Freshwater Biology (1983) **13**, 177–183

The influence of elevated nitrate concentration on rate of leaf decomposition in a stream

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SUMMARY. 1. Leaf decomposition was compared in two streams at the Coweeta Hydrologic Laboratory, North Carolina, U.S.A. One stream drains an undisturbed hardwood watershed, while the other drains a successional watershed subject to an insect outbreak. The successional watershed has elevated nitrate concentrations in the streamwater.

2. Both black locust (*Robinia pseudo-acacia*) and sweet birch (*Betula lenta*) leaf litter decomposed 2.8 times more rapidly in the stream with high nitrate concentrations.

3. The more rapid decay rates appeared to be partly due to accelerated microbial processing in response to nitrate enrichment, because microbial biomass (as ATP) was higher in the nitrate-enriched stream.

4. At each point in time, nitrogen and phosphorus content of the litter was lower in the high nitrate stream; however, there was no significant difference in nitrogen or phosphorus content at the same state of leaf decay in the two streams.

Introduction

Decomposing leaves are an important organic carbon source in small forested streams (e.g. Anderson & Sedell, 1979). In addition to intrinsic differences between leaf species, leaf pack decomposition rate is influenced by environmental factors such as temperature, nutrient content of the water, sediment deposition rate, and densities of leaf-shredding invertebrates (e.g. Meyer, 1980; Elwood *et al.*, 1981; Wallace, Webster & Cuffney, 1982). By altering these aspects of the stream environment, watershed disturbances such as clear-cutting can have an impact on leaf decomposition in streams (Webster & Waide, 1982).

In this study we investigated the effect of a natural watershed perturbation on leaf decomposition in streams by comparing decomposition rate, microbial colonization of the leaves, and leaf nutrient content in two streams: Grady Branch and Sawmill Branch. Both streams are in the Coweeta Hydrologic Laboratory, Macon County, North Carolina, U.S.A. Gady Branch drains a watershed (WS18) undisturbed by man since at least 1924. Sawmill Branch drains a watershed (WS6) that was clear-cut, planted with grass and fertilized in 1958, treated with herbicide in 1966–67, and allowed to begin old field succesion in 1968. After these disturbances, Sawmill Branch had significantly higher nitrate concentrations than Grady Branch. In 1979 black locust (*Robinia pseudo-acacia*), the dominant tree species on WS6, showed increased infestation with locust borers (*Megacyllene robiniae* Forst), which killed or weakened many trees. One correlate of this infestation was that streamwater nitrate concentration was further elevated (Table 1); a similar increase in nitrate

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0046-5070/83/0400-0177 $02.00

TABLE 1. Monthly mean concentration (weighted for discharge) of nutrients in streamwater during the period of this study. Grady Branch (GB) drains the reference watershed while Sawmill Branch (SB) drains the disturbed watershed. All values are as mg/l. Data are from the Coweeta Hydrologic Laboratory files.

	NO_3-N	NH_4-N	PO_4-P	Ca	K	Na	Cl	Mg	SO_4	SiO_2
November 1980										
GB	0.001	0.004	<0.001	0.65	0.49	0.96	0.61	0.30	0.81	7.62
SB	0.624	0.010	<0.001	1.10	0.76	1.00	1.41	0.60	0.81	6.90
December 1980										
GB	0.009	0.002	<0.001	0.52	0.36	0.90	0.52	0.24	0.75	7.27
SB	0.767	0.007	<0.001	0.88	0.57	1.04	1.03	0.54	0.70	6.52
January 1981										
GB	0.018	0.003	<0.001	0.51	0.33	0.83	0.54	0.27	0.71	7.36
SB	0.837	0.002	<0.001	0.90	0.54	1.03	0.98	0.55	0.64	6.64
February 1981										
GB	0.008	0.002	<0.001	0.54	0.35	0.87	0.62	0.28	0.64	7.08
SB	0.882	0.004	<0.001	0.91	0.61	1.10	1.00	0.55	0.70	6.58
March 1981										
GB	0.002	0.002	<0.001	0.54	0.35	0.87	0.51	0.27	0.52	7.29
SB	0.817	0.004	<0.001	0.90	0.56	1.00	0.84	0.53	0.57	6.59
Mean over 5 month period										
GB	0.006	0.002	<0.001	0.56	0.38	0.90	0.57	0.28	0.66	7.31
SB	0.773	0.006	<0.001	0.93	0.63	1.04	1.08	0.56	0.69	6.67
Ratio of Sawmill Branch concentration to Grady Branch concentration										
	129	3.0	1.0	1.6	1.6	1.2	1.9	2.0	1.0	0.9

concentration has been observed in other Coweeta streams asociated with insect infestations and defoliation (Swank *et al.*, 1981).

We examined the effect of this watershed perturbation on litter decomposition rate in the stream using leaves from two abundant tree species: black locust and sweet birch (*Betula lenta* L.). Black locust litter has considerably higher nitrogen content than sweet birch litter. Hence it was also possible to determine if litter nitrogen content affected the manner in which decomposition rate responded to the nitrate enrichment.

Methods

Leaves were collected just prior to abscission from black locust and sweet birch trees and air dried at room temperature for 2 weeks. Leaflets without rachises were used from black locust. 5 g of leaves were put into bags (17 × 35 cm, 5 mm mesh opening), and twenty-five bags of leaves from each species were placed in similar riffle habitats in Grady Branch and Sawmill Branch on 13 November 1980.

On each sampling date (10, 30, 58, 87 and 115 days after placement in the stream), five randomly selected bags of each species were removed from each stream. Leaf bags were refrigerated until invertebrates could be removed from the bags (usually within 24 h of collection) and stored in 95% ethanol for later identification. The remaining leaf material was washed to remove excess sediment, and the washings were passed through a mesh bag to avoid loss of leaf particles > 5 mm. Leaves were then dried to constant weight at 50°C. Samples were ground in a Wiley mill (40 mesh), and subsamples were ashed (450°C for 6 h) to determine ash-free dry weight (AFDW). Other subsamples were frozen for later N and P analyses using a modified Kjehldahl procedure (Technicon AutoAnalyzer II, 1977).

Within 1 h of removal from the stream, a disc (1.5 cm diameter) was cut from a leaf in each bag. After ATP extraction, the discs were dried and ashed for weight measurements. Discs were always taken from an area of the leaf equidistant from the midrib and leaf edge. ATP was extracted from each disc by 5 min exposure to 5 ml boiling $NaHCO_3$ (0.1 M) (Bancroft, Paul & Wiebe, 1976). The extract was then added to 5 ml frozen Tris buffer for rapid cooling and immediately frozen. The ATP content of each extract was analysed in triplicate at a later date (Bancroft *et al.*, 1976). Each sample was checked for interference from fulvic acids by spiking with a known amount of ATP (Cunningham & Wetzel, 1978).

Results

For litter from both species, decay rates were 2.8 times greater in the disturbed stream (Sawmill Branch) than in the reference stream (Grady Branch) (Table 2). The temporal pattern of each species' weight loss was significantly different in the two streams (analysis of variance, $P<0.01$). In both streams locust leaflets decayed about 1.5 times more rapidly than birch leaves (Table 2).

Although locust leaflets had a higher %N (mg total Kjehldahl N/mg AFDW) than birch leaves at all times, both species showed a decline in %N over time in the disturbed stream (Fig. 1). In the reference stream, locust leaflets showed a slight increase in %N over the first 2 months, followed by a decline, while birch leaves showed a continuous slow decline in %N (Fig. 1). Values of %N for leaves were significantly different in the two streams for each species over time (two-way analysis of variance, $P<0.05$). Because %N varied as the leaves decomposed, and because they decomposed at different rates in the two streams, a comparison of %N over time in the two streams does not answer the question of whether %N in leaves at the same state of decay was the same in the two streams. To answer this question, %N was plotted as a function of % of original AFDW remaining (Fig. 2). For both species, leaves from the reference stream appeared to have a slightly higher N content at the same state of decay; however, these differences were not significant (analysis of variance, $P>0.05$).

We used nM ATP/g AFDW as an indicator of microbial biomass on the leaves and observed a general trend of increasing microbial biomass with time (Fig. 3) and with increasing leaf decay. Microbial biomass on leaves in the two streams was compared for each species over time; the disturbed stream had significantly greater microbial biomass (two-way analysis of variance, $P<0.05$). We also compared microbial biomass on the two leaf species. In the reference stream, birch leaves had significantly lower microbial biomass than locust leaves sampled at the same time (analysis of variance, $P<0.05$), whereas in the disturbed stream, there was no significant difference in microbial biomass between species.

As discussed above for nitrogen, these comparisons do not reveal whether microbial biomass at the same state of leaf decay was similar in the two streams. A plot of microbial biomass *v.* % original AFDW remaining revealed considerable overlap between the two streams for both species, and an analysis of variance showed no significant difference ($P>0.05$) in microbial biomass at similar states of leaf decay in the two streams.

TABLE 2. Exponential decay coefficients (k) for locust and birch leaves in two streams. Values are the negative slopes ± 1 SE of the regression of the natural logarithm of (% AFDW remaining) *v.* number of days leaves were in the streams.

	k (day^{-1}) in Sawmill Branch	k (day^{-1}) in Grady Branch
Black locust	0.0153 ± 0.0015	0.0053 ± 0.0007
Sweet birch	0.0100 ± 0.0012	0.0036 ± 0.0005

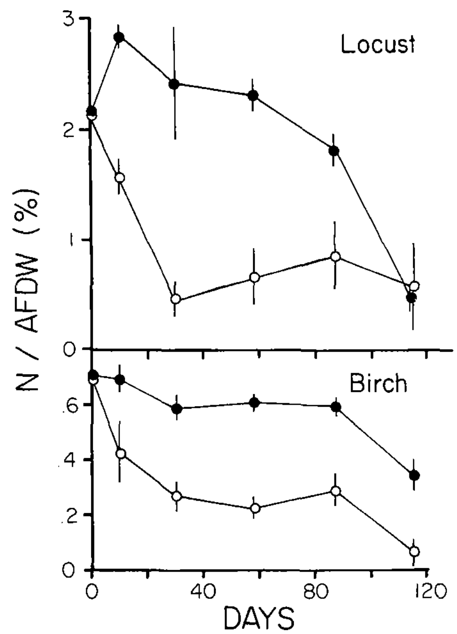

FIG. 1. Nitrogen content (100 × mg N/mg AFDW) of locust and birch leaves as a function of number of days they were in the reference (•) or disturbed (○) stream. Values are the means from five leaf packs with one standard error indicated.

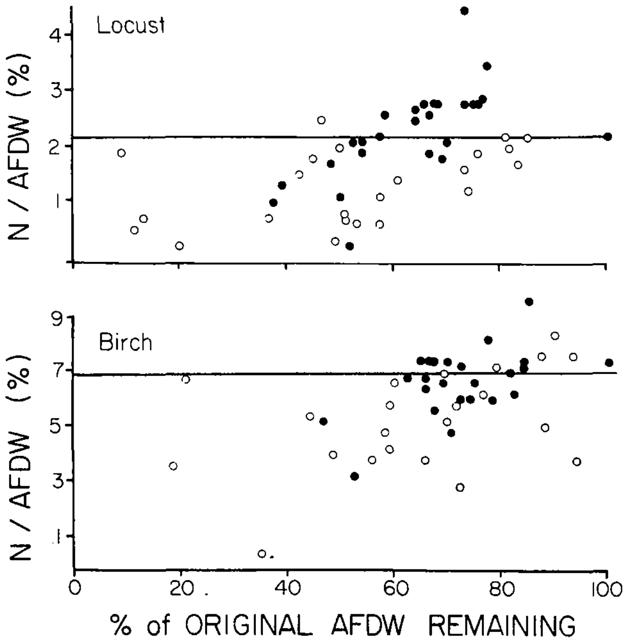

FIG. 2. Nitrogen content (100 × mg N/mg AFDW) of locust and birch leaves as a function of state of decay of the leaves, i.e. percentage of original AFDW remaining in the leaf pack. Data from both reference (•) and disturbed (○) streams are shown. The solid line indicates original nitrogen content of the leaves.

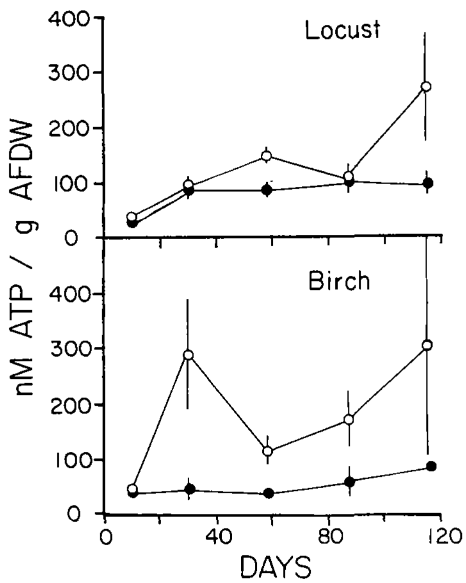

FIG. 3. Microbial biomass measured as ATP on locust and birch leaves as a function of number of days they were in the reference (•) or disturbed (○) stream. Values are the means from five leaf packs with one standard error indicated.

Four genera of leaf-shredding insects were common in the leaf packs: *Peltoperla, Nemoura, Pycnopsyche* and *Tipula*. There was no significant difference between absolute abundances of shredders found in leaf packs from the two streams. However, when abundance was expressed per g of leaf material, the relative abundance of shredders was slightly greater in the disturbed stream (Fig. 4, analysis of variance, $P<0.05$).

Discussion

The two streams studied drain watersheds that have very different treatment histories, and that differ in area by 40% and in discharge over the period of the experiment by 27%; Sawmill Branch is the smaller stream. The two streams have slightly different temperature regimes; Sawmill Branch was about 1°C warmer in the winter (J. O'Hop, pers. comm.). There are also differences in the cationic composition of streamwater (Table 1). The leaf packs from the disturbed stream had a significantly higher

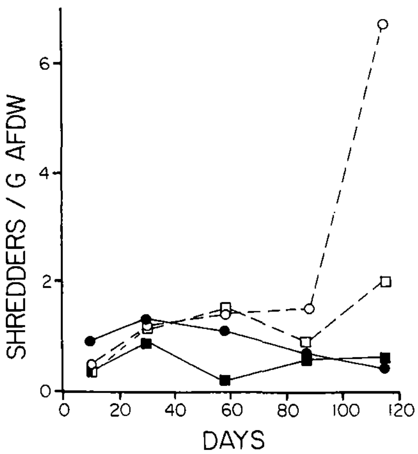

FIG. 4. Mean number of shredders per g AFDW of litter on each sampling day. Squares and circles represent locust and birch leaf packs respectively; closed symbols and solid lines represent the reference stream, while open symbols and dashed lines represent the disturbed stream.

ash content (analysis of variance, $P<0.05$), indicating a higher sediment load in that stream. Preliminary seston data also showed higher seston concentrations there (J. R. Webster, pers. comm.). The current velocity in similar habitat types and the standing stock of fine detritus are not significantly different in the two streams (Haefner & Wallace, 1981).

Other studies have demonstrated significantly lower shredder densities in the disturbed stream (Woodall & Wallace, 1972; Haefner & Wallace, 1981). Our data (Fig. 4) indicated slightly higher numbers of shredders/g leaf in the disturbed stream. It is likely that our leaf packs were islands of a resource that was in limited abundance elsewhere in the habitat, since the standing stock of leaf detritus was half that in the undisturbed stream (Haefner & Wallace, 1981).

Higher shredder densities in the leaf packs and the warmer water temperature in the disturbed stream would accelerate leaf decay in this stream. The temperature difference amounted to about 115 degree-days over the course of this study. If 1000 degree-days are necessary for 90% of weight loss to occur (B. J. Hanson and K. W. Cummins, pers. comm.), then the difference in accumulated degree days is insufficient to account for the nearly threefold increase in decay rates observed. The most striking difference between the two streams is in nitrate concentration, which is 129 times higher in the disturbed stream (Table 1). Hence, the differences observed in leaf decomposition rate appear to be primarily a consequence of N enrichment in the disturbed stream. A higher leaf decay rate has also been observed in a stream draining a clear-cut watershed at Coweeta, although factors in addition to elevated nitrate concentrations (temperature and availability of leaves to shredders) were probably important in that study (Webster & Waide, 1982).

Other studies do not consistently show a stimulatory effect of N enrichment on leaf decay (e.g. Kaushik & Hynes, 1971; Triska & Sedell, 1976). Some of the inconsistency may be due to the leaf species involved. Cellulose degradation appears to be enhanced by N enrichment, while lignin decay is not (Egglishaw, 1972; Federle & Vestal, 1980). Hence a species with a high lignin content might be expected to show less response to N enrichment. Another more important factor leading to inconsistent results in field studies is that streams may differ in ways other than just nitrate concentration. In laboratory or controlled stream studies where other factors influencing leaf decay were held constant, the stimulatory effect of N enrichment was apparent when adequately high concentrations (>0.2 mg NO_3-N/l) were used (e.g. Howarth & Fisher, 1976).

In the disturbed stream, both species of leaf litter showed a general decline in %N over time; in the reference stream, birch litter showed a decline, while locust litter initially increased in %N, even though its original N content was about 3 times that of birch. These results are perplexing. Most studies of leaf decomposition in streams have reported an increase in %N during at least the initial stages of leaf decay (e.g. Triska, Sedell & Buckley, 1975; Suberkropp, Godshalk & Klug, 1976; Triska & Sedell, 1976; Triska & Buckley, 1978) although some workers have noted little change in %N with leaf decay (e.g. Hart & Howmiller, 1975; Howarth & Fisher, 1976), and others have observed a decline in %N (Hart & Howmiller, 1975). The changes we observed in %P with leaf decay were very similar to those observed for %N (Fig. 5), although another study reported an increase in P content rather than the decline reported here (Meyer, 1980). The inconsistency between published results with respect to the N and P content

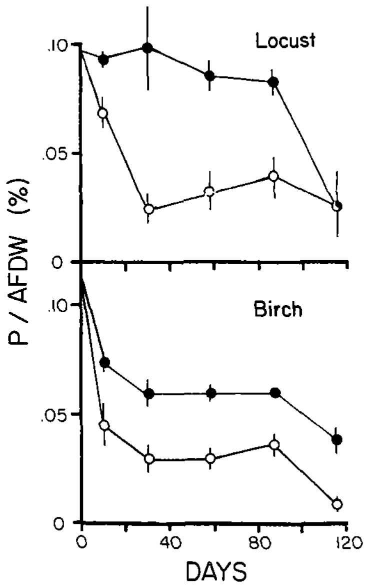

FIG. 5. Phosphorus content (100 × mg P/mg AFDW) of locust and birch leaves as a function of number of days the leaves were in the reference (•) or disturbed (○) stream. Values are means from five leaf packs with one standard error indicated.

of leaves during decomposition implies that there are both species and environmentally-caused (e.g. microbial flora in the habitat) differences in the behaviour of the nutrient content of leaves during the decay process.

Both leaf species showed consistently lower %N and %P in the disturbed, high nitrate stream. This is surprising because most workers have reported either higher %N or no significant difference in %N with N enrichment (e.g. Kaushik & Hynes, 1971; Triska & Sedell, 1976). The differences between streams in %N over time appear to be a consequence of differences in decay rate in the two streams, because %N is similar at the same state of decay in both streams (Fig. 2). Due to the accelerated leaf decay rates, leaf material that had been in the disturbed stream for 1 month had a N content comparable to leaves that had been in the reference stream for 4 months.

The more rapid leaf decay in the disturbed stream appeared to be partly due to more rapid microbial processing of the leaves. Other workers have noted greater respiration rates on decaying leaves in N-enriched systems (Almazan & Boyd, 1978; Anderson, 1978; Durbin, Nixon & Oviatt, 1979). We noted greater microbial biomass. Microbial biomass accumulation appeared to be sensitive to N availability. If N stimulated microbial growth, one would expect to see a greater effect of high ambient nitrate concentrations in the species with lowest tissue N. That is precisely what was observed. In the reference stream, birch leaves had significantly lower microbial biomass than locust leaves; but with the higher ambient nitrate concentrations in the disturbed stream, microbial biomass on the two species was not significantly different. Greater ambient N compensated for lower tissue N in birch leaves; this has also been observed in decomposing macrophytes (Carpenter & Adams, 1979) and has been suggested as an explanation for the accelerated decay of rhododendron litter after clear-cutting (Webster & Waide, 1982).

Microbial biomass appears to be a good indicator of detrital food quality (Ward & Cummins, 1979) and therefore the higher microbial biomass on leaves in the disturbed stream has interesting consequences for leaf-shredding insects. *Peltoperla maria* Needham and Smith, an abundant shredder species, is larger and has a shorter generation time in the disturbed stream (J. O'Hop & J. B. Wallace, pers. comm.). This may be partly due to higher quality food in that stream, since the quantity of food available to shredders is lower (Haefner & Wallace, 1981).

The data presented here indicate that natural watershed disturbances such as insect infestations or anthropogenic disturbances such as clear cutting, by increasing the nitrate content of stream water, can alter rates of detrital processing in streams. In a detritus-based ecosystem like a stream, a change in detrital processing rates could profoundly affect stream ecosystem function.

Acknowledgments

We thank L. R. Pomeroy for the use of his ATP photometer and E. Schultz for assistance with the chemical analyses. L. Boring, D. A. Crossley, Jr, B. Haines, W. T. Swank, J. B. Wallace and J. R. Webster provided useful comments on an earlier version of this manuscript. This research was funded by grant 80-12093 from the Ecosystem Studies Program of NSF.

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(Manuscript accepted 14 September 1982)