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LEAF MATERIAL IN THREE REPLICATE  
STREAMS WITH DIFFERENT NITRATE INPUT**

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INTRODUCTION

Since Teal (1957), numerous workers have confirmed the role of allochthonous organic material as a major energy input to forest stream communities (Nelson and Scott 1962, Hynes 1963, Egglshaw 1964, Minshall 1967, Fisher 1971, Fisher and Likens 1972). General acceptance of the heterotrophic nature of small woodland streams has resulted in investigations concerning the biological processing of such particulate organic matter and its relationship to the biotic community (Kaushik and Hynes 1968, 1971, Hynes and Kaushik 1969, Triska 1970, Cummins, et al. 1972, 1973, Iversen 1973, Sedell et al. 1974a, Barlocher and Kendrick 1973a, b, Peterson and Cummins 1974).

These studies of organic debris processing have been emphasized in small streams where terrestrial inputs of organic debris constitute the stream's energy base. Insights into the processing of coarse particulate leaf litter by microbes have been fundamental to the understanding of biotic processes in small stream ecosystems. To test the effect of nitrate addition on litter processing, a controlled experiment was undertaken in three replicate stream channels. The magnitude of nitrate addition simulated the effect of nitrogen fertilization or logging on small watershed streams. The objectives of the research were threefold:

1. To determine if decomposition rates in streams are increased by nitrate additions such as might occur in clearcut or fertilized watersheds.
2. To monitor the change in chemical composition of the plant material as

related to microbial activity.

3. To compare respiration rates of decomposing litter between treatment and control streams, for four litter species.

#### STUDY SITE

The study area is located at the Weyerhaeuser Company's St. Helens Tree Farm in the Cascades, 8 miles northwest of Cougar, Washington. The site covers about 5 acres adjacent to the Kalama River. The soil is of volcanic origin with large pyroclastic rocks overlying finely divided pumice.

The source of stream water is a large natural spring whose flow is approximately 16 cubic feet per second. The flow may get as low as 11 cfs in the fall and as high as 18 cfs in the winter when augmented by rain and snow.

The streams are all 1.2 meters wide and range in length from 120 to 215 meters. An effort was made to make them as nearly replicates as possible. The stream beds were filled with small, smooth stones varying in size between 2 and 4 centimeters. All of the vegetation of any size surrounding the streams has been removed.

The temperature of all three streams is very constant throughout the year,  $6 \pm .5^{\circ}$  C. The ambient air temperature exercises only a slight effect on the water temperature as the water flows downstream. The current velocities of the riffles under study are between 0.3 and 0.4 meters in all three streams. Water chemistry is the same for all three streams (Table 1). Water chemistry ( $\text{NO}_3$ ,  $\text{PO}_4$ , P, Na, K, Ca, Mg) analyses are conducted by Weyerhaeuser personnel every month and analyses for 17 trace metals are done on a quarterly basis. Between 1973 and July 1974, Weyerhaeuser Co. conducted a nitrogen experiment using these streams. One stream received a chronic input of 100 ppb nitrate above base level, one stream received nitrate of 100 ppb when precipitation amounted to 0.65 cm or

more, and one stream was left as a control at the base 30 ppb nitrate. During the course of the study actual nitrate concentrations fluctuated around a mean of 37 ppb for the control stream, 59 ppb for the intermittent nitrate input stream, and 138 ppb for the continuous nitrate input stream (Fig. 1). The 3-4 fold increase in nitrates in the continuous input channel although small, simulated the increase in nitrates following such perturbations as nitrogen fertilization and clearcut logging practices. The intermittent nitrate addition further simulated a clearcut watershed where nitrates would enter the stream as runoff from precipitation.

These small changes in nitrate concentration are particularly important in timber producing regions of the Cascade mountains of the Pacific Northwest, since nitrate levels of undisturbed systems are extraordinarily low compared to the geologically older regions of the eastern United States and Canada. Fredrickson (1971, 1975) found a mean annual increase of 100 ppb in a clearcut watershed of the H. J. Andrews forest, where some shrubbery remained, to 250 ppb, on a slope where all vegetation had been removed. Both cases represent concentration increases from barely detectable levels in unperturbed streams. Thus, treatment levels of nitrate input in this study were substantially lower than even the control levels (approximately 400 ppb) of comparable laboratory studies in eastern North America (Hynes and Kaushik 1969, Howarth and Fisher, personal communication).

The Weyerhaeuser site was ideal to test the effects of small nitrate additions on the breakdown of organic debris for four reasons: (1) The streams were well studied both biologically and in terms of water chemistry; (2) the temperature was constant, thereby removing it as a variable; (3) the current and water volume was also constant, thus removing another two variables. The fact that these streams do not flood in the winter greatly minimizes the fragmentation of litter due to mechanical means e.g., current and physical abrasion by suspended sediment; (4) since there is no vegetative material falling into these streams,

there are no insects present which feed primarily on intact leaves. This absence of leaf shredders also permitted focusing on the decomposition processes in the streams.

#### METHODS

To accomplish the objectives, leaf litter of four common species, vine maple (*Acer circinatum*), big leaf maple (*Acer macrophyllum*), red alder (*Alnus rubra*), and Douglas-fir (*Pseudotsuga menziesii*) was collected at abscission and dried at 50° C. Leaf material was tied into packs with monofilament nylon line, anchored to bricks, and incubated in the three streams to simulate natural leaf accumulations. Leaf packs were placed in the three study streams 16 November 1973, at the time of autumnal introduction of organic matter. Leaf packs were removed periodically until all leaf material disappeared. Three leaf packs of each litter species were removed from each stream on each sampling date. Leaf material was re-dried for estimates of weight loss. Loss rates were estimated by fitting data to the exponential model  $Y_t = Y_0 e^{-kt}$  derived from Olson (1963) and Peterson and Cummins (1974). Lines were fitted by linear regression and logarithmic transformation. Leaf packs were then ground through a 40 mesh screen on a Wiley mill for chemical analysis.

In addition to weight loss, changes in lignin composition were compared with changes in acid detergent fiber according to the method of Van Soest (1963). A 500 mg sample of leaf material is pretreated with a dilute acid detergent solution followed by acetone extraction to remove the most readily decomposable components of litter, the acid detergent cell wall (ADCW) fraction. This fraction consists of the most labile constituents, including soluble carbohydrates, soluble protein, organic acids, nonprotein nitrogen, hemicellulose and additional soluble organic material. The remainder is essentially a cellulose-lignin residue.

Cellulose is then hydrolyzed by treatment with 72% sulfuric acid to produce a lignin residue. Lignin is in turn ashed at 500° C for 4 hours to correct for ash content. This method, when undertaken on a series of leaf packs through time, provides an important index of decomposition. Decomposition is noted by a percentage increase in refractory lignin composition, since the more labile acid detergent fiber is decomposed. Changes in absolute amount of lignin and ADCW were also calculated, based on an initial leaf pack weight of 10 grams.

The capacity of leaf material to retain and hold nitrogen as decomposition proceeds was also examined. Absolute change in total nitrogen is a comparison of nitrogen content on each sampling date to nitrogen content prior to stream incubation. Measurement of nitrogen concentration was undertaken by the Kjeldahl method.

Following collection, litter packs were returned to the laboratory where insect larvae and filamentous green algae associated with the packs were removed by hand. Leaf detritus was then cut into disks and placed in a 14-station Gilson respirometer (Gilson 1963) for measurement of oxygen consumption. Respiration was measured at the ambient year round temperature of 6° C. Due to limited capacity of the respirometer, measurements were conducted on red alder, vine maple, and Douglas-fir needles.

## RESULTS AND DISCUSSION

### Weight Loss

Changes of weight loss were determined for all four leaf species in all three streams, and analyzed by regression analysis (Fig. 2). The regression analysis was based on data from two to three leaf packs of each species from all eleven sampling dates. The slopes of the regression lines for all four species were not significantly different. Only by the 200th day did differences

between the control stream and the two treatment streams begin to emerge in vine maple and big-leaf maple. The high total nitrogen concentration in alder (Table 2) which is maintained throughout decomposition may have been responsible for the absence of change in decomposition rate by small additions in nitrate in this species. The high total fiber content of Douglas-fir may have caused a slow decomposition rate not easily accelerated by small additions of nitrate. Recent studies by Kaushik and Hynes (1971), and by Howarth and Fisher (personal communication) in eastern North America, have reported contrary results. Both studies demonstrated a significant effect of nitrate addition on weight loss at levels 100 times those used in this study. At temperature of 10° C, Hynes and Kauskik (1969) found the difference in weight loss between control leaves (water 0.4 mg/l nitrate) and nitrate treated leaves (10 mg/l) highly significant ( $p = .001 - .01$ ) on elm, *Ulmus americana* and non-significant on alder, *Alnus rugosa* and the more refractory oak, *Quercus alba*. In a microcosm study by Howarth and Fisher (personal communication), leaf discs of maple, *Acer saccharum* did exhibit significantly greater weight loss at nitrate levels of 10 mg/l than did control leaves. Since the levels of nitrate concentration were far higher than are ever experienced in the Cascade Mountains, the previous studies are of limited value in the assessment of land management impacts in the Cascade Region of the northwestern United States.

Comparison of decomposition rates between species indicated vine maple disappeared fastest, followed by red alder. Vine maple lost 50% of its initial weight in 40-70 days in all three streams compared to 70-90 days for red alder. Big-leaf maple and, finally, Douglas-fir were the slowest decomposing species. Packs of big-leaf maple required 75-100 days to lose 50% of its weight, compared to 120-140 days for Douglas-fir. The same order of leaf decomposition for these four species has been reported by Sedell et al. (1975) for other Cascade streams.

### Acid Detergent Analysis for Lignin

Many previous terrestrial studies of litter breakdown (Peevy and Norman 1948, Pinck, Allison and Sherman 1950, and Alexander 1961) have indicated that differences in lignin composition may provide a good relative prediction of decomposition rates. Cromack (1973) working in a wet hardwood forest and white pine plantation of the southwestern United States, found the rate of change of lignin content the best single criterion of litter decay rates. In terms of initial carbon quality this was also true in our studies (Table 2). Vine maple and red alder, the litter species which decompose most rapidly, had the highest acid detergent cell wall component (ADCW) and lowest concentration of lignin. Big-leaf maple and Douglas-fir, characterized by higher lignin content and lower ADCW fraction, were more slowly decomposed.

Since increase in percentage lignin content was a reliable index of decomposition, a comparison of lignin increase was chosen as a second decomposition parameter. As expected, lignin composition increased and acid detergent fiber decreased as decomposition proceeded (Fig. 3). The two litter species which decomposed fastest, vine maple and alder showed the greatest increase in lignin and decrease in acid detergent cell wall. The two slowest decomposing species, big-leaf maple and Douglas-fir, exhibited the least increase in lignin content and decrease in acid detergent cell wall. Differences in percent composition between the nitrate treated streams and the control stream were not observed.

When the four leaf species were compared in terms of absolute content of ADCW and lignin, a similar picture emerged (Fig. 4). Acid detergent cell wall, the most labile constituent, decomposed more rapidly than the lignin fraction. This is particularly apparent in alder and Douglas-fir, the two litter species which exhibited the least weight loss differences as a result of nitrate addition.

In big-leaf maple and vine maple, the lignin fraction decomposed slower in the control stream than in the treatment stream. This trend is especially apparent in big-leaf maple which had a higher lignin content in the control stream than in the treatment streams for the full decomposition cycle. In vine maple, the same trend may also be observed, particularly on the last two sampling dates. The ability of the microbial community to break down the lignin fraction of the two maple species may have been responsible in part for the weight loss differences observed between treatment and control streams in the two species.

#### Absolute Nitrogen Content

As a third test for nitrate effect on decomposition, absolute nitrogen composition of the litter was compared between the two treatment streams and the control stream. Increase in nitrogen concentration is a well known phenomena in decomposing litter. Such a capacity to retain and hold nitrogen may be based on four possible processes. (1) Nitrogen immobilization. Nitrogen may be immobilized by incorporation into fungal and microbial protein as carbon is mineralized. Nitrogen immobilization has commonly been demonstrated in agricultural investigations (Richards and Norman 1931, Waksman and Gerretsen 1931, Bremner 1955, and Alexander 1961). Increases in nitrogen concentration or immobilization have also been observed for many species of leaf litter and wood in soil studies (Melin 1930, Caldwell and DeLong 1950, Saito 1957, Ivarson and Sowden 1959, Bock, et al. 1960, Allison and Klein 1961, Bock 1963, and Swift 1973). When nitrogen immobilization occurs, the nitrogen concentration increases while the absolute amount of nitrogen remains unchanged. (2) Uptake of nitrate from stream water. If absolute amounts of nitrogen increase by uptake of nitrate from the water, then litter from the two streams with nitrate treatments might be expected to contain higher absolute amounts of nitrogen than the control

stream. (3) Nitrogen fixation. This microbial process results in an increase in nitrogen content by fixation of molecular nitrogen. Both of the latter two processes could result in an absolute increase in the amount of leaf pack nitrogen.

(4) Exchange of ammonia or ammonium on organic substrates. The last possible source of nitrogen was considered non-significant since ammonia was not detectable in the spring water source and increased to only 1.5 ppb at the foot of the experimental channels. The small increase in ammonia was presumably mediated by periphyton and aquatic invertebrate activity.

Absolute increases in nitrogen content, in addition to increases in concentration, as litter decomposes on soils have been reported by Gilbert and Bock (1960) and Bock (1964). In flowing water systems, Mathews and Kowalczewski (1969) reported increases in nitrogen concentration throughout decomposition, and absolute nitrogen increases during initial stages of decomposition of willow and sycamore litter in coarse mesh bags from the River Thames. Iversen (1973) has also found both absolute increase and increase in concentration of nitrogen in beech leaves decomposing in a Danish stream.

In this study, absolute increases in nitrogen content were used since they establish a basis of comparison with leaf litter at the time of initial incubation and were normalized to an initial leaf pack of 10 grams dry weight. All species of litter leached approximately 50% of their initial nitrogen content during the first four days (Fig. 5). Within 40 to 70 days, leaf packs contained a greater nitrogen content than when the packs were initially incubated in the respective streams. Nonetheless, the final absolute content of nitrogen was not related to nitrate treatment.

Absolute content of nitrogen was considered in relation to weight loss, to determine the capacity of leaf packs to retain nitrogen as the weight of the leaf packs decreased (Fig. 6). More than 100% of the initial nitrogen remained

although approximately 60% of the pack weight had decomposed in vine maple and 45% in alder. In the slower decomposing big-leaf maple and Douglas-fir, 100% of the initial nitrogen content remained although more than 50% of the pack had disappeared. For alder and vine maple, peak nitrogen content was attained within 40 days of leaf pack incubation. Packs of big-leaf maple attained maximum nitrogen content within 110 days, and conifer within 173 days. Peak nitrogen content occurred at incubation times near those at which litter material became palatable to aquatic detritus consuming invertebrates in previous studies (Sedell, et al. 1975, Anderson and Grafius 1975).

Despite massive overall change in nitrogen content in all leaf species, there was no difference in nitrogen increase between either of the nitrate treated streams and the control stream. Nitrate uptake from the water column already occurs against such a high gradient that small (3-4 fold) increases seemingly do not significantly affect the rate of nitrate uptake. These findings are consistent with laboratory studies of Hynes and Kaushik (1969). Their investigations indicated a highly significant increase in nitrogen content of incubated elm leaves when levels of nitrate ( $\text{KNO}_3$ ) in cultured water were augmented to levels of 100, or 10 mg/l. However, the control leaves in natural streamwater at .4 mg/l did not exhibit a significant change in nitrogen content. In more refractory oak litter, significant nitrogen increase was not observed at levels of 5-10 mg/l of nitrate. Considering the natural nitrate level of .037 mg/l, absence of a nitrogen increase in litter due to a small increase in nitrogen concentration in water was consistent with their results. Seemingly, decomposing leaf material does have a capacity to remove nitrate from the water to attain and hold a nitrogen level near or even slightly higher than the starting concentration. The capacity to achieve nitrogen concentration significantly

above this level, however, would depend on a massive influx of nitrogen far above levels usually experienced as a result of land management practices.

#### Respiration

Respiration activities tracked well with time in all three streams regardless of nitrogen treatment (Fig. 7). Respiration rates increased as leaf packs became colonized in autumn and decreased as packs were reduced to refractory lignified residues by spring. Since stream temperature remained essentially constant, temperature effects have been eliminated. One confounding aspect of the litter decomposition was increased day length, which triggered a bloom of filamentous green algae in all three streams during spring. The algae consisted primarily of *Zygnema sp.* and *Tribonema bombycincime* which formed large mats that eventually broke free from the bottom gravel. Mats of algae completely covered leaf packs during the latter stages of decomposition and resulted in the presence of high populations of copopods, chironimids and turbellarians. High surface activity of microbes, as a result of breakdown of algal mats contributed to respiration rates on later sampling dates, but was not related to the breakdown of litter material. Utilization of litter as an inert surface by microbes or chemical oxygen demand accounted for approximately one-third of the observed oxygen consumption. This figure was calculated by assuming a respiration quotient (R.Q.) of 1 and back calculating at each sampling date to determine the amount of oxygen required to decompose the observed weight loss of leaf material.

Leaf packs of vine maple, which lost weight most rapidly also had the highest rates of oxygen consumption. Needle packs of Douglas-fir which disappeared most slowly had the lowest rates of oxygen consumption, but over a more extended time period. At 123 days in place, when vine maple packs had the highest oxygen consumption rates, an average of 25% of the leaf material remained in all three

streams. In Douglas-fir maximum oxygen consumption of needle packs varied over a wide range (73-160) days depending on nitrogen treatment. After 235 days approximately 30% of the needle packs still remained in all three streams. For alder, approximately 38% of the leaf pack remained after 123 days, the time of maximum oxygen consumption. In alder, measurements of oxygen consumption were lower in the control stream than in the intermittent or continuous nitrate input stream, however for vine maple or Douglas-fir this trend was not observed. Overall, no significant effects of nitrate addition could be concluded from the respiration measurement of oxygen consumption. As in the other measured parameters species differences of litter material were greater than treatment differences in all three streams.

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#### SUMMARY AND CONCLUSIONS

Inputs of leaf litter and other allochthonous debris constitute the biological energy base of small watershed streams. The relationship of decomposition rate to nitrate inputs of a 3 - 4 fold magnitude was tested in three replicate experimental streams. Such a magnitude of nitrate inputs simulated possible input effects of nitrogen fertilization or logging, two common forest practices. At the levels tested, no significant increase in decomposition rate was observed on any of four litter species as a result of nitrate addition.

Common parameters measured included weight loss, changes in carbon quality, absolute changes in nitrogen content, and microbial respiration measured as oxygen consumption. Results were partially confounded by growth of filamentous green algae during spring. As a result of chemical oxygen demand or leaf pack envelopment by the decaying algal mat, approximately one-third of the total oxygen consumption was not related to litter decomposition. Phosphate in conjunction with nitrate addition was not tested since phosphate did not appear limiting.

Although not related to increases in nitrate concentration in water, decomposing litter did exhibit a twofold increase in nitrogen content following initial leaching. The mechanism of this increase, whether by biological or chemical means, remains to be investigated. Also, the impact of nutrient chemistry in such refractory materials as wood and bark, should also be the subject of future experimentation.

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Table 1. Water chemistry (mean concentration of the Weyerhaeuser  
Experimental Streams.

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TDS	50 ppm
PO <sub>4</sub> <sup>-P</sup>	18-24 µg/l
pH	6.5-6.8
CO <sub>2</sub>	5 mg/l
O <sub>2</sub>	11.5 mg/l
NO <sub>3</sub> <sup>-N</sup>	37 µg/l Control stream
	59 µg/l Intermittant input stream
	138 µg/l Continuous input stream

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Table 2. Determination of acid detergent cell wall (ADCW), cellulose (C), lignin (L) ash (A), total fiber (TF) and nitrogen (N) prior to decomposition.

	<u>%ADCW</u>	<u>%L</u>	<u>%C</u>	<u>%TF</u>	<u>%A</u>	<u>%N</u>
Alder	80.6	9.5	9.0	18.5	1.0	2.16
Vine maple	78.9	8.5	14.7	23.2	2.9	.51
Big-leaf maple	64.8	17.3	16.3	33.6	1.6	.71
Douglas-fir	59.4	24.2	14.5	38.7	1.9	.64

Fig. 1. Actual nitrate concentrations of the water for the control and treatment streams. Control stream ( $\Delta \cdots \Delta$ ), continuous nitrate input stream ( $\circ \text{---} \circ$ ), intermittent nitrate input stream ( $\square \text{---} \square$ ). Mean nitrate concentrations are on the right axis.

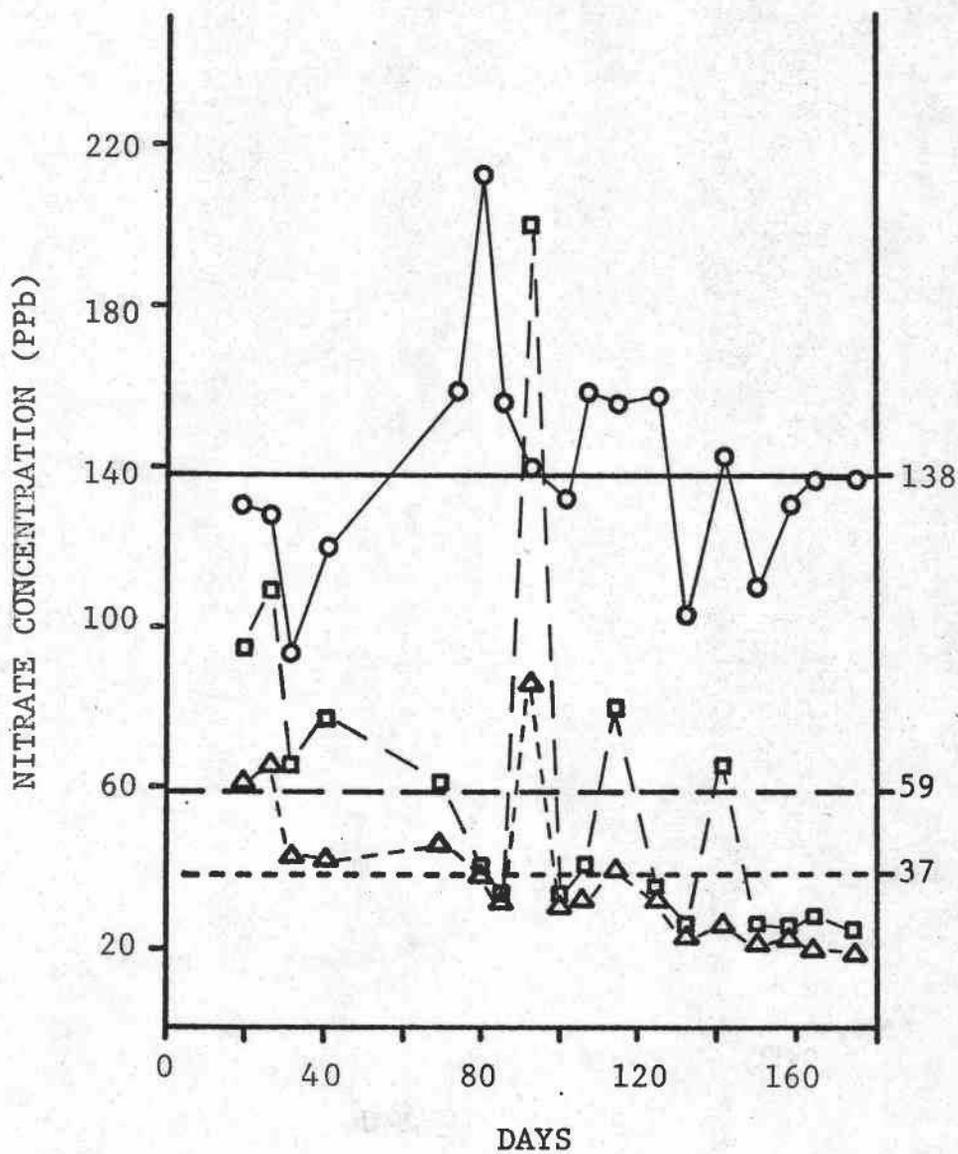


Fig. 2. Linear regression following log transformation for leaf pack weight retained by: (a) alder; (b) vine maple; (c) Douglas-fir; and (d) big-leaf maple. Control stream ( $\Delta$ ), continuous nitrate input stream ( $\circ$ ) and intermittent nitrate input stream ( $\square$ ).

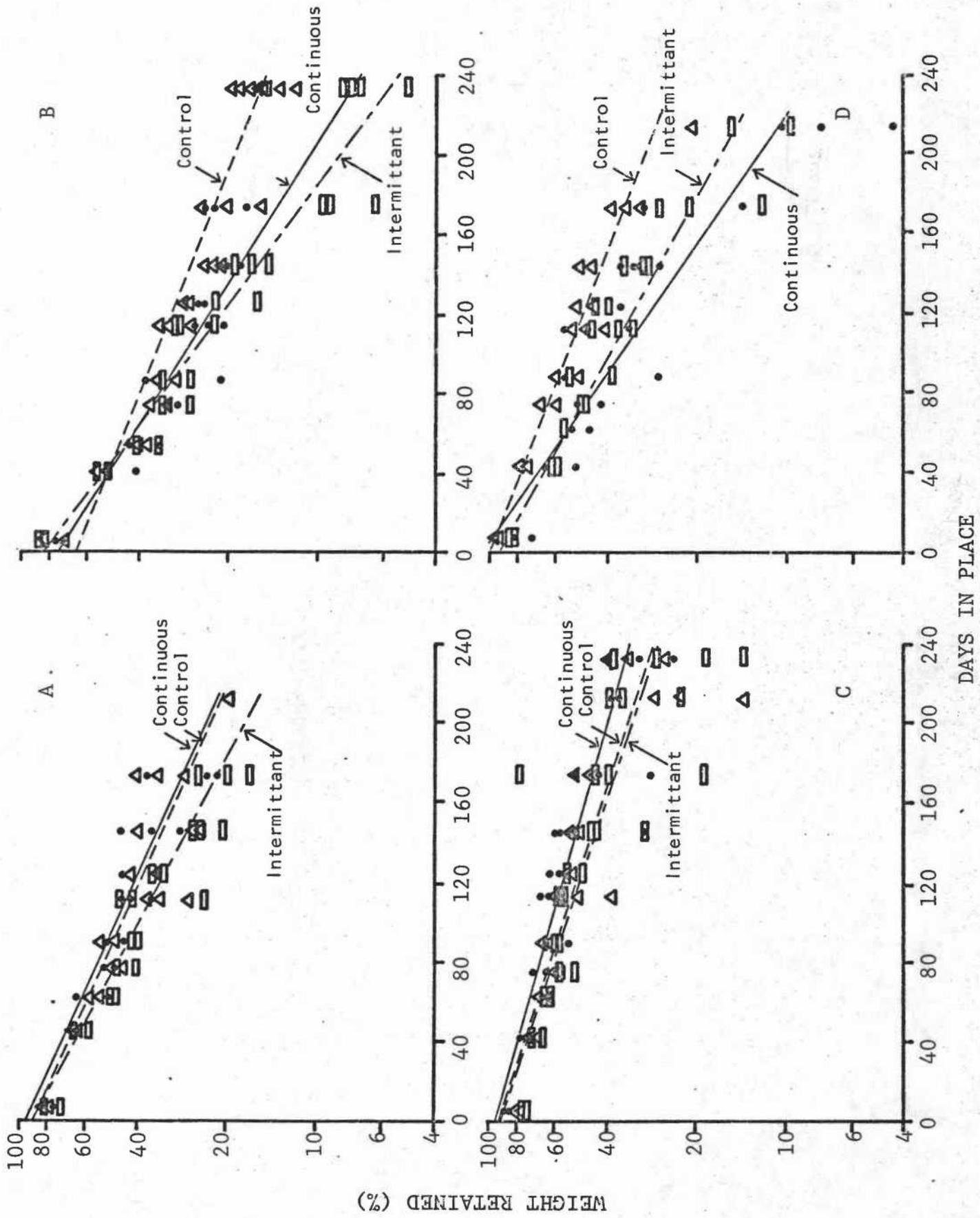
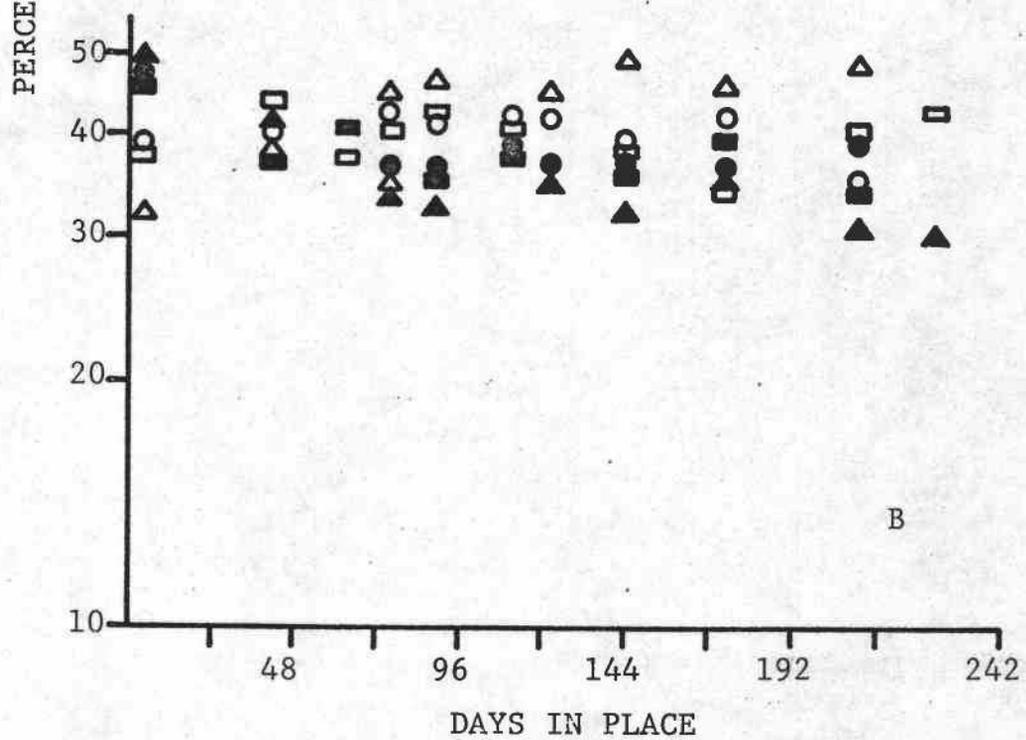
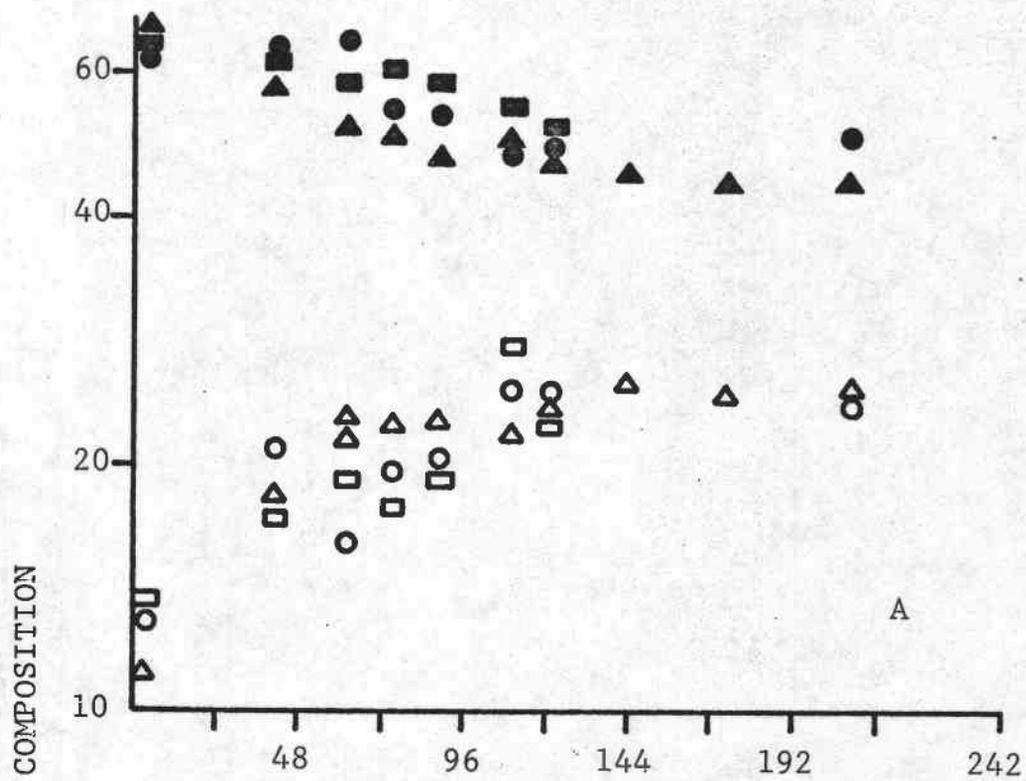


Fig. 3. Increase in lignin content (open symbols) and decrease in acid detergent cell wall (solid symbols) for leaf packs of (a) vine maple, (b) big-leaf maple, (c) red alder, and (d) Douglas-fir, undergoing decomposition in three streams with different nitrate input. Control stream ( $\Delta$ ), continuous nitrate input stream ( $\circ$ ) and intermittent nitrate input stream ( $\square$ ).



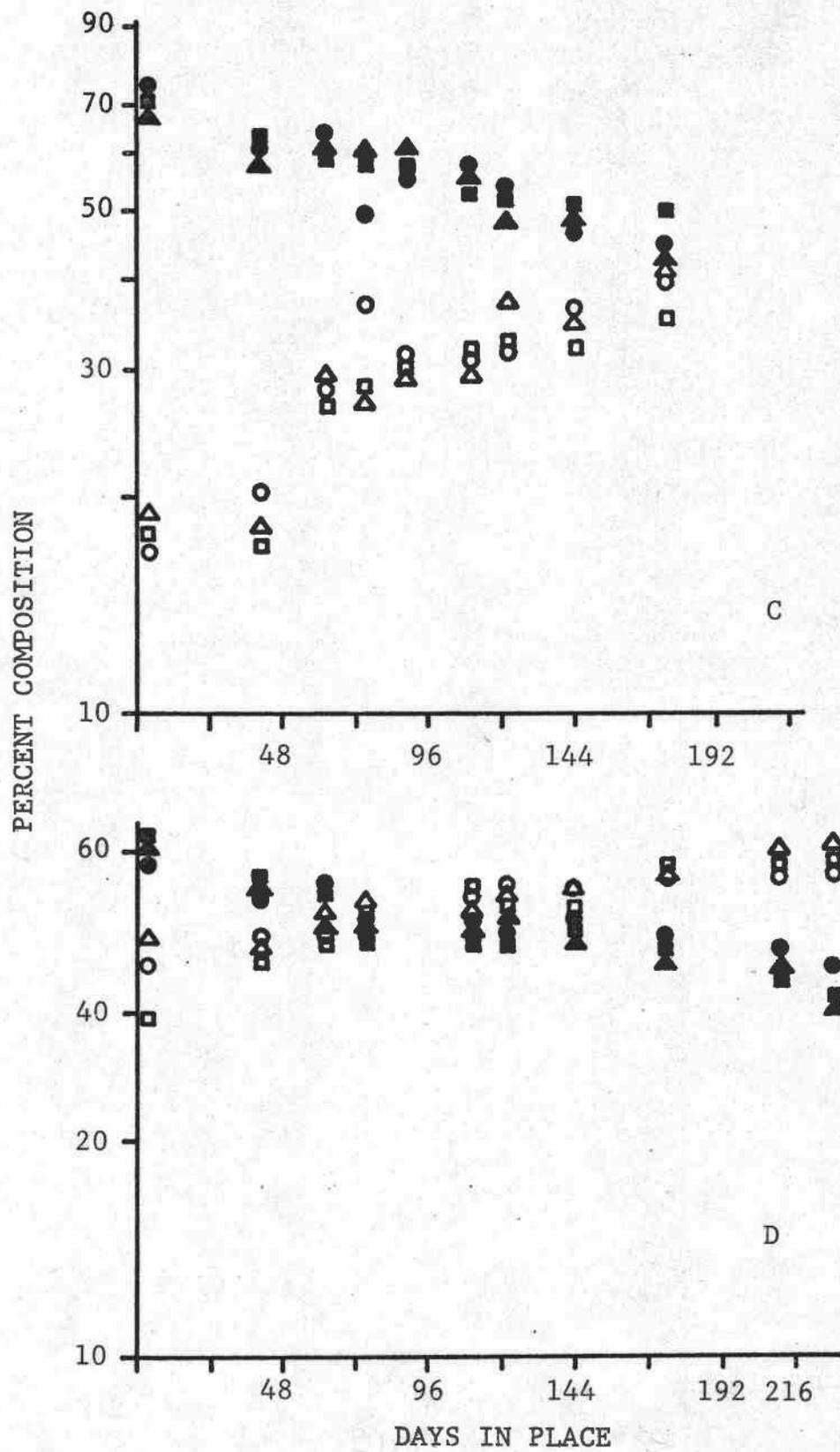
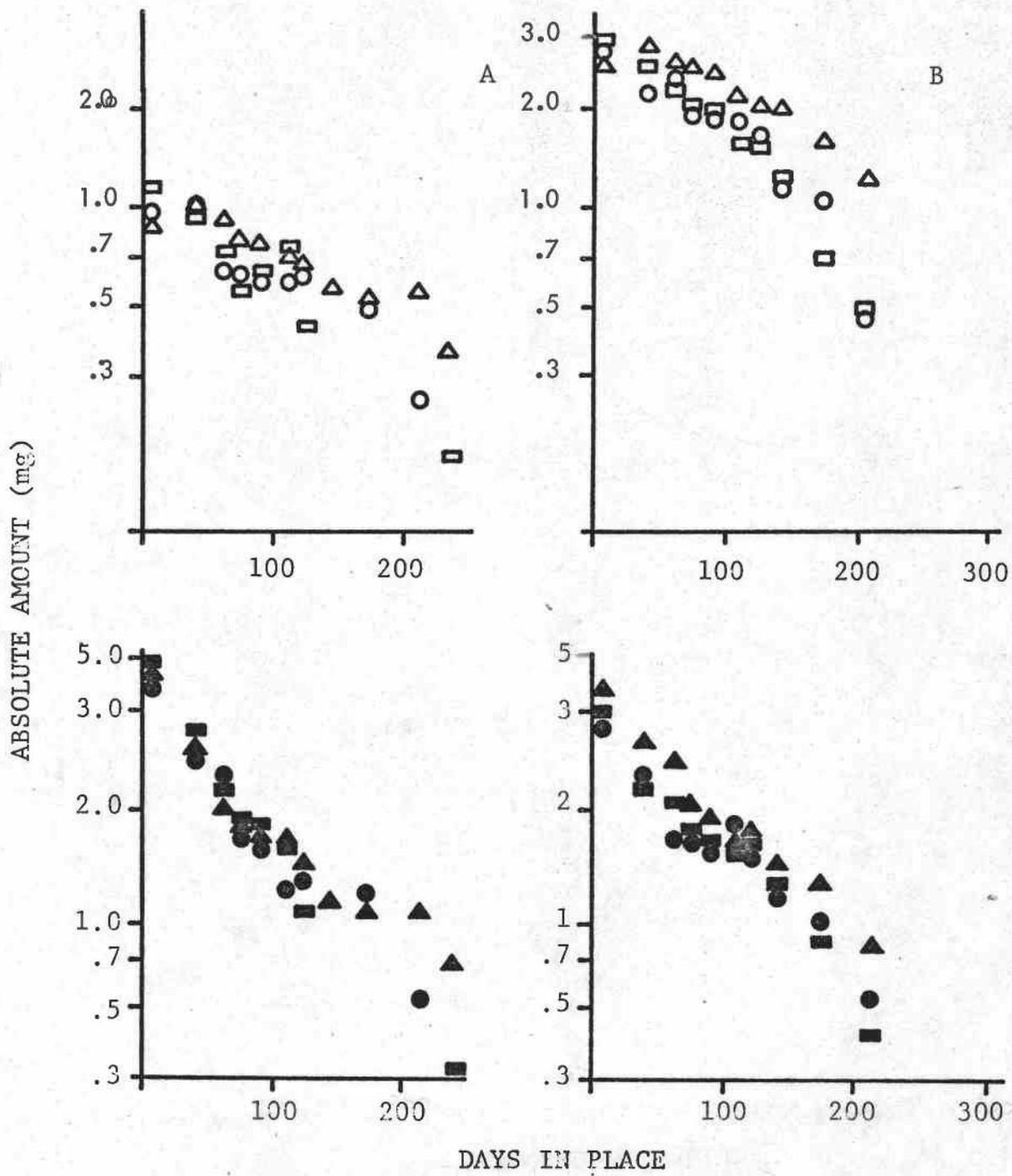


Fig. 4. Decline in absolute amount of lignin (open symbols) and acid detergent cell wall (closed symbols) for leaf packs of (a) vine maple, (b) big-leaf maple, (c) red alder, and (d) Douglas-fir, undergoing decomposition in three streams with different nitrate input. Control stream ( $\Delta$ ), continuous nitrate input stream ( $\circ$ ), and intermittent nitrate input stream ( $\square$ ). Initial leaf pack weight normalized to 10 grams.



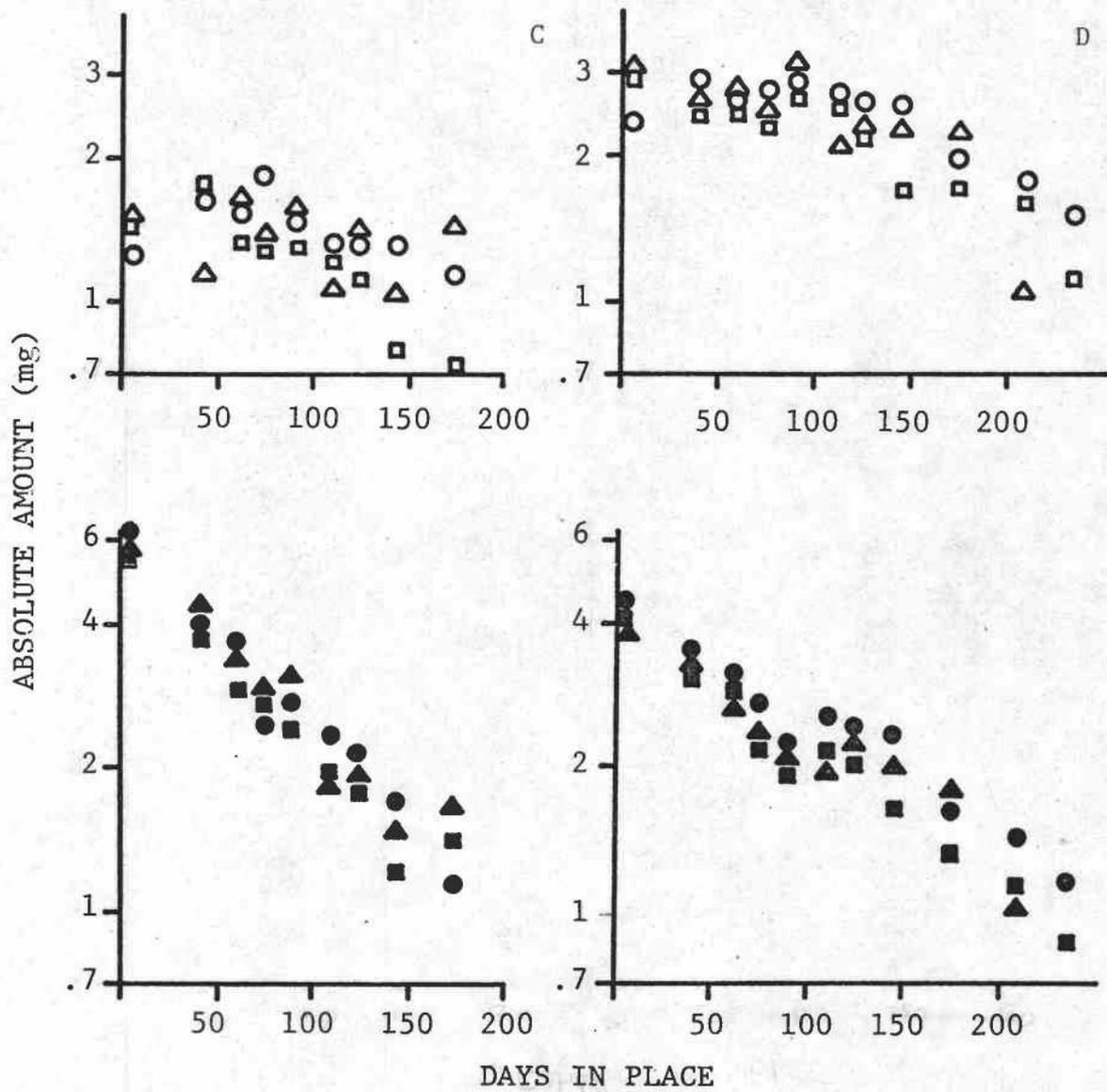


Fig. 5. Absolute change (%) in nitrogen concentration for leaf packs of: (a) vine maple, (b) big-leaf maple, (c) alder, and (d) Douglas-fir; from three streams with different nitrate input. Control stream ( $\Delta$ ), continuous nitrate input stream ( $\circ$ ), and intermittent nitrate input stream ( $\square$ ).

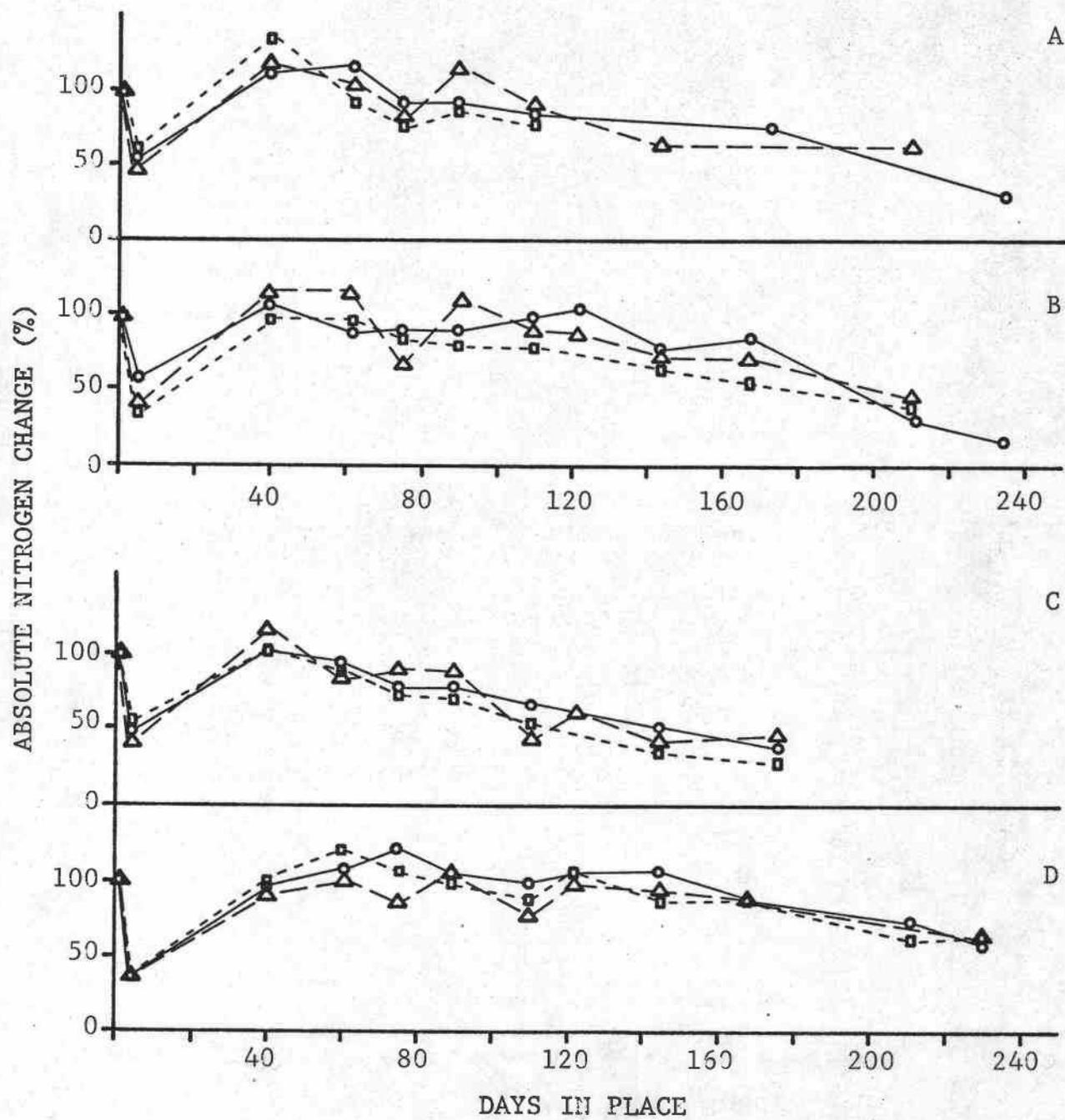
Figs  
1A-D

Fig. 6. Absolute nitrogen content (mg) for leaf packs of: (a) vine maple, (b) big-leaf maple, (c) alder, and (d) Douglas-fir; in relation to weight loss resulting from litter disappearance in three streams with different nitrate input. Control stream ( $\Delta$ ), continuous nitrate input stream ( $\circ$ ), and intermittent nitrate input stream ( $\square$ ). Initial leaf pack weight normalized to 10 grams.

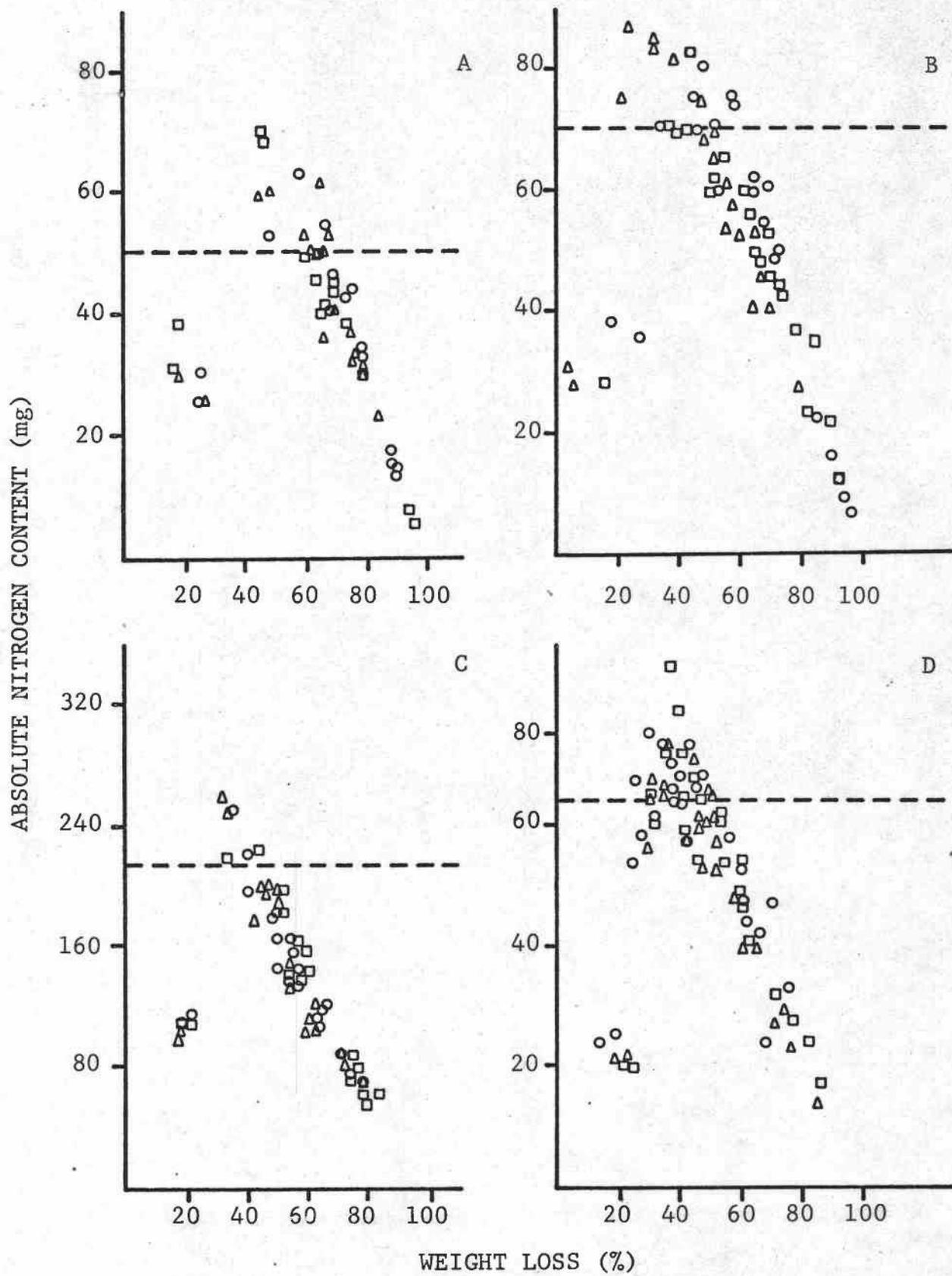


Fig. 7. Measurements of oxygen consumption for leaf packs of (a) vine maple, (b) alder, and (c) Douglas-fir; from 3 streams with different nitrate input. Control stream ( $\Delta$ ), continuous nitrate input stream ( $\circ$ ), and intermittent nitrate input stream ( $\square$ ).

