HYPHOMYCETES AND MACROINVERTEBRATES COLONIZING LEAF LITTER IN TWO BELGIAN STREAMS WITH CONTRASTING WATER QUALITY

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SUMMARY

Breakdown, hyphomycete and invertebrate colonization of beech (*Fagus sylvatica* L.) and poplar (*Populus nigra* **x** *euromerican* Dode juinier) leaves were ctudied in two headwater streams with contrasting water chemistry. Breakdown of both species was slower in stream B, a headwater stream containing herbicides and low concentrations of metal ions. This was related to lower conidial production of aquatic hyphomycetes and lower biomass of *Gammarus* sp., a shredder that dominated invertebrate assemblages on leaf packs. Differences in processing rates, colonization by aquatic hyphomycetes, and shredder assemblages appeared to be related to stream water chemistry. Although the herbicide and metal concentrations in stream B were rather moderate, the activity of aquatic hyphomycetes and shredderc may have been adversely affected.

INTRODUCTION

Plant organic matter, consisting of autumn-ched leaves, represents the main energy input to stream ecocyctems (KAUSHIK & HYNES 1968; CUMMINS, 1974). During the last three decades, numerous studies have assessed allochthonous leaf litter inputs to streams and rivers in different parts oí the world (KAUSHIK & HYNES. 1968: BARLOCHER & KENDRICK, 1974; CUMMINS, 1974; SUBERKROPP & KLUG, 1976; IQBAL *et al.*, 1979; CHAMIER, 1987; CHAUVET. 1987: CHERGUI & PATTEE, 1991; GESSNER & CHAUVET. 1994; POZO *et al.*, 1997). Although most observations were carried out in unpolluted streams, some studies suggected ihat water chemistry may influence microbial activity and shredder colonization (TRISKA & SEDELL. 1976: WALLACE *et al.*, 1982; HOWELLS *et al.*, 1983; ABEL & BARLOCHER, 1984: CIJFFNEY *et al.*, 1984; **AU** *et al.*. 1992; MAUND *et al.*, 1992: GRIFFITH & PERRY, 1993; MALTBY & CRANE, 1994; SUBERKROPP & CHAUVET, 1995; BERMINGHAM *et al.*, 1996a. b). Factors which affect leaf decomposition in streams may have a vast impact on the energy and nutrient budgets of downstream areas.

In this study, we measured mass losses of leaves in litterbags placed in two streams diffcring in water chemistry (concentration of herbicides and metal ions). Concurrently, dynamics of aquatic hyphomycetes, and density and biomacs of macroinvertebrates colonizing the leaf bags were measured. The rnain objective was to assess whether or not slight changes in water quality affect ratec of leaf litter breakdown.

MATERIALS AND METHODS

Study sites

The ctudy area is located in the Bois de Lauzelle, a 200 ha forested watershed (50°40'N, 4°37'W) in Louvain-la-Neuve, Belgium (fig. 1), drained by a second-order stream (Blanc-Ry). Several studies indicated that the tributaries reaching the Blanc-Ry from the south differ in water chemistry from those coming from the north, and are slightly polluted with some herbicides (CHENG. 1994; LAMBERT. 1994; HALLAUX. 1995; CHENC, 1996). CHABOT (1994) described the hydrogeology of this tone: the general gradient of the groundwater runs frorn south-east to north-west in a geological layer of *Bruxelliens*

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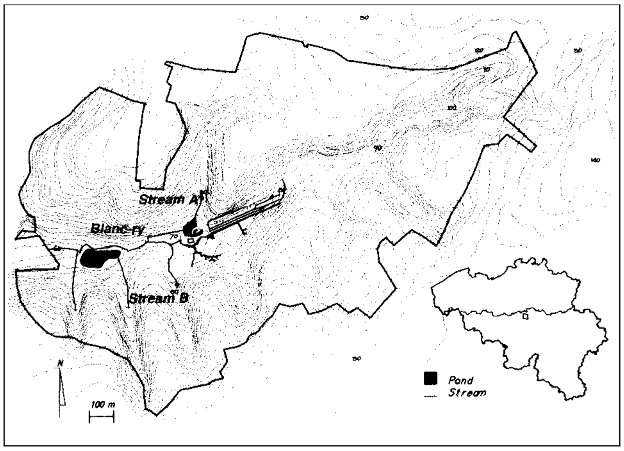

Figure 1. Location of the two study streams in the Bois de Lauzelle

sand. In addition, the groundwater layer in this zone is particularly vulnerable to deterioration because the *Bruxelliens* sand appears on the surface in many places without a surface silt cover (fig. 2). Herbicides and other pollutants could easily infiltrate through the surface of the southern part, where agricultural fields and scientific-industrial parks, potential sources of pollution, are located. Pollutants leached into the groundwater could eventually reach the Blanc-Ry.

Two first-order streams were selected for the study. Stream A, with a length of 110 m, was located in the northem bank of Blanc-Ry and runs through a niixed stand of oak, maple and alder. Stream B, with a lenght of 250 m, was located several hundred meters south from stream A, and is surrounded mainly by beech, oak and maple forest. No difference of the forest canopy can be observed between these two sites, according to standing basal area measurements: 24 m^2 ha^{-1} and 25 m^2 ha^{-1} respectively for the catchment of stream A and stream B (DEGROOTE, 1997).

Water quality

Water samples were taken at the two sites, three times per month, from April to September 1997, a period when higher concentrations of herbicides in stream B had been reported. Field analyses included temperature (mercury thermometer), pH (pH-meter WTW pH 91), and conductivity (conductivity meter JENWAY 4070). Water samples were frozen and stored for further determination of anions (Cl, NO_3, SO_4) by HPLC, and cations (Ca, Mg, K, Na, Cd, B, Fe, Mn, Cu, Ni, Al, Cr, Zn) by Flame Emission Spectrometry with Inductively Coupled Plasma (ICP) within a short period. Once a month, we tested additiorial samples for the presence of the herbicides atrazine, simazine and diuron. Water was paper filtered immediately after collection, the herbicides were concentrated by solid phase extraction (J.T. BAKER Inc., 1990), and analysed by HPLC.

Litter degradation

Leaf breakdown of two common tree species, beech (*Fagus sylvatica* L.) and poplar (*Populus nigra nigru* x *euromerican* Dode juinier.) was studied. Leaves were collected on the ground after abscission, in late autumn of 1996, and air dried. We placed 4-g samples into nylon bags (25 x 16 cm), with a mesh size of 7 x 7 mm, large enough to permit free entry of any macroinvertebrates. Twenty-four bags of each species, tied to

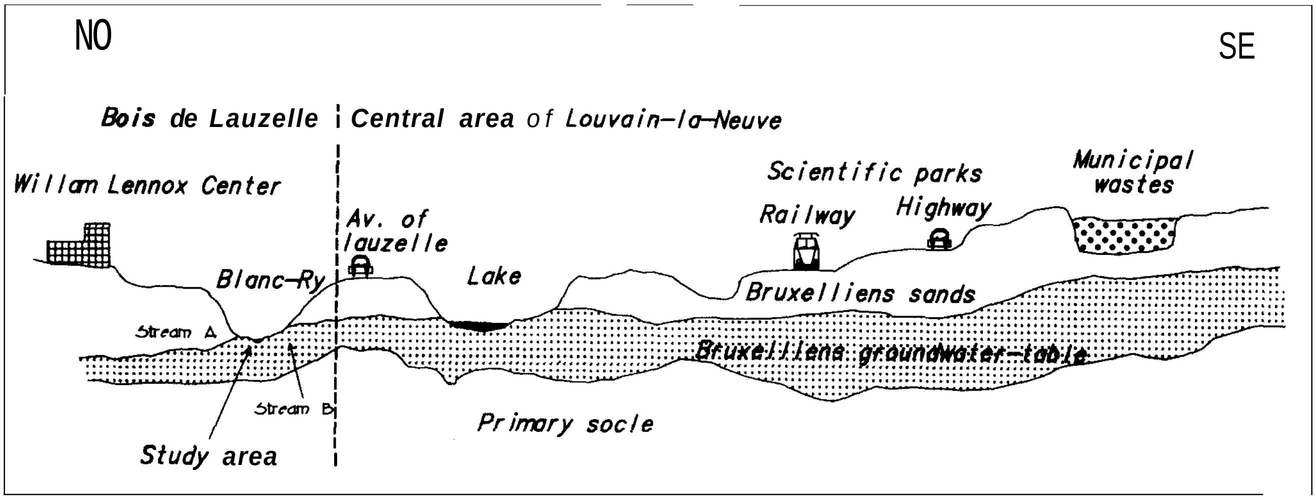

Figure 2. Hydrogeologic **conditions** of Louvain-la-Neuve, Belgium (After CHABOT, 1994)

bricks, were submerged at each study cite on 7 April 1997.

Four bags of each cpecies were collected from each study site after 2 days, 2 weeks, 4 weeks, and then at 4-week intervals until Juiy 1997. Of these four bags. one was placed in a plastic bag with stream water for analysis of aquatic hyphomycetes, which was carried within 3 hours after sampling. The remaining three mesh bags were also placed in plastic bags but not necessarily with stream water. Back in the laboratory, the leaves in the last three bags were carefully washed under flowing tap water through a 800 μm sieve to remove sediment and macroinvertebrates. The macroinvertebrates were preserved in 70 % ethanol for further identification. The leaves were weighed to the nearest 0.1 mg, after oven-drying at 50 °C for 48 hours. Ash-free dry mass (AFDM) determinations were made by burning a subsample of the dried remaining leaf material at 550 °C for three hours.

Aquatic hyphomycetes

The method to analyse aquatic hyphomycetes on leaf samples was modified from WOOD-EGGENSCHWILER & BARLOCHER (1983). At each sampling time, twenty 95 mm^2 discs were cut from each species with a stainless cork borer, placed separately in flasks (250 ml, filled with 100 ml distilled water) and aerated for 72 h at 20 °C. Each leaf disc was collected, dried and weighed, and the supernatant was filtered through 5 μm membrane filters. Conidia of aquatic hyphomycetes trapped on the filter were stained with 0.1 % lactophenol cotton blue. The number of conidia produced per mg dry weight of leaf was determined by counting all conidia on a small section of the filters under light microscope (magnification 310 X). The key references used for identification were NILSSON (1964), GONCZOL (1971), INCOLD (1975) and WEBSTER & DESCALS (1981).

Macroinvertabrates

Macroinvertebrates associated with the leaves were sorted and identified to family, genus or species. They were assigiied to functional feeding groups according to TACHET *et al.* (1987). Dry weight of macroinvertebrates was determined after oven drying to constant weight at 60 °C. The trichopterans were removed from their caces before weighing.

Breakdown rates and data analysis

The breakdown coefficient (k) was estimated for each leaf species in each site by regressing leaf pack mass versus time according to the exponential decay modei:

$$Wt = Wo\, e^{-kt} \quad (1)$$

where Wo was the initial leaf mass, Wt was leaf mass remaining at time t, and **k** the specific breakdown coefficient (PETERSEN & CUMMINS, 1974). Analysis of covariance (ANCOVA) was used to compare regressions ($p < 0.05$) (ZAR, 1984). Analysis of variance (ANOVA) was used to compare macroinvertebrates and aquatic hyphomycetes in different leaf bags (ZAR, 1984). Statistical calculations were performed with SYSTAT software (WILKINSON, 1990).

RESULTS

Streamwater quality

Table 1 shows the physico-chemical characteristics of stream water during the experiment. Water temperature and pH were similar in both streams. However, there were differences in the concentrations of some compounds such as herbicides, especially diuron, and some metal ions. Although the average of

Tahle 1. Physical and chemical characteristics of the water in the two study streams. Average values with standard deviation (SD) from April 1994 to July 1997 for herbicides, and from April to July 1997 for others.

	Stream A	Stream B
Temperature (°C)	10.0 (0.57)	10.6 (0.98)
pH	7.31 (0.09)	7.35 (0.17)
Conductivity (μs cm^{-1})	385 (12)	444 (10)
Ca^{2+} (mg l^{-1})	66.5 (2.1)	63.9 (7.7)
Mg^{2+} (mg l^{-1})	9.87 (0.2)	9.81 (0.5)
Na^{+} (mg l^{-1})	13.8 (2.1)	24.3 (2.1)
K^{+} (mg l^{-1})	2.58 (0.8)	4.37 (0.8)
Cl^{-} (mg l^{-1})	36.2 (8.0)	48.6 (7.7)
NO_3^{-} (mg l^{-1})	18.2 (5.3)	18.4 (5.7)
SO_4^{2-} (mg l^{-1})	56.7 (11.5)	53.6 (13.9)
Atrazine (ppb)	<0.05 (0.00)	0.45 (0.32)
Simazine (ppb)	<0.05 (0.00)	0.45 (0.36)
Diuron (ppb)	<0.05 (0.00)	1.43 (0.70)
Al (μg l^{-1})	0.0 (0.0)	2.3 (6.4)
B (μg l^{-1})	17.9 (14.8)	30.2 (14.0)
Cd (μg l^{-1})	11.3 (5.3)	11.7 (4.2)
Cu (μg l^{-1})	6.4 (4.1)	9.3 (6.5)
Cr (μg l^{-1})	59.9 (21.1)	77.8 (36.2)
Fe (μg l^{-1})	3.6 (2.9)	4.3 (2.9)
Mn (μg l^{-1})	4.3 (3.9)	4.9 (6.3)
Ni (μg l^{-1})	10.2 (15.5)	21.4 (27.6)
Zn (μg l^{-1})	23.3 (11.3)	24.1 (20.3)

Table 2. Periodical "peak values" for metal ion concentrations (μg l^{-1}) at 3 sampling dates.

Date	Metal ions	Stream A	Stream B
01/04/97	Cr	52	131
	Cu	6	19
	Mn	4	12
	Ni	0	67
04/06/97	Cr	57	133
	Cu	9	18
	Mn	9	19
	Ni	18	73
02/07/97	Cr	69	129
	Cu	9	19
	Mn	4	12
	Ni	15	71

some metal concentrations was not significantly different, peak values were often measured in stream B, as shown in table 2. In addition, conductivity was also higher in stream B.

Breakdown rates

Fig. 3 shows mass losses of leaves over time. Breakdown of both species was slower in stream B than that in stream A. In both streams, poplar decayed faster than beech. Beech mass remaining after 3 months was 57 % in stream B and 16 % in stream A, contrasting with only 23 % and 6 % for black poplar in streams B and A, respectively, after nearly 2 months of incubation. This difference between streams was noticeable after 14 days for poplar leaves, but only after 28 dayc for beech leaves.

All data fitted to the negative exponential model and regression coefficientc were highly significant ($p < 0.01$). Breakdown rates were higher in stream A for both leaf species: 0.0533 d^{-1} for poplar and 0.0209 d^{-1} for beech, versus 0.0287 d^{-1} and 0.0051 d^{-1} in stream B. Analysis of covariance (ANCOVA) showed that all four breakdown rates were significantly different (table 3).

Aquatic hyphomycetes

A total of 20 species belonging to 12 genera of aquatic hyphomycetes were found on leaf bags. The five dominant species in both streams were *Alatospora acuminata, Clavariopsis aquatica, Articulospora tetracladia, Anguillospora longissima*, and *Dactylella submersa*. The conidial production on leaves was significantly higher in stream A than in B (ANOVA, $p < 0.05$, fig. 4). Fungal colonization was slower on beech leaves, but yielded higher conidial production. The highest conidial production for both species occurred after 28 days of stream expocure.

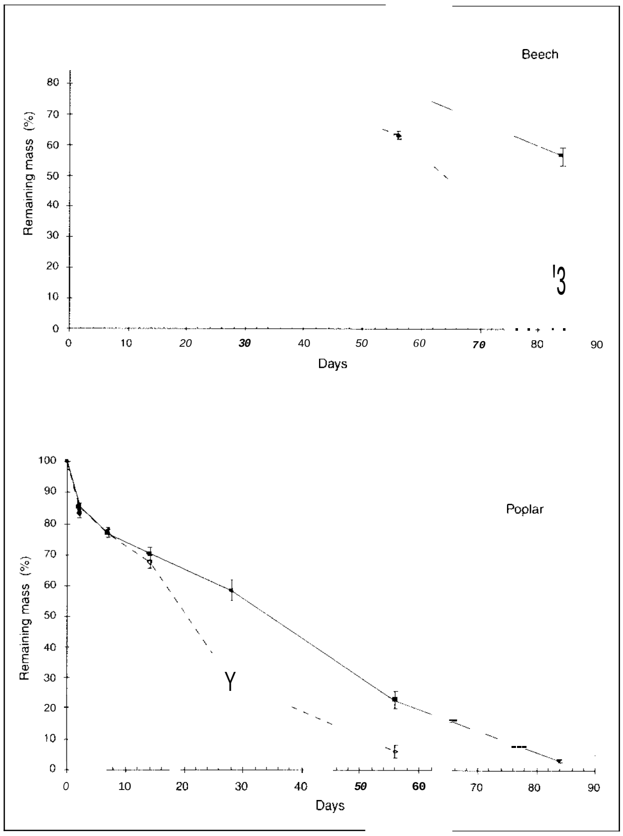

Figure 3. Remaining mass (%) of leaf bags in the study streams: --- Stream A, — Stream B. Error bars represent SD (n = 3).

Table 3. Breakdown rates (*k*, d^{-1}) of leaf litter in the two study streams. All four rates are significantly different (ANCOVA, Tukey's comparison).

Streams	Beech	Poplar
A	0.0209	0.0533
B	0.0051	0.0287

Macroinvertebrates

Macroinvertebrates were more abundant in litter bags from stream A (fig. 5), and followed similar patterns for both leaf species. All invertebrates found were shredders, and *Gammarus pulex* L. amounted to more than 95 % of the total. Limnephilidae, Nemouridae and *Asellus* sp. were also found at both sites, but in very small numbers.

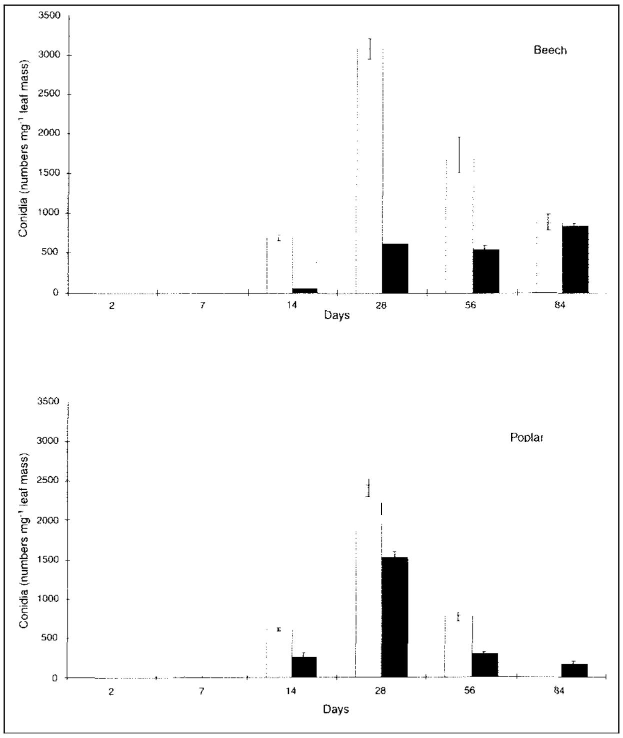

Figure **4.** Average conidial numbers of aquatic hyphomycetes produced per mg of leaves in the study streama: ❐ Stream A, ■ Stream B. Error bars represent SD ($n = 3$).

Patterns of macroinvertebrate biomass were similar to thoae of density (fig. 6). Differences between streams were significant for both dencity and biomass on the two leaf species (ANOVA, $p < 0.05$).

DISCUSSION

The decomposition process has been subdivided into three distinct phases: leaching, microbial colonization and invertebrate feeding (PETERSEN & CUMMINS, 1974). Leaching occurs mainly during the first days of incubation. In the present study, macc loss during the first two days of incubation was 6.2 % for beech and 16.7 % for poplar in stream **A,** and 6.0 % and 14.9%. respectively, in stream B.

The lower conidial production of aquatic hyphomycetes on both leaf species in stream B suggests that microbial colonization was inhibited. The chemical composition of stream B may induce a light contamination by herbicides and by some metal ions which are not favourable for these fungi. AU *et al.* (1992) found that conidial production on leaves decreased in reaches subjected to sewage inputs. CHAUVET ***et** al.* (1997) reported that the activity of aquatic hyphomycetes appeared to be more

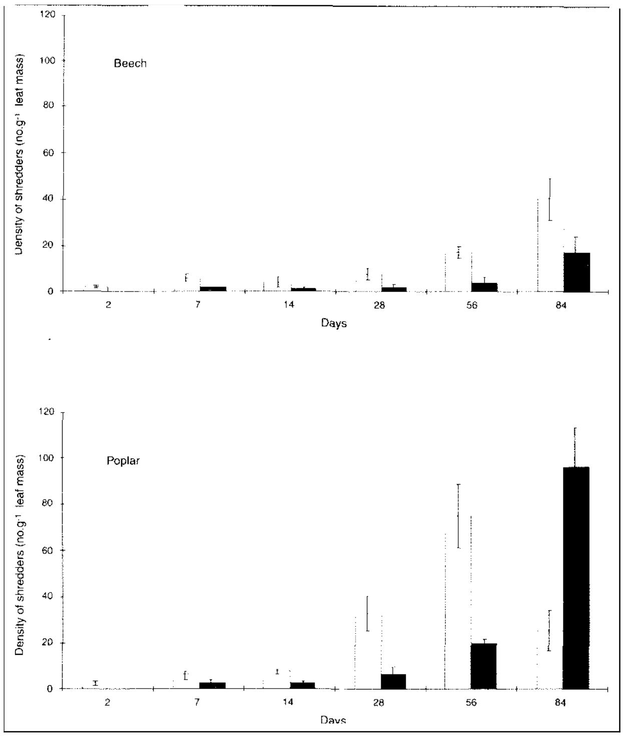

Figure 5. Density of shredders (number g⁻¹ leaf mass) in leaf bags in the study streams: ❐ Stream **A,** ■ Stream B. Error bars represent SD ($n = 3$).

affected by stream chemictry than by leaf cpecies. BERMINGHAM *et al.* (1996a) demonstrated that some species of aquatic hyphomycetes were absent on ***Alnus*** and ***Acer*** leaves incubated downstream of coal mine effluent and the rate of processing of both leaves therefore was reduced. It seems likely, that water chemistry instead of other factors was responsible for the lower conidial production of aquatic hyphomycetes on leaves in stream B. Direct or indirect biochemical influences of water quality, specially the chronic effect of herbicides, on the dynamics of aquatic hyphomycetes need further investigation.

Some works suggested that macroinvertebrates select leaf litter as a food source mainly in function of the microbial colonization, and more specifically of the microflora (KAUSHIK & HYNES, 1971; MACKAY & KALFF, 1973; BARLOCHER, 1985). Results of the present study agree with this finding. For both leaf species, the highest densities and biomass of macroinvertebrates were found in stream **A,** where bags showed higher colonization by aquatic hyphomycetes. On the other hand, in stream B, where higher amounts of herbicides and metal ions are periodically observed, colonization by aquatic hyphomycetes and macroinvertebrates were much lower.

However, although we found conidial production to be higher on beech than on poplar leaves, the density and biomass of

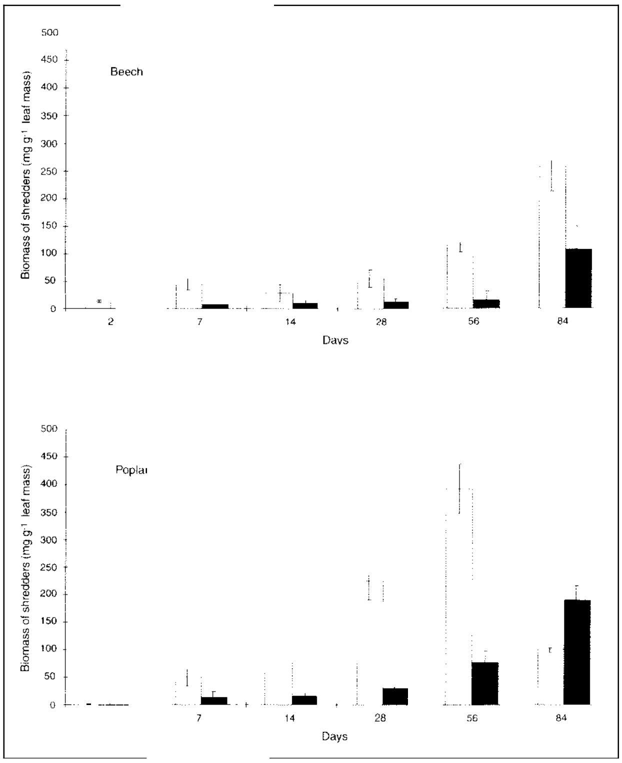

Figure 6. Biomass **of** shredders (mg g^{-1} leaf mass) in leaf bags in the study streams: ❐ Streani A. ■ Streani B. Error bars represent SD (n = *3).*

macroinvertebrates were higher on poplar. This phenomenon is probably related to the initial leaf quality.

Chronic exposure even to low concentrations of some metal ions decreases the growth and curvival of *Gammarus* sp. (MAUND *et al.*, 1992; CRANE, 1995; PLENET, 1995). Some toxicity studies (WALLACE *et al.*, 1982: CUFFNEY *et al.*, 1984; DEWEY, 1986) demonstrated the potential hazards of pesticides to the aquatic insect community. Therefore, although herbicide and metal ion concentrations in stream B are not high, the colonization of leaf bags by *Gammarus* may be affected.

Because of hydrogeological constraints, it is possible that some periodic increases **of** contaminants, though not clearly detected in our analyses, occur in stream B. Furthermore, water quality in stream B has certainly been influenced by agricultural wastec and public activities nearby, and probably experienced periodic pollution over several years (CHENG, 1994). These longtime chronic exposures, even to moderate pollution, could explain the slower leaf breakdown **at** this site.

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