12.2	1.9	22.1	1.8	7.9	2.7	23.6	4.6	33.4	89.0	42.2	6.4
21	15	60.8	58.6	27.9	5.9	1.7	0.3	0	18.5	14.2	16.7	90.4	64.8

[1]The leaf uptake and respiration data are not significantly different if presented on a per unit weight basis rather than by leaf disc sets (3 per flask)

[2]Obtained by difference

take and oxygen consumption data together suggest natural colonization and utilization cycles involving organisms of the leaf matrix rather than increased colonization by microorganisms responding to leachate treatment. Near the end of the experimental period (20 and 21 days of incubation) when the disc's matrix has been reduced from an average of 22.6 mg to 17.1 mg (after 14-15 days incubation) utilization and respiration were apparently dominated by surface organisms, as indicated by much greater glucose uptake at the second measurement (Table 1).

Microscopic examination of hickory leaves during the experiment revealed a diverse fungal flora. Some of the dominant forms were the aquatic hyphomycetes *Tetracladium marchalianum* DeWild and *Lemoniera aquatica* DeW. and the predominantly terrestrial fungal genera *Alternaria, epicoccum, Cladosporium* and *Fusarium.* A general reduction in bacterial diversity (based on colony type) accompanied increased numbers of bacteria in transport. However, the dramatic increase of a single genus, *Sphaerotilis,* as reported by Warren et al. (1964) in response to continuous sucrose enrichment, was not observed. Dominant bacteria were characterized as members of the genera *Flavobacterium, Aeromonas, Achromobacter* and other unclassified gram negative rods (Breek, Murray and Smith 1957).

Dissolved organic nitrogen compounds leached rapidly from the leaves but only LDON levels dropped with RDON remaining high throughout the period. The greatly increased and sustained level of DON in the treatment channel, coupled with reduction of DOC during the period following peak leachate levels, resulted in significant changes in the dissolved organic C/N ratios. Although the ratio averaged about 20 throughout the period for the control channel, it remained below 5 in the treatment stream from four days to 24 days after the introduction of leachate. The C/N ratio may have been underestimated due to insensitivity of the analysis technique to highly resistant carbon molecules.

There was no significant change in macrobenthic species composition or density in leaf packs and sediments sampled 6 days before and 15 days after leachate introduction. Of the 25 genera represented, the caddisfly *Hydropsyche* and the psephenid beetle *Ectopria* were numerically dominant in the sediments. The caddisflies *Hydropsyche* and *Pycnopsyche* together with the stonefly *Taeniopteryx* were most abundant in the leaf packs. Total animal drift was similar in both channels except on the day of maximum leachate levels (day 1; Fig. 2). This peak in animal drift resulted from hydropsychid larvae and adult elmid beetles. Although the hydropsychids dominated the drift throughout the period of measurement, elmid adults were encountered in significant numbers only in the treatment channel on the day of maximum leachate levels. No sustained increases in mortality were observed in either channel during the experiment; mortality ranged from 0% to 26% with no significant difference between channels. A high mortality was observed only once, two weeks after leachate introduction, and consisted of corixids in both channels.

The significant capacity of the experimental stream to process dissolved organic carbon seems clearly indicated by the rapid decrease in leachate concentration and return to pretreatment conditions; for example bacterial counts in the water were reduced to original levels 7 days after the maximum. High instantaneous decay constants (Riggs 1963) were calculated for POC, DOC and bacteria (Fig. 3). Both the removal of DOC and the associated reduction in bacterial

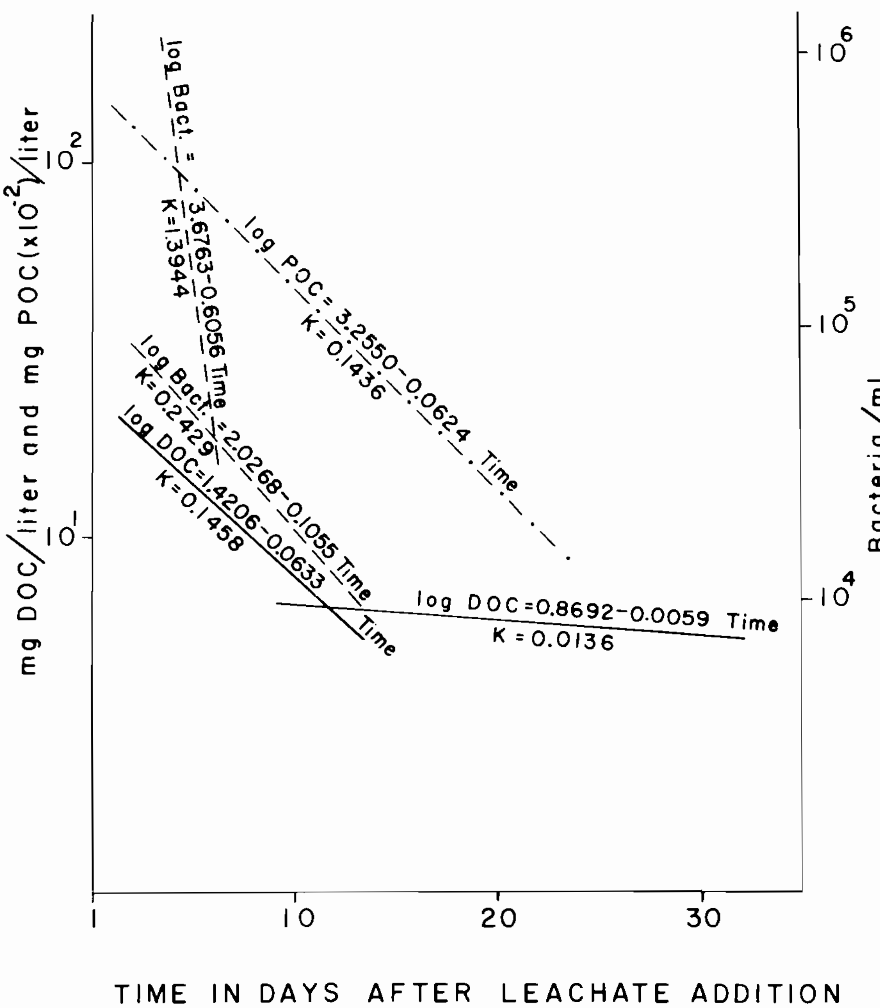

Fig. 3. Exponential decay and instantaneous decay constants (K) of DOC (mg/ℓ), POC (mg x 10^{-2}/ℓ) and bacteria (cells/mℓ) after leachate introduction. DOC and bacteria shown as having two rates. Lines fitted by linear regression; all simple linear regression coefficients significant at the 95% level.

densities can be partitioned into two rates; an initial rapid depletion of biologically labile dissolved carbon compounds together with a precipitous decline in suspended bacteria, and a slower rate of utilization of more resistant organic compounds by the bacteria. However, since the data also conform to a parabolic decay curve ($P < 0.05$), the pattern may be the result of a gradual removal of increasingly resistant carbon compounnds rather than the degradation of two, generally distinct, carbon pools. Because dramatic changes in bacterial colony types were not observed during the period of decline in densities, the interpretation of two rates of disappearance would imply that physiological rather than morphological bacterial differences were involved. POC, which includes the bacteria, fits a single rate of exponential decay.

The data indicate that the processing of dissolved organic carbon was primarily by bacteria in transport rather than organisms inhabiting the matrix of coarse particulate organic matter substrates such as leaf litter as indicated by leaf disc respiration and organic carbon uptake (Fig. 2, Table 1). Reduction in substrate levels of dissolved organic carbon compounds together with utilization of the increased bacterial densities by "collectors" in the animal benthos were most probably the major factors that produced the decline in bacterial numbers rather than nitrogen depletion. However, the possible role of phosphorus was not evaluated. Bretthauer (1971) found the disappearance of amino acids leached from beech leaves in aerated tanks was reduced to 25% of the maximum level after 7 days (temperature not given) which corresponds to the pattern for DOC reported here. He also found no correlation between amino acid removal and protozoan cell numbers, although bacterial numbers (not given) presumably would have shown a correlation as described in this study.

The enrichment with dissolved organic matter represented about a ten-fold increase over initial levels in the greenhouse channels (Fig. 2) and the natural stream (Augusta Creek) for the same period; this is comparable to levels reported for heavily polluted streams (e.g. Wuhrmann et al. 1967, 25-30 mg/ℓ soluble organic carbon). If the total leaf litter used in the treatment channel (321 g/m^2) is taken as the monthly input to the stream, it represents a 3 to 6-fold increase over levels calculated from data reported for natural streams (Fisher 1971 = 50 g/m^2/month, R. L. Vannote, Stroud Water Resources Center, Avondale, Pa., personal communication = 67 g/m^2/mo; R. Murless, Dept. Zool., Univ. Georgia, personal communication = 125 g/m^2/mo). Although the leaves were not made available for processing by the stream community, the DOM was. Dissolved organic carbon levels of less than one fifth of those produced in the treatment channel are the rule in Augusta Creek and the untreated greenhouse channels. The results indicate the rapidity and thoroughness with which natural leachate can be processed in "healthy" streams with the exception of a low residue of resistant organic carbon and nitrogen compounds (3-4 mg/ℓ DON) which in natural streams is most likely exported and metabolized in some other system. The observed DOM reduction was probably attributable to: 1) physical flocculation, 2) increased production of animal benthos mediated through increased bacterial densities and, primarily, 3) microbial metabolism.

Fisher (1971, Fisher and Likens 1972) reported that 66% of the annual energy input (6032 Kcal/m^2/yr = approximately 1400 g/m^2/yr) to Bear Brook was transported downstream, about three quarters of it as DOM. The 85% (1.3 mg/ℓ/day or approximately 1 g/m^2/day) removal of DOM observed in the present experiment is in sharp contrast to the 25% processing previously reported. If Augusta Creek discharge is equated to recirculation times in the experimental channels, the carbon processing observed during the first 24 hours after leachate introduction should occur in approximately one mile of stream length, a relationship we plan to test in the field. The measurement of relatively constant total DOM levels, even at weekly intervals, may obscure rapid turnover rates of the biologically labile DOM. Clearly, one of the keys to successful and comprehensive stream management that allows for the maintenance of organism diversity lies in qualitative, quantitative and rate manipulations of dissolved and particulate organic matter. To be successful, it appears that this must be accomplished by taking advantage of the extensive processing capacity that is a basic feature of diverse heterotrophic streams without destroying that diversity upon which the efficiency of such streams depends.

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