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Biofilm development and invertebrate colonization of wood in four New Zealand streams of contrasting pH

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SUMMARY

1. Biofilm development and activity on wood substrata (*Nothofagus menziesii*) were examined at four forested sites in a South Island, New Zealand, river catchment over a period of 6 months. Two of the sites had brown waters and mean pH of 3.7 and 4.5, whereas the other two had clear waters and mean pH of 6.3 and 6.8.
2. Fungi and other filamentous heterotrophs were the dominant colonizers of wood at all sites; few algal cells were present. Incorporation of ^{14}C-glucose by biofilms was greatest in all four streams after 3 months, whereas endocellulase activity fluctuated over time and temporal patterns differed among streams.
3. No clear relationship was found between the incorporation of ^{14}C-glucose or endocellulase activity of biofilms and pH, although at one near-neutral pH site ^{14}C-glucose uptake increased in response to nutrient (N + P) additions.
4. After 6 months, incorporation of ^{14}C-glucose and endocellulase activity of biofilms on *Pinus radiata* dowels buried vertically in the stream beds did not differ at depths of 3–9 cm and 19–25 cm in each stream.
5. Radiotracer experiments with a grazing amphipod (*Paraleptamphopus* sp.) demonstrated that biofilms on wood from all four sites could be ingested and at least partially assimilated. Chironomid larvae and harpacticoid copepods were the most abundant invertebrates colonizing wood substrata at all sites. Different chironomid species dominated at acidic and near-neutral pH sites.
6. Overall, our findings provide little support for the hypothesis that microbial activity on organic substrata is necessarily lower in streams of low pH.

Introduction

Wood is an ubiquitous structural component of forest streams. Fallen logs, branches and roots help to define channel morphology and form the structure of debris dams where particulate organic matter accumulates. Wood also provides habitat and cover for invertebrates and fish, and in some sandy streams it is of major importance as a habitat for insect larvae (Benke *et al.*, 1985). Invertebrates that colonize wood may also use it directly or indirectly as a source of food. Filter feeders may use wood as a net-spinning site, whereas grazers and gougers feed on the surface biofilms or ingest the decomposing wood itself (Anderson, Steedman & Dudley, 1984; Benke *et al.*, 1984).

Biofilms occur on most surfaces in streams, including stones, leaves and wood. They consist of bacteria, fungi, algae, detrital particles and exoenzymes often incorporated in a gelatinous glycocalyx (Rounick & Winterbourn, 1983; Lock *et al.*, 1984). The biological components of biofilms on leaves and wood differ fundamentally from stone-surface organic layers because they are involved in the decomposition of their substratum. Sinsabaugh, Golladay & Linkins

(1991a) demonstrated that epilithic (stone surface) and epixylic (wood surface) biofilms that developed on substrata placed in a fourth-order boreal river differed from each other and that most of the microbial biomass and metabolic parameters they measured were greater on wood. In the same river, microbial biomass was also greater on wood than leaves (Golladay & Sinsabaugh, 1991). This was consistent with the findings of Tank, Webster & Benfield (1993) that respiration rates for decaying sticks in a forest stream were up to ten times greater than those for leaves. Golladay & Sinsabaugh (1991) suggested that more extensive biofilms can develop on wood because of its greater physical stability and persistence.

In New Zealand forest streams, the standing stock of wood is frequently sparse compared with that reported elsewhere (Winterbourn, Rounick & Cowie, 1981; Anderson, 1982; Evans, Townsend & Crowl, 1993). A major reason for this appears to be the flood-prone, non-retentive nature of these streams, especially in the mountains. Nevertheless, where wood occurs in New Zealand streams, it provides a habitat for invertebrates, most of which also occur on adjacent stony substrata (Anderson, 1982). Very few of these wood-associated invertebrates appear to ingest the wood itself, but some almost certainly feed on epixylic biofilms (Anderson, 1982) and associated fine detritus (Winterbourn, 1982).

Biofilm communities and rates of decomposition of organic matter in streams can be influenced by stream pH. For example, Collier & Winterbourn (1987a) found that leaf breakdown was slower in acid, brown-water streams (pH 4–5) than in clear streams of circum-neutral pH. Mulholland *et al.* (1987) reported that microbial ATP, bacterial production and respiration on decomposing leaves differed in four mountain streams of pH 4.5–6.4. Groom & Hildrew (1989) found that microbial biomass and respiration rates of alder (*Alnus glutinosa* (L.) Gaertn.) and beech (*Fagus silvatica* L.) leaves conditioned in circum-neutral water (pH 6.5–7.2) were greater than those conditioned in acidic water (pH 3.8–5.2). Nevertheless, it is difficult to separate the effects of pH from those of other factors. Some common hyphomycete species, for example, occur over a wide pH range (Suberkropp, 1992). Comparative studies of the structure and activity of epixylic microbial communities in relation to pH do not appear to have been undertaken, but the work with leaves suggests that differences can be expected.

The colonization of decaying leaves by aquatic invertebrates may also reflect their quality as food, itself a consequence, at least in part, of the physico-chemical environment. The findings of Groom & Hildrew (1989) support this hypothesis. Thus, alder leaves conditioned in a circum-neutral pH stream, and then transferred to an acid stream, were colonized more rapidly by animals than leaves conditioned in the acid stream. Microbial enzymes degrade leaf material (Findlay, Meyer & Smith, 1986), and if enzyme activity is lower at low pH, then the rate at which leaf carbon is made available to consumers in a digestible form may be reduced.

The aims of the present study were to compare the development of epixylic biofilms and the colonization of wood substrata by invertebrates in four small forest streams differing in pH. Because logs and branches are commonly buried by shifting bed materials in unstable, South Island streams, we set out sets of experimental substrata at the surface and within the beds of each stream. We predicted that microbial community development would be greater in the higher pH streams and that a less abundant, taxonomically distinct fauna would occur at the acidic sites.

Materials and methods

Study sites

Four study sites were located on small, forested streams in the Inangahua River catchment, Westland, New Zealand. This is a high rainfall area (65-year annual mean rainfall at Reefton near the middle of the catchment, 1920 mm; New Zealand Meteorological Service, 1973), and all four streams are subject to frequent spates, scouring and deposition of bed materials.

All streams drain subcatchments dominated by southern beech (*Nothofagus* spp.), which provide heavy shading throughout the year. Stream channels were well defined and < 1 m wide at all sites. Stream beds were dominated by sandstone gravels and cobbles, except at Site A1 where pools with coarse sand beds alternated with short reaches dominated by moss-covered boulders. Small amounts of wood debris were present in the channels. Sites A1 and A2 were on acid brown-water streams, whereas Sites N1 and N2 were on clearer tributaries with pH close to neutral. The experimental design therefore incorporated replicate sites in acidic and near-neutral pH regimes. The

benthic invertebrate fauna at N1 was described by Cowie (1985: his Site 1), and detailed accounts of the hydrology, water chemistry and fauna of A2 were given by Jackson (1987), Collier, Jackson & Winterbourn (1989), Collier, Winterbourn & Jackson (1989) and Moore & Jackson (1989) (their Site LA2).

(a) Water chemistry

Water samples were collected in polyethylene bottles on all sampling days and kept cold prior to analysis. Conductivity and pH were measured with appropriate meters, and total alkalinity was determined by acid titration to pH 4.2. Samples for ion analysis collected on three occasions were filtered (Gelman type A/E, pore size $\approx 0.45\ \mu m$), and 20-ml subsamples were preserved with two drops of either concentrated nitric acid or formalin prior to analysis by atomic absorption spectroscopy. These samples were also used to measure plant nutrients NO_3-N and PO_4-P by ion chromatography.

Stream temperatures were measured with a calibrated, thermister–thermometer on each sampling day. Continuous recording with maximum–minimum thermometers was abandoned following losses of instruments during floods.

(b) Wood colonization

Wooden substrata were placed in all four streams on 13 April 1993. Sampling units were heat-sterilized tongue depressors ($140 \times 16 \times 1$ mm), hereafter referred to as sticks, cut from untreated silver beech (*Nothofagus menziesii* (Hook. f.) Oerst.), one of the common forest trees present at all sites. Twelve sets of six sticks were attached to plastic-mesh sheets with cable ties and either staked firmly to the stream bed or buried beneath gravel to a depth of ≈ 10 cm. The broad surfaces of the sticks faced up and down, and their long axes were orientated parallel to the flow. Sets of surface and buried sticks were placed where physical conditions were as similar as possible.

Sets of six sticks (one surface and one buried set when available) were collected for examination of biofilms from each stream after 30, 61, 93 and 177 days. Individual sticks were not taken randomly from among the sets; this was not possible without severely disturbing the remaining buried sticks in particular. Each set was taken to the laboratory in a container of cold stream water. Examination by scanning electron microscopy indicated that variability in microbial colonization on each stick was at least as great as variability between sticks from a set of six. Therefore, taking into account complications in randomizing collection from buried sticks (i.e. one stick collected from each set), we believe that it was appropriate to use sticks as sampling replicates. Additional sets of sticks were collected on days 30 and 93 to examine colonization by invertebrates. Upon removal from the stream, these sticks were placed individually in glass vials and preserved with 70% ethanol.

Biofilm development was also examined on 10-mm-diameter *Pinus radiata* (D. Don) dowels that were driven vertically into the stream beds. After 177 days, three dowels were carefully removed from each stream, and 3-cm-long sections were cut from each with secateurs at distances of 3–6, 6–9, 19–22 and 22–25 cm below the bed surface. They were returned to the laboratory in vials of stream water kept on ice.

To determine the effect of nutrient additions (nitrogen and phosphorus) on the development of epixylic biofilms, nutrient-diffusing substrata were incubated in each stream for 30 days commencing on 13 April. The substrata were 60-ml cups containing 2% agar, identical to those described by Winterbourn (1990), except that the diffusion surface on which biofilms developed was formed by a very thin disc (12.5 cm^2) of wooden veneer (untreated rimu, *Dacrydium cupressinum* Lamb.). Three treatments were used: no-nutrient controls, nitrogen additions (0.66 M $NaNO_3$) and nitrogen plus phosphorus additions (0.66 M $NaNO_3$ + 0.06 M KH_2PO_4). Twelve cups (four replicates of each treatment) were glued into plastic trays (see Winterbourn, 1990), one of which was anchored to the bed in a pool in each stream.

(c) Laboratory assays

We measured the incorporation of ^{14}C-glucose into biofilms as a comparative measure of heterotrophic activity of the microbial biofilm (Sawyer & King, 1993; Chappell & Goulder, 1994) and endocellulase activity as an index of the cellulose degrading potential of biofilm organisms. Chlorophyll *a* concentration provided a measure of algal biomass. Visual comparisons of the intact structure and composition of biofilms were made with scanning electron microscopy (SEM).

All enzyme assays and incubations were conducted

at 5 °C (close to the prevailing winter stream temperatures), using water from the appropriate stream and/or buffers with pH 4 (low pH sites) or pH 7 (higher pH sites). Cellulase assays were done on biofilms scraped from sticks but not ground in any way. Our protocols therefore differ from those of Sinsabaugh, Benfield & Linkins (1981) and Sinsabaugh *et al.* (1991a, 1993) who used homogenized, macerated or pulverized samples.

Intact biofilm samples (2 cm^2) for examination by SEM were fixed in 3% gluteraldehyde in 0.1 M Na cacodylate buffer, post-fixed in 2% buffered OsO_4, rinsed in buffer and dehydrated in an ethanol series. Samples were vacuum-dried, mounted on stubs, coated with gold and viewed with a Cambridge Stereoscan 600 scanning electron microscope.

Photosynthetic pigments were extracted from sections of stick in 2 ml 90% acetone at 5 °C (24 h); concentrations of chlorophyll *a* and phaeopigments were determined spectrophotometrically (Moss, 1967a,b).

Uptake of ^{14}C-glucose by intact biofilms was determined by incubating substrata for 5 h at 5 °C in 250 ml of filtered stream water to which 25 µl of ^{14}C-glucose (10.8 GBq $mmol^{-1}$, 1.85 MBq ml^{-1}) was added. Our incubation time was slightly shorter than that used by Sawyer & King (1993) (6 h), but longer than the 3 h used by Chappell & Goulder (1994). After incubation, substrata were rinsed with distilled water, and biofilm samples (5 cm^2) were digested with 2 ml hyamine hydroxide for 20 h at 60 °C. We are assuming that abiotic uptake of ^{14}C-glucose is minimal and similar for all substrates. After cooling, one drop of H_2O_2, and 0.5 ml of glacial acetic acid were added to each vial (Fox, 1976), and 1 ml of this digestant mixture was added to 12 ml of scintillation cocktail. Samples were dark-adapted for 12 h before radioactivity was counted on a Beckman LS 2800 scintillation counter. Quench was determined using the relationship between counting efficiency and H number.

Endocellulase activity was measured by the viscometric method of Almin & Eriksson (1967). Biofilm from 5 cm^2 of substrate was scraped into a vial containing 1 ml of 0.1 M acetate (pH 4) or phosphate (pH 7) buffer to which 2 ml of 1% carboxymethylcellulose (CMC) was added. After thorough mixing, vials were incubated on a shaker table at 5 °C for 12 h, and then centrifuged. The viscosity of two subsamples of supernatant per vial was measured by determining fall-velocity in a 0.1-ml glass pipette. Enzyme activity (units cm^{-2} h^{-1}) was calculated from the difference in fall velocities of samples and 'no-biofilm' controls run simultaneously.

(d) Invertebrates

Separate sets of sticks were examined for invertebrates on days 30 and 93. Adherent material was scraped from all surfaces of sticks and transferred to Bogorov counting trays in 70% ethanol. Invertebrates were identified and counted under a dissecting microscope at 16×. Subsamples of chironomid larvae and oligochaetes were mounted on slides to facilitate identification.

To determine whether components of heterotrophic biofilms could be ingested and assimilated, feeding trials were conducted with an amphipod, *Paraleptamphopus* sp. (Eusiridae) collected at site A2, where it was abundant. Biofilms on sticks collected from each stream after 93 days were labelled with ^{14}C-glucose as described above, rinsed and placed in jars of stream water to which six amphipods (mean wet weight for six = 3.98 mg) were added. They were allowed to feed overnight for 12 h (5 °C) before being transferred to new jars without food for a further 12 h. During this period, gut contents were fully evacuated as indicated by the examination of individuals under a microscope. Amphipods were weighed (+ 0.1 mg), and digested with hyamine hydroxide so that ^{14}C accumulated in body tissues could be counted. Dead amphipods (killed by gentle squeezing) placed in separate containers with labelled sticks, and treated identically to live individuals were used as controls to account for any passive uptake of ^{14}C.

Results

Water chemistry

Physical and chemical features of the four sites are summarized in Table 1. In addition to low pH, the brown-water sites were characterized by low conductivity and negligible alkalinity. The moderately high calcium concentration at N2 suggests that some limestone was present in the catchment, and the concentration of total aluminium was higher in the brown-water streams, as in other West Coast brown-water streams (Collier & Winterbourn, 1987b; Stenzel &

Table 1 Physical and chemical characteristics of the four study sites in the Inangahua River drainage, April–August 1993. Values are means and ranges (*single value only)

	Sites			
Variable	A1	A2	N1	N2
Altitude (m)	700	300	450	250
Temperature (°C)	6.0 (4–10)	7.6 (4–7.5)	7.8 (6.2–9)	8.4 (6–11)
pH	4.5 (4.2–4.7)	3.7 (3.5–3.8)	6.3 (6.0–6.7)	6.8 (6.5–7.1)
Conductivity ($\mu S\ cm^{-1}$) at 25 °C	19 (17–21)	31 (28–35)	64 (60–71)	165 (131–224)
Alkalinity ($mg\ l^{-1}$) as $CaCO_3$	2.0 (1.7–3.2)	0.7 (0.7–0.7)	27 (23–31)	57 (31–76)
Na ($mg\ l^{-1}$)	2.7 (2.0–3.0)	2.1 (2.0–2.2)	5.1 (4.6–5.4)	3.9 (3.0–5.3)
Mg ($mg\ l^{-1}$)	0.2 (0.1–0.3)	0.3 (0.3–0.4)	5.4 (4.0–9.4)	6.1 (6.0–6.2)
K ($mg\ l^{-1}$)	0.5 (0.4–0.5)	0.5 (0.3–0.8)	0.9 (0.7–1.1)	1.2 (0.9–1.7)
Ca ($mg\ l^{-1}$)	0.7 (0.3–1.0)	1.0 (0.8–1.2)	2.4 (2.0–3.3)	19.0 (15–23)
Al ($mg\ l^{-1}$)	0.3 (0.3–0.4)	0.3 (0.2–0.5)	0.03 (< 0.01–0.05)	0.09 (0.04–0.12)
NO_3-N ($mg\ l^{-1}$)	0.05 *	0.07 (0.06–0.08)	0.05 (0.04–0.07)	0.03 (0.02–0.05)
PO_4-P ($mg\ l^{-1}$)	< 0.04	< 0.04	< 0.04	< 0.04

Herrmann, 1990). Mean water temperatures recorded at the four sites over the 6-month period (autumn–winter) ranged from 6.0 to 8.4 °C.

Biofilm development and composition

During the study period, a major storm (40-year event) resulted in substantial scouring and deposition of bed materials at all sites. Some buried sticks were exposed or lost, and some surface-incubated sticks were washed away. Consequently, comparisons between buried and surface sticks were made only after 30 days incubation. Because no significant differences in measured parameters were obtained between the two treatments at that time (see below), initial stick placement was not considered further in making comparisons among streams.

At all sites, microbial communities that developed on sticks consisted predominantly of filamentous organisms (Fig. 1). These included septate and coenocytic fungal hyphae and fine filaments ($\approx 1\ \mu m$) presumed to be actinomycetes. Fruiting bodies of fungi were rarely found. Some bacterial rods were seen, but no shift towards a bacteria-dominated microbial community was apparent over the 6 months. Our SEM work (Fig. 1) indicates that the overall density of microorganisms increased over time, although colonization was patchy on both surface-incubated and buried sticks. Few hyphae appeared to have penetrated the wood surface. Dense hyphal mats associated with thick (2–3 mm) layers of mucilage were present on sticks in the most acidic stream (A2) on Day 93 and to a lesser extent on Day 177. Apparently identical fungal mats were also observed on sticks in a circumneutral beech-forest stream in another part of the South Island, indicating that these fungal mats were not restricted to acidic brown-water streams (our unpublished data).

As anticipated, algal colonization of sticks in the four heavily shaded streams was very low. This was indicated by the sparse representation of diatoms in fields viewed by SEM, and by chlorophyll *a* (plus phaeopigment) values that averaged only 0.07 $\mu g\ cm^{-2}$ in all four streams (range 0.01–0.24 $\mu g\ cm^{-2}$) over the course of the study.

Incorporation of ^{14}C-glucose by biofilms

Incorporation of ^{14}C-glucose was used to provide a comparative measure of heterotrophic activity in the developing biofilms. No significant differences were found between buried and surface sticks at individual sites after 30 days (one-way ANOVA, $n = 10$ for each stream, $P > 0.05$). Radioactive counts increased progressively over the first 3 months in all four streams, but no further increases were found in the three streams sampled another 3 months later (Fig. 2). On each sampling day, both the highest and lowest counts were obtained from sticks from the acid sites.

The ^{14}C data are consistent with the evidence pro-

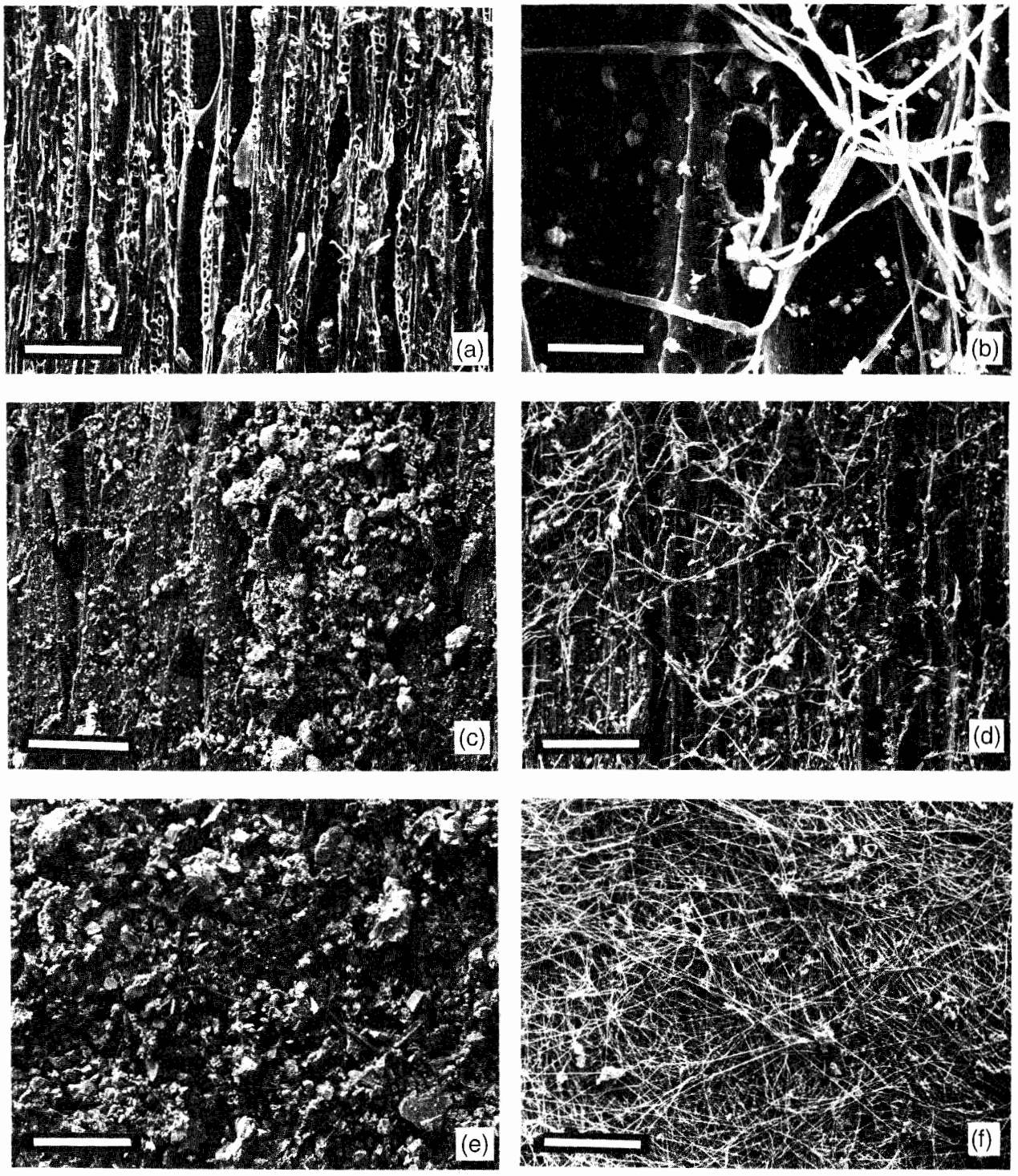

Fig. 1 Scanning electron micrographs of wood substrata (*Nothofagus menziesii*): (a) pre-incubation (scale bar = 200 μm); (b) after 61 days in A2 showing hyphae and bacteria in greater detail (scale bar = 20 μm); (c) after 30 days in N2 (scale bar = 200 μm); (d) after 93 days in N2 (scale bar = 200 μm); (e) after 30 days in A2 (scale bar = 200 μm); (f) after 93 days in A2 showing the thick fungal mat associated with mucilage (scale bar = 200 μm).

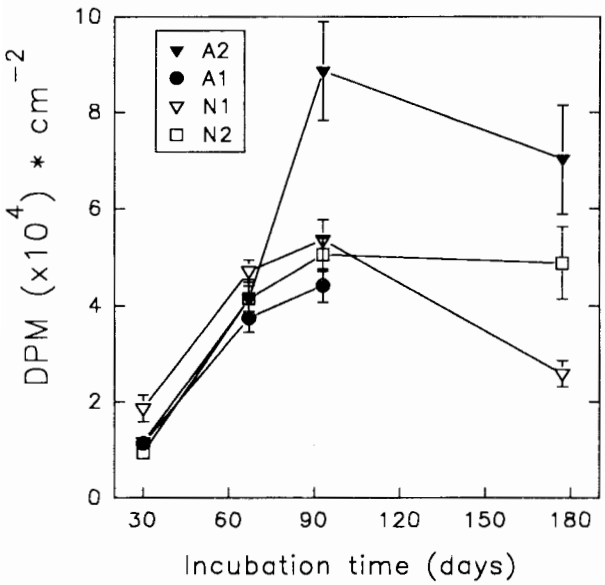

Fig. 2 Incorporation of ^{14}C by biofilms on sticks held in the four streams for up to 177 days. Mean DPM ± standard errors (SE) are plotted. No data were obtained for A2 on Day 177.

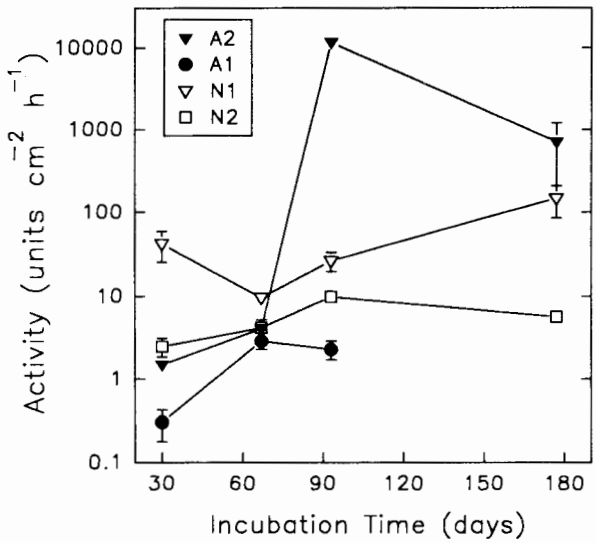

Fig. 3 Endocellulase activity of biofilm samples scraped from sticks held in the four streams for up to 177 days. Mean activities ± standard errors (SE) are plotted.

vided by SEM that microbial density continued to increase up to Day 93. The significantly higher ^{14}C counts obtained from sticks at A2 on Days 93 and 177 (two-way ANOVA, least-squares means, $F = 16.41$, 109.84, d.f. = 3,3, $n = 117$, $P < 0.05$) coincided with the presence of the thick, mucilaginous biofilms.

Endocellulase activity

After 30 days incubation, endocellulase activity was highest at N1(Fig. 3) (one-way ANOVA, Tukey's test, $F = 6.49$, d.f. = 3, $n = 40$, $P < 0.05$) and N1 was the only stream to show significant differences between surface and buried sticks with higher endocellulase activity on surface sticks (one-way ANOVA, $F = 5.79$, d.f. = 1, $n = 10$). Endocellulase activity was always lowest at A1, but very strong enzyme activity (Fig. 3) was recorded at the other acidic site (A2) when the mucilaginous biofilm was present. A significant correlation was found between ^{14}C counts and cellulase activity in the full data set (Pearson correlation, $r = 0.35$, $n = 117$, $P < 0.0001$) using individual sticks as replicates.

Microbial activity and depth of burial

Incorporation of ^{14}C and endocellulase activity of biofilm organisms were measured at two depths within the stream beds (3–9 and 19–25 cm) on dowels that had been buried vertically in the streams for 177 days (Fig. 4). Neither parameter differed with depth on individual dowels (Wilcoxon signed rank paired comparison, $n = 14$, $P > 0.05$), although incorporation of ^{14}C was significantly lower at A1 than in the other three streams (one-way ANOVA, least-squares means, $F = 11.15$, d.f. = 3, $n = 28$, $P < 0.05$). Considerable small-scale variability in endocellulase activity was evident on dowels from A1, but no significant differences (two-way ANOVA, $n = 28$, $P > 0.05$) were found among streams.

Response to nutrient additions

The responses of wood-colonizing microflora to additions of nitrogen and phosphorus are shown in Fig. 5. Although mean ^{14}C counts on substrates exposed to supplements of N and N + P were over twice those of controls at two sites, variation in counts among replicates was high and the differences over control values were significant only at N2 (one-way ANOVA on $\log x + 1$ transformed data, $F = 8.51$, d.f. = 2, $n = 11$, $P < 0.05$). Stream water samples from N2 also had the lowest mean NO_3-N concentration (Table 1).

Invertebrate colonization

Tube-dwelling chironomid larvae and harpacticoid copepods were the most abundant colonists of sticks

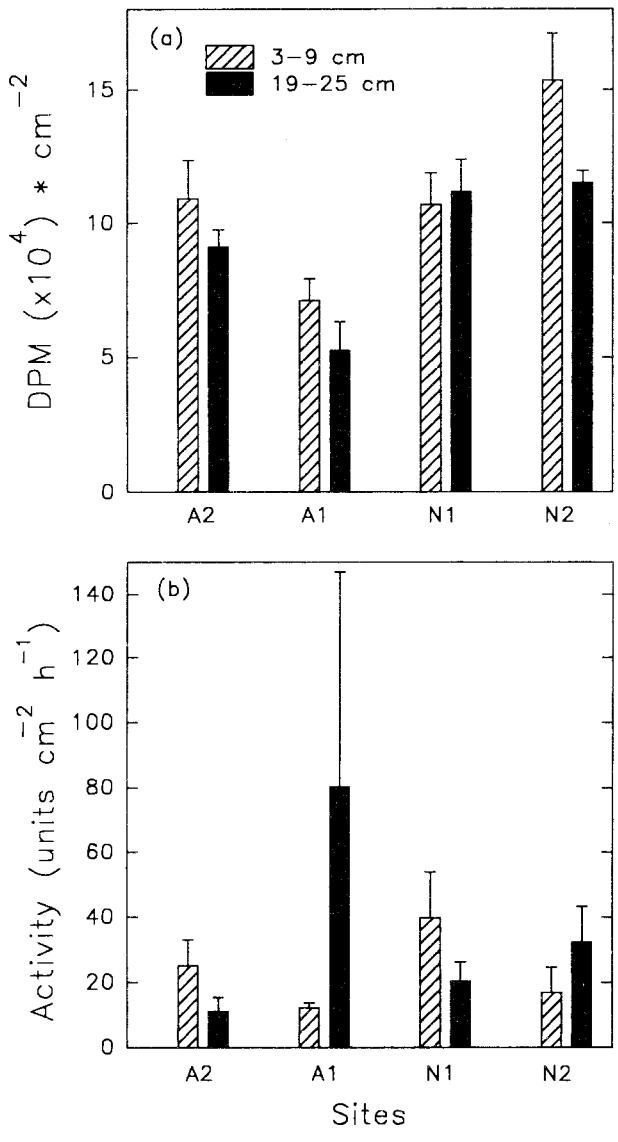

Fig. 4 Incorporation of (a) ^{14}C and (b) endocellulase activity of biofilm samples from sections of dowel 3–9 cm and 19–25 cm into the stream bed after 177 days. Assay conditions as for Figs 2 and 3. Means ± standard errors (SE) are plotted.

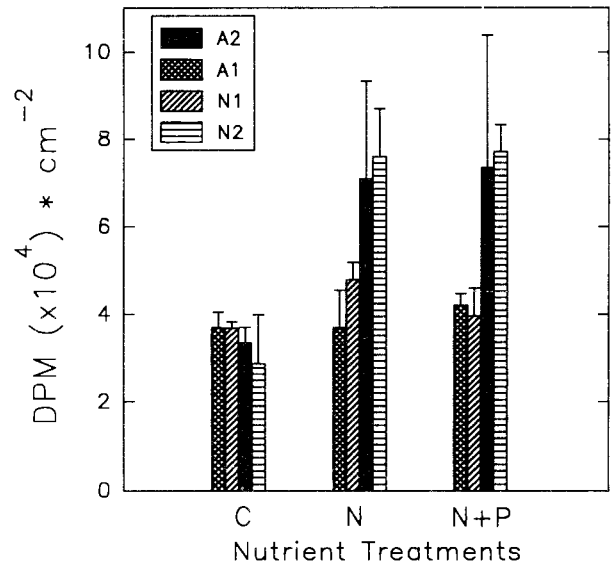

Fig. 5 Incorporation of ^{14}C by biofilms that developed on wood veneer discs overlying nutrient-releasing agar in the four streams after 30 days. Nutrient treatments: C = no-nutrient controls, N = NO_3 added, N + P = NO_3 + PO_4 added. Mean DPM ± standard errors (SE) are plotted.

in all four streams on Days 30 and 93 (65–90% of individuals per stream; Table 2). Nine taxa colonized sticks in each acidic stream, and eight and eleven taxa colonized sticks in N1 and N2, respectively. With the exception of the predatory larvae of *Philorheithrus agilis* (Hudson) and Ceratopogonidae, all species found were suspected to be consumers of wood biofilms. The main difference between the two pairs of sites was that the species composition of the chironomid fauna in the acid and near-neutral streams was distinct. In contrast, faunal abundances among the pairs of sites showed no clear pattern (Table 2).

Amphipods (*Paraleptamphopus* sp.) kept with sticks taken from each of the four streams incorporated ^{14}C-labelled biofilm material into their body tissues (Fig. 6). Dead larvae accumulated very little ^{14}C into tissues passively (less than 10% of feeding larvae). The stream in which sticks had been incubated had no significant effect on levels of radioactivity (DPM per mg amphipod) present in amphipod tissue (one-way ANOVA, $F = 0.96$, d.f. = 3, $n = 18$, $P > 0.05$). However, the low uptake by all amphipods kept with sticks from A2 may indicate that the thick mucilaginous biofilm present reduced feeding efficiency.

Discussion

The present study was conducted in low-light environments in small, forested streams so that epixylic biofilms that developed on introduced substrata would be primarily heterotrophic. The very low chlorophyll *a* values recorded at all sites (mean 0.07 μg cm^{-2}) and the presence of few algal cells in SEM fields indicated that this was achieved. In all four forested streams, epixylic biofilms were composed predominantly of filamentous fungi, along with actinomycetes, bacteria, amorphous detrital aggregates and trapped silt

Table 2 Invertebrate taxa found on sticks taken from the four streams. x = present. The relative abundance (%) of Chironomidae and Harpacticoida (combined data for days 30 and 93) and total numbers of invertebrates per stick are also shown (— = no data)

	Sites			
Taxa	A1	A2	N1	N2
Ephemeroptera				
Deleatidium sp.				x
Atalophlebioides cromwelli (Phillips)				x
Plecoptera				
Zelandobius confusus (Hare)	x	x	x	
Spaniocerca zelandica Tillyard			x	
Trichoptera				
Philorheithrus agilis (Hudson)	x			
Coleoptera				
Homalaena sp.		x		
Podaena sp.	x			
Diptera – Chironomidae	25	74	46	38
Eukiefferiella sp.	x	x		
Naonella sp.			x	x
Orthocladiinae sp.	x			
Podonomus sp.			x	x
Paucispinigera approximata Freeman		x		
Macropelopiini sp.		x		
Other Diptera				
Empididae sp.	x	x		x
Ceratopogonidae sp.		x		x
Hydracarina	x		x	x
Tardigrada			x	x
Copepoda				
Harpacticoida	40	16	44	35
Oligochaeta				
Telmatodrilus multiprostatus Brinkhurst	x	x		
Slavina sp.			x	x
Gastropoda				
Potamopyrgus antipodarum (Gray)				x
Number of taxa	9	9	8	11
Individuals per stick (mean and range, n = 6)				
(a) After 30 days	3.2 (0–5)	5.3 (1–11)	12.9 (5–20)	3.4 (2–9)
(b) After 93 days	1.5 (0–4)	10.2 (2–15)	—	4.7 (1–7)

particles. However, the methods we used did not enable identification of hyphomycete species or other fungal mycelia. Our SEM work indicated few obvious differences in composition of biofilms between sites, despite differences in streamwater pH and other aspects of water chemistry. In contrast, Collier & Winterbourn (1987a) found that fungi were common on kamahi (*Weinmannia racemosa* Linn. f.) leaves incubated in New Zealand brown-water streams, whereas bacteria were more common at sites with circum-

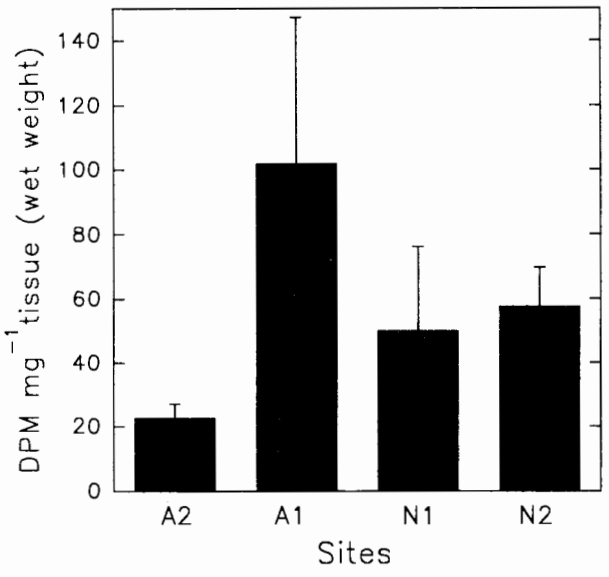

Fig. 6 Incorporation of ^{14}C by amphipods (*Paraleptamphopus* sp.) allowed to graze on labelled biofilms. Mean DPM ± standard errors (SE) are plotted.

neutral pH. They also found that many fungal hyphae were associated with amorphous detrital matter that accumulated on leaf surfaces, a phenomenon also noted on wood in the present study.

A number of enzymes have been considered in comparative studies of biofilms in streams and other freshwater systems (Jones & Lock, 1989; Sinsabaugh *et al.*, 1991b; Scholz & Boon, 1993) including several involved in the cellulose-digesting complex. Sinsabaugh & Linkins (1993) found a strong linear relationship between cumulative mass loss of white birch (*Betula papyrifera* Marsh.) sticks and cumulative activity of endocellulase and of four other enzymes involved in lignocellulose degradation. Therefore, our use of an endocellulase assay as a convenient comparative indicator of overall cellulose-digesting activity was considered reasonable even though endocellulases alone cannot degrade crystalline cellulose.

Within individual streams, no differences in values of measured parameters were found between surface and buried sticks after 30 days. Disturbance of wood substrata during severe flooding in the following month prevented such comparisons being made subsequently, but measurements made on dowels driven vertically into the stream beds indicated that burial had little effect on microbial metabolism (glucose uptake) or endocellulase activity after 177 days. This finding emphasizes the three-dimensional nature of the stream bed and the need to recognize this when considering ecosystem-level processes concerning organic matter.

Furthermore, we found few differences in biofilm development and enzyme activity among sites. Both the highest and lowest endocellulase activity and ^{14}C-fixation values were found at the two acid sites, the former being associated with a period of high mucilage production. Maximum values for both parameters were recorded at or after 13 weeks at all sites, compared with an endocellulase peak after 6 weeks on wood in a North American river, where water temperature was much higher (> 15 °C; Golladay & Sinsabaugh, 1991; Sinsabaugh *et al.*, 1991b).

Our results contrast with those reported for several leaf decomposition studies in which comparisons were made among streams differing in pH. Bacterial production associated with decomposing leaves was positively correlated with streamwater pH in several studies, and lower rates of leaf decomposition at pH < 6 have been attributed primarily to reduced microbial activity (Mulholland *et al.*, 1992). However, faster breakdown of leaves in circum-neutral, compared with acidic brown-water streams in New Zealand was attributable largely to shredder feeding (Collier & Winterbourn, 1987a). Chamier (1985) suggested that the disruption of microbial enzyme systems involved in leaf degradation may result in slower leaf breakdown in acidified streams, but the findings of the present study and those of Collier & Winterbourn (1987a) indicate this is not the case in naturally acidic (pH 4–5), brown-water streams in New Zealand.

Why wood-surface biofilms appear to be less affected by stream pH than leaf biofilms is not immediately evident. The greater persistence and physical stability of wood may favour the establishment of microbial communities dominated by pH-tolerant fungi and actinomycetes that can slowly metabolize the refractory ligno-cellulose substrates provided by wood. In contrast, the more decomposable nature of leaves may provide a greater pool of breakdown products that can be used by micro-organisms, including bacteria, with more specific water quality requirements.

Alternatively, the superficial similarity of the microbial communities we observed on wood at different sites (by SEM) may mask functional (i.e. enzymatic) differences in the organisms concerned. For example, Bengtsson (1983) suggested that the different chemical compositions of beech (*Fagus*) and alder (*Alnus*) leaves

may have selected for different guilds of fungal communities. In our present state of knowledge, we do not have sufficient information concerning such (functional) differences to understand how they might affect the success of fungi in streams (Suberkropp, 1992), or how physico-chemical factors influence the relationships between micro-organisms and their substrates.

The results of our 30-day nutrient-diffusion experiments also suggest that nutrient availability can limit microbial biomass on wood in acidic and near-neutral streams and, by implication, decomposition rates. In subsequent experiments in which cellulose-fibre cloth rather than wood veneer was used as the nutrient-diffusion surface, substantially greater bacterial colonization and decomposition were obtained when N and P were provided (our unpublished data). Howarth & Fisher (1976) also found that the addition of N + P accelerated leaf weight loss and increased rates of respiration associated with leaves. Although not reported by them, it is probably reasonable to infer that microbial biomass was also greater under nutrient-enriched conditions.

In contrast to the microbial biofilm parameters, faunal similarity was greatest among sites that were most similar in pH. Although small, tube-dwelling Chironomidae (predominantly Orthocladiinae) and harpacticoid copepods were numerically dominant on wood at all sites, species composition differed between the two acid and the two neutral streams. Anderson (1989) found that two orthoclad species were the dominant miners of wood in an Oregon stream and that a significant proportion of the entire midge community exploited wood to some extent. Hax & Golladay (1993) also reported that Chironomidae were abundant on wood substrata (white birch ice-cream sticks similar to our silver beech tongue depressors) throughout the 5 months of their study in a boreal river. However, population densities they reported (up to 250 000 m^{-2}) were much higher than the 30- and 60-day means of 200–3000 m^{-2} found at the four New Zealand sites considered here.

We found no shredding, gouging, mining or wood-boring insects, and most of the fauna was assumed to be feeding on wood-surface biofilms and associated fine detritus. Similarly, gut analyses of larvae belonging to at least seven chironomid genera taken from the surfaces of woody substrata in other New Zealand streams provided no evidence that wood was ingested (Anderson, 1982). It is also interesting that the other abundant meiofaunal group we found, the harpacticoid Copepoda, was the most numerous invertebrate group associated with fine detritus in debris-created habitats of steep, first-order streams in the Cascade Range, Oregon (Anderson & Sedell, 1979). Little seems to be known about the feeding biology of harpacticoids in streams, although they were categorized as collector-gatherers by Wallace, Webster & Lowe (1992). Our laboratory experiments with *Paraleptamphopus* and ^{14}C-labelled biofilms indicated that at least some heterotrophic components of the epixylic communities in all four streams were readily ingested and incorporated into amphipod tissue. Similarly, heterotrophic (non-algal) stone-surface organic layers can be used by larvae of the New Zealand mayfly *Deleatidium* (Rounick & Winterbourn, 1983), and insects belonging to several orders (Plecoptera, Trichoptera, Diptera) incorporated ^{14}C from labelled fungi and bacteria colonizing decaying leaves (e.g. Winterbourn & Davis, 1976; Findlay *et al.*, 1986; van Frankenhuyzen & Geen, 1986).

In conclusion, the results of our wood colonization study are not consistent with the hypothesis that microbial biomass and activity on organic substrata are necessarily lower in streams of low pH. In the South Island of New Zealand, the low pH of brown-water streams is a consequence of naturally high organic acid concentrations rather than anthropogenically induced acidification. Therefore, the wood-colonizing microflora in New Zealand brown-water streams is probably well adapted for life in a low-pH environment. Furthermore, nutrient-poor conditions and high levels of physical disturbance, such as were found at all four of our sites, can be expected to minimize differences in microbial community biomass and production among chemically diverse streams.

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References

Almin K.E. & Eriksson K.E. (1967) Enzymic degradation of polymers. I. Viscometric method for the determination of enzyme activity. *Biochimica Biophysica Acta*, **139**, 238–247.

Anderson N.H. (1982) A survey of aquatic insects associated with wood debris in New Zealand streams. *Mauri Ora*, **10**, 21–33.

Anderson N.H. (1989) Xylophagous Chironomidae from Oregon streams. *Aquatic Insects*, **11**, 33–45.

Anderson N.H. & Sedell J.R. (1979) Detritus processing by macroinvertebrates in stream ecosystems. *Annual Review of Entomology*, **24**, 351–77.

Anderson N.H., Steedman R.J. & Dudley T. (1984) Patterns of exploitation by stream invertebrates of wood debris (xylophagy). *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie*, **22**, 1847–1852.

Bengtsson G. (1983) Habitat selection in two species of aquatic hyphomycetes. *Microbial Ecology*, **9**, 15–26.

Benke A.C., van Arsdall T.C., Gillespie D.M. & Parrish F.K. (1984) Invertebrate productivity in a subtropical blackwater river: the importance of habitat and life history. *Ecological Monographs*, **54**, 25–63.

Benke A.C., Henry R.L., Gillespie D.M. & Hunter R.J. (1985) Importance of snag habitat for animal production in southeastern streams. *Fisheries*, **10**, 8–13.

Chamier, A-C. (1985) Cell-wall-degrading enzymes of aquatic hyphomycetes: a review. *Botanical Journal of the Linnaean Society*, **91**, 67–81.

Chappell K.R. & Goulder R. (1994) Seasonal variation of epilithic extracellular enzyme activity in three diverse headstreams. *Archiv für Hydrobiologie*, **130**, 195–214.

Collier K.J. & Winterbourn M.J. (1987a) Breakdown of kamahi leaves in four South Westland streams. *Mauri Ora*, **14**, 33–42.

Collier K.J. & Winterbourn M.J. (1987b) Faunal and chemical dynamics of some acid and alkaline New Zealand streams. *Freshwater Biology*, **18**, 227–240.

Collier K.J., Jackson R.J. & Winterbourn M.J. (1989a) Dissolved organic carbon dynamics of developed and undeveloped wetland catchments in Westland, New Zealand. *Archiv für Hydrobiologie*, **117**, 21–38.

Collier K.J., Winterbourn M.J. & Jackson R.J. (1989b) Impacts of wetland afforestation on the distribution of benthic invertebrates in acid streams of Westland, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, **23**, 479–490.

Cowie B. (1985) An analysis of changes in the invertebrate community along a southern New Zealand montane stream. *Hydobiologia*, **120**, 35–46.

Evans B.F., Townsend C.R. & Crowl T.A. (1993) The retention of woody debris structures in a small stream following a large flood. *New Zealand Natural Sciences*, **20**, 35–39.

Findlay S., Meyer J.L. & Smith P.J. (1986) Incorporation of microbial biomass by *Peltoperla* sp. (Plecoptera) and *Tipula* sp. (Diptera). *Journal of the North American Benthological Society*, **5**, 306–310.

Fox B.W. (1976) *Techniques of Sample Preparation for Liquid Scintillation Counting*. North Holland, Amsterdam.

van Frankenhuyzen K. & Geen G.H. (1986) Microbe-mediated effects of low pH on availability of detrital energy to a shredder, *Clistoronia magnifica* (Trichoptera: Limnephilidae). *Canadian Journal of Zoology*, **64**, 421–426.

Golladay S.W. & Sinsabaugh R.L. (1991) Biofilm development on leaf and wood surfaces in a boreal river. *Freshwater Biology*, **25**, 437–450.

Groom A.P. & Hildrew A.G. (1989) Food quality for detritivores in streams of contrasting pH. *Journal of Animal Ecology*, **58**, 863–881.

Hax C.L. & Golladay S.W. (1993) Macroinvertebrate colonization and biofilm development on leaves and wood in a boreal river. *Freshwater Biology*, **29**, 79–87.

Howarth R.W. & Fisher S.G. (1976) Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. *Freshwater Biology*, **6**, 221–228.

Jackson R.J. (1987) Hydrology of an acid wetland before and after draining for afforestation, western New Zealand. *Forest Hydrology and Watershed Management* (Proceedings of the Vancouver Symposium, August 1987). I.H.A.S. publication, **167**, 465–474.

Jones S.E. & Lock M.A. (1989) Hydrolytic extracellular enzyme activity in heterotrophic biofilms from two contrasting streams. *Freshwater Biology*, **22**, 289–296.

Lock M.A., Wallace R.L., Costerton J.W., Ventullo R.M. & Charlton S.E. (1984) River epilithon: toward a structural–functional model. *Oikos*, **42**, 10–22.

Moore T.R. & Jackson R.J. (1989) Dynamics of dissolved organic carbon in forested and disturbed catchments, Westland, New Zealand 2. Larry River. *Water Resources Research*, **25**, 1331–1339.

Moss B. (1967a) A spectrophotometric method for the estimation of percentage degradation of chlorophylls to pheo-pigments in extracts of algae. *Limnology and Oceanography*, **12**, 335–340.

Moss B. (1967b) A note on the estimation of chlorophyll

a in freshwater algal communities. *Limnology and Oceanography*, **12**, 340–342.

Mulholland P.J., Driscoll C.T., Elwood J.W., Osgood M.P., Palumbo A.V., Rosemond A.D., Smith M.E. & Schofield C. (1992) Relationships between stream acidity and bacteria, macroinvertebrates, and fish: a comparison of north temperate and south temperate mountain streams, U.S.A. *Hydrobiologia*, **239**, 7–24.

Mulholland P.J., Palumbo A.V., Elwood J.W. & Rosemond A.D. (1987) Effects of acidification on leaf decomposition in streams. *Journal of the North American Benthological Society*, **6**, 147–158.

New Zealand Meterological Service (1973) Rainfall normals for New Zealand 1941–1970. *New Zealand Meteorological Service Miscellaneous Publications*, **145**.

Rounick J.S. & Winterbourn M.J. (1983) The formation, structure and utilization of stone surface organic layers in two New Zealand streams. *Freshwater Biology*, **13**, 57–72.

Sawyer T.E. & King G.M. (1993) Glucose uptake and end product formation in an intertidal marine sediment. *Applied and Environmental Microbiology*, **59**, 120–128.

Scholz O. & Boon P.I. (1993) Biofilm development and extracellular enzyme activities on wood in billabongs of south-eastern Australia. *Freshwater Biology*, **30**, 359–368.

Sinsabaugh R.L., Antibus R.K., Linkins A.E., McClaugherty C.A., Rayburn L., Repert D. & Weiland T. (1993) Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology*, **74**, 1586–1593.

Sinsabaugh R.L., Benfield E.F. & Linkins A.E. (1981) Cellulase activity associated with the decomposition of leaf litter in a woodland stream. *Oikos*, **36**, 184–190.

Sinsabaugh R.L., Golladay S.W. & Linkins A.E. (1991a) Comparison of epilithic and epixylic biofilm development in a boreal river. *Freshwater Biology*, **25**, 179–187.

Sinsabaugh R.L. & Linkins A.E. (1993) Statistical modelling of litter decomposition from integrated cellulose activity. *Ecology*, **74**, 1594–1597.

Sinsabaugh R.L., Repert D., Weiland T., Golladay S.W. & Linkins A.E. (1991b) Exoenzyme accumulation in epilithic biofilms. *Hydrobiologia*, **222**, 29–37.

Stenzel A. & Herrmann R. (1990) Comparing the effects of acidic deposition on the chemistry of small streams in the South Island of New Zealand with those in the Fichtelgebirge, F.R.G. *Catena*, **17**, 69–83.

Suberkropp K. (1992) Aquatic hyphomycete communities. *The Fungal Community. Its Organization and Role in the Ecosystem*, 2nd edn (eds G. C. Carroll and D.T. Wicklow), pp. 729–747. Marcel Dekker Inc., New York.

Tank J.L., Webster J.R. & Benfield E.F. (1993) Microbial respiration on decaying leaves and sticks in a southern Appalachian stream. *Journal of the North American Benthological Society*, **12**, 394–405.

Wallace J.B., Webster J.R. & Lowe R.L. (1992) High-gradient streams of the Appalachians. *Biodiversity of Southeastern United States/Aquatic Communities* (eds C. T. Hackney, S. M. Adams and W. A. Martin), pp. 133–191. John Wiley, New York.

Winterbourn M.J. (1982) The invertebrate fauna of a forest stream and its association with fine particulate matter. *New Zealand Journal of Marine and Freshwater Research*, **16**, 271–281.

Winterbourn M.J. (1990) Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream. *Freshwater Biology*, **23**, 463–474.

Winterbourn M.J. & Davis S.F. (1976) Ecological role of *Zelandopsyche ingens* (Trichoptera, Oeconesidae) in a beech forest stream ecosystem. *Australian Journal of Marine and Freshwater Research*, **27**, 192–215.

Winterbourn M.J., Rounick J.S. & Cowie B. (1981) Are New Zealand stream ecosystems really different? *New Zealand Journal of Marine and Freshwater Research*, **15**, 321–328.

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