Microbial Activity on Wood in Streams:
Exploring Abiotic and Biotic Factors Affecting the Structure and Function of Wood Biofilms.

by

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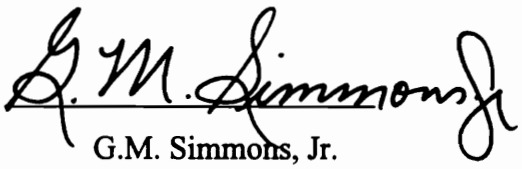

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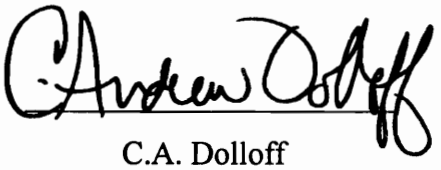

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(ABSTRACT)

In this examination of microbial colonization of wood and factors affecting the structure and function of wood biofilms in streams, the first two chapters summarize research conducted in New Zealand and compare the composition of biofilm colonizing wood to that colonizing rocks and leaves. Similar biofilms developed on wood veneer, natural twigs, and beech leaves, but fungi did not colonize stones where diatoms were the predominant colonizer. Comparing wood incubated on the streambed surface to wood buried beneath the streambed, fungal hyphae dominated the biofilm but actinomycetes and bacteria were also present. An assay of microbial activity (^{14}C glucose uptake) indicated that surface biofilms were more active than biofilms on buried wood. There was no relationship between ^{14}C glucose uptake and stream pH indicating that acidity did not affect wood biofilm activity in these streams. Biofilm activity on wood buried at 3-9 cm in the streambed was not significantly different than that buried at 19-25 cm.

Chapters 3, 4, and 5 examine the processes governing wood biofilms in the presence and absence of leaf litter in 2 small mountain streams at Coweeta Hydrologic Laboratory in the southern Appalachians. Microbial respiration, fungal biomass,

extracellular enzyme activity, and the effect of nutrient addition were used as descriptors of wood biofilms. Exclusion of leaf litter from a headwater stream enhanced extracellular enzyme activity, and fungal biomass was 7 times higher than that in the reference stream. Relative activities of selected extracellular enzyme activities suggested that the biofilm in the reference stream was nutrient limited. Also, nutrient releasing substrates placed beneath wood veneers indicated co-limitation of nitrogen and phosphorus on biofilms in the reference stream. Competition for nutrients by microbial biofilms may play a regulatory role in detrital processing in these streams.

Laboratory feeding studies using *Tallaperla* sp. were conducted to explore the suitability of wood biofilms as a food resource for shredders. There were no differences in *Tallaperla* growth rates on wood and leaves, and *Tallaperla* grew equally well on wood incubated for 1 or 2 months. At the end of each study, fungal biomass on wood in the feeding chambers was not different from fungal biomass at the beginning indicating that *Tallaperla* nymphs were not food limited and fungal production was able to compensate for invertebrate grazing. In the absence of leaf litter, stream shredders such as *Tallaperla* can survive and grow on the microbial biofilm on wood. The carbon stored in wood in streams is transferred to higher trophic levels via microbial biofilms.

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practice, grand ideas, and plans for the future. Thank you for your candid advice and support.

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General Introduction

> "If I could do it all over again, and relive my vision in the twenty-first century, I would be a microbial ecologist. ...Into that world I would go... I would cut my way through clonal forests sprawled across grains of sand, travel in an imagined submarine through drops of water proportionately the size of lakes, and track predators and prey in order to discover new life ways and alien food webs."
>
> E.O. Wilson, from his autobiography, *Naturalist.*

Microbial organisms play an important role in streams. These microbial assemblages are both autotrophic and heterotrophic and occur attached to all surfaces within streams including rocks (epilithon), sediments (epipelon), and wood (epixylon). The attached microbial communities are commonly referred to as "biofilms" (Lock 1993). Lock et. al. (1984) proposed a structural and functional model of the microbial biofilm in which heterotrophic (bacteria and fungi) and autotrophic (algae) components are attached to surfaces by a microbially produced extracellular polysaccharide matrix. The matrix is credited for helping to entrap soluble and particulate organic matter as well as retaining extracellular enzymes and the products of enzymatic processes. The matrix provides biofilm stability, like a "glue" that allows for multistory colonization. This model was developed to describe epilithic biofilms in streams, but research is now focusing on other wetted surfaces such as wood and leaves (e.g., Golladay and Sinsabaugh 1991, Suberkropp 1992).

Over the past 20 years, the contribution and processing of terrestrial leaves entering lotic systems has been studied extensively. Much of the ecology associated with litter processing has involved the taxonomy, distribution, and function of aquatic

hyphomycete fungi, which play the major role in leaf litter decomposition (Suberkropp and Klug 1976, Suberkropp 1992). While dominant species rankings may change on a resource through time, generally there is no successional replacement pattern observed in aquatic fungal assemblages on leaves (Suberkropp 1992). The lack of succession may be due to the relatively short-term nature of leaf resources (generally on the order of months).

In contrast, a significant portion of the allochthonous organic matter entering streams is wood, in the form of twigs, branches and logs, that is much longer lasting. Unlike leaves, wood provides a slow-decomposing, long-term substrate for microbial colonization (Shearer and Webster 1991). However, there have been few studies of the microbial community involved in wood decomposition (Shearer and Von Bodman 1983). Colonization of wood by aquatic hyphomycetes in freshwater habitats was first described by Jones & Oliver (1964). Until then, microbial colonization of wood had only been examined in terrestrial and marine environments, and reports of aquatic hyphomycetes in freshwater had been limited to Ingold's work describing the species found on decomposing leaf litter (1952). Microbial colonization of wood is primarily limited to the wood surface because of poor oxygen diffusion into dense woody tissue thereby inhibiting microbial respiration below the surface (Aumen et al. 1983). Diversity of fungi on wood surfaces can be explained by the constant sloughing of rotted wood particles by abrasion in addition to grazing or gouging insect feeding. In this way, uncolonized material is exposed and new species can colonize depending on environmental

conditions, thereby creating a "time-release" substrate (Shearer and Webster 1991). Shearer and Webster (1991) studied aquatic hyphomycete colonization on twigs in the river Teign throughout 6 months of submersion. Results indicated that 3 species occurred frequently at all sites along the pH gradient, while 13-26 species occurred at each individual site. Additionally, they found no evidence for fungal preference between 2 different wood types.

Although fungi are the major component of wood biofilms, bacteria and algae also colonize wood in streams. Couch and Meyer (1992) studied the development and composition of epixylic biofilms in a blackwater river (Ogeechee River, Georgia, USA), however, they only studied the interaction between algae and bacteria. Although they could find no correlation between algal and bacterial biomass, this study was one of the first to examine biofilms found in a habitat other than low-order, high-gradient, mountain streams. They also showed that at least 9 times more carbon was stored in the extracellular polysaccharide matrix than in bacterial biomass (Couch and Meyer 1992). These results emphasized potential of the biofilm as a food resource to higher trophic levels and demonstrated that significant amounts of carbon are stored in the biofilm in forms other than live bacterial biomass.

In the following 5 chapters I explore a variety of factors affecting the structure and function of wood biofilms in streams. I begin by describing research conducted in New Zealand where I examine the wood biofilm in relation to other substrates, both organic and inorganic, found in streams (chapter 1) and the effect of pH on biofilm

activity on surface and buried wood (chapter 2). The forest streams of the South Island of New Zealand are physically harsh, characterized by fluctuating flows, high sediment loads, and unstable beds (Winterbourn 1995) and retention of course particulate organic matter is low (e.g., Winterbourn et al. 1988). Wood biofilms provide a carbon source and because of their inherent stability may play a significant role in supporting stream community metabolism.

In contrast to New Zealand streams, small mountain streams in the southern Appalachians of the United States are characterized by substantial inputs and retention of both leaves and wood. At Coweeta Hydrologic Laboratory, Macon Co., North Carolina, I examined some of the processes governing wood biofilms in the presence and absence of leaf litter. I studied the effect of leaf litter exclusion on wood biofilms in a first-order stream where a 180-m canopy was used to exclude leaves. I used microbial respiration, fungal biomass, extracellular enzyme activity, and the effect of nutrient addition as descriptors of wood biofilms in this and a reference stream (chapters 3 and 4). I also explored the suitability of wood biofilms as a food resource in a laboratory feeding study using a common macroinvertebrate shredder, *Tallaperla* sp. (chapter 5).

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Chapter 1:

Microbial activity and invertebrate colonisation of wood in a New Zealand forest stream.[1]

[1]Tank, J.L. and M.J. Winterbourn. 1996. New Zealand Journal of Marine and Freshwater Research. 30:271-280.

Abstract Breakdown and colonisation of buried and surface-incubated wood was examined in a South Island, beech forest stream. Comparisons were also made of microbial activity on wood, leaves, and stones. Weight loss of sticks was initially slow, but after 11.5 months 39% of their initial dry weight had been lost. Microbial colonists were mainly fungal hyphae, fine actinomycete-like filaments and unicellular bacteria. Chironomid larvae, oligochaetes, and harpacticoid copepods were the most abundant animal colonisers. Incorporation of ^{14}C-glucose by wood-surface biofilms increased in the first 3 months and was greater on surface-incubated than buried sticks. Endocellulase activity varied over time, and very high values after 2 months were associated with dense patches of filamentous micro-organisms. Similar microbial assemblages developed on surface-incubated sticks, leaves and twigs, but fungi were not found on stones where diatoms predominated and endocellulase activity was negligible. Results of preliminary experiments with diffusion substrata suggested that the heterotrophic biofilm microflora was nutrient limited. Our findings indicate that wood surfaces can be important sites of organic matter uptake and transfer in forest streams.

Keywords Streams; wood; biofilms; endocellulase; Chironomidae; New Zealand

INTRODUCTION

Although wood is a characteristic feature of forest streams, its functional role in stream ecosystems has received much less attention than allochthonous leaf litter (Harmon et al. 1986; Maser & Sedell 1994). One reason for this is the refractory nature of wood resulting in its slow rate of breakdown, and consequent lack of attractiveness to stream ecologists interested in decomposition.

Nevertheless, it is well known that wood can be an important determinant of the structure and complexity of stream channels (Maser & Sedell 1994), and that it is colonised by a wide range of organisms. At least in its early stages, wood decomposition in aquatic environments is primarily a surface phenomenon involving fungi, actinomycetes, and other bacteria (Triska & Cromack 1980; Aumen et al. 1983; Harmon et al. 1986). Some microbial colonists are involved in the breakdown of ligno-cellulose (Golladay & Sinsabaugh 1991), and the micro-organisms themselves provide food for numerous aquatic invertebrates (Anderson et al. 1978,1984). Hax & Golladay (1993) found a positive correlation between macroinvertebrate density on wood and the degree of microbial biofilm development, Phillips & Kilambi (1994) noted that degree of decay was an important microhabitat factor to which the larvae of some chironomid taxa responded, whereas Dudley & Anderson (1982) found that other insects colonised wood simply as a substrate.

The amount of woody debris in New Zealand forest streams tends to be relatively small compared with that in North America (Winterbourn et al. 1981; Anderson 1982;

Evans et al. 1993a,1993b). Nevertheless, Evans et al. (1993a) found that wood incorporated into the substrate of a native forest stream influenced bed stability and they suggested it may provide a carbon and nutrient store.

Little is known about the colonisation and decomposition of wood in New Zealand streams. Anderson (1982) recorded a broad spectrum of aquatic insects on wood, and noted that most of them also occurred on stony substrata. He found only two species that burrowed into submerged wood: the larvae of a tipulid Limonia nigrescens (Hutton) and a chironomid Harrisius pallidus (Freeman), both of which occurred only at a late stage in substrate decomposition. Tank & Winterbourn (1995) described the development of biofilm communities dominated by narrow filamentous micro-organisms on strips of wood incubated in four West Coast streams differing in pH. Substrata were also colonised by low densities of invertebrates, the most common being tube-dwelling larvae of Chironomidae, and harpacticoid copepods.

The present study investigates the breakdown and colonisation of buried and surface-incubated wood by micro-organisms and invertebrates in a small beech forest stream. We compare microbial communities and their activity on wood, leaves, and stones, and make some preliminary observations on their responses to nutrient additions.

STUDY SITE

Fieldwork was conducted in Middle Bush Stream, a tributary of the Cass River in the Waimakariri Basin, South Island, New Zealand (43° 02'S, 171° 45'E). At the study site

(650 m a.s.l.) the stream flows through mountain beech forest (Nothofagus solandri var. cliffortioides (Hook.f.) Poole), which provided heavy, year-round shading and inputs of plant litter. Middle Bush Stream is about 1 m wide with a stony bed and baseflow discharge of about 4 l s^{-1}. Mean alkalinity (± 1 SE) of stream water during the study was 38 ± 2 mg $CaCO_3$ l^{-1}, pH was 6.6 ± 0.1, and conductivity was 103 ± 7 µS cm^{-1} (at 25°C). Water temperature was monitored with maximum-minimum thermometers for 11 months and averaged 6°C (range 2-10°C).

Middle Bush Stream has been the site of numerous ecological studies over the last two decades. For more detailed descriptions of the stream, its catchment and biota see Winterbourn (1987), Death (1991), and references therein.

METHODS

Colonisation and breakdown of wood

Wooden substrata were placed in a shaded, forested reach of Middle Bush Stream on 13 April 1993. Sampling units were heat-sterilised tongue depressors (140 X 16 X 1 mm), hereafter referred to as "sticks", cut from untreated silver beech (Nothofagus menziesii (Hook.f.) Oerst.). Sets of sticks were attached to plastic-mesh sheets with cable ties, and either staked firmly to the stream bed, or buried beneath about 10 cm of gravel. Long axes of sticks were oriented parallel to the direction of flow.

Sets (6 sticks/set) of surface-incubated and buried sticks were collected after 30, 61, and 93 days (buried sticks were also collected after 189 days), and returned to the

laboratory in containers of cold stream water, and sticks (5 replicates/treatment) were assayed for chlorophyll a, ^{14}C-glucose incorporation, and endocellulase activity (see below). Intact epixylic biofilms were examined by scanning electron microscopy. Biofilm development was also examined on 35 cm long, 10 mm diameter Pinus radiata (D.Don) dowels that were driven vertically into the stream bed. After 189 days, three dowels were carefully removed from the stream and 3 cm long sections were cut from each of them at distances of 3-9 and 19-25 cm below the bed surface. They were taken to the laboratory in vials of cold stream water.

Additional sets of sticks were collected on days 30, 61, 93, 189, 238, and 348, and used to examine colonisation by benthic invertebrates. Buried sticks were obtained on all dates but surface-incubated sticks were taken only on days 30 and 93. Sticks were placed individually in glass vials and preserved with 70% ethanol. In the laboratory, adherent material was scraped from each stick and placed in a Bogorov counting cell. Invertebrates were identified and counted at 16X magnification. Subsamples of chironomid larvae and oligochaetes were mounted on slides to enable identification to generic or species level.

The sets of buried sticks used to examine invertebrates were also used to assess changes in stick weight over time. They were thoroughly washed and brushed before being dried to constant weight at 50^{o}C. Sticks were cooled in air overnight before weighing to the nearest 0.01 g. Because sticks were not weighed individually before placement in the stream, changes in mass were estimated by making comparisons with an unused control sample of sticks (n = 12) that were dried and weighed in the same way.

Substrate comparisons

Biofilms on organic and inorganic substrata were compared after 60 and 90 days incubation starting on 21 August 1993. Inorganic substrata consisting of greywacke sandstone (the natural stream bed material) as used by Rounick & Winterbourn (1983) were glued to plastic trays with Silaflex, a clear, non-toxic sealant containing no anti-fungal agent. Three kinds of organic substrata were compared: silver beech "sticks", mountain beech twigs (1-2 cm diameter) collected from the stream banks, and recently fallen mountain beech leaves. Twigs were tied together in rafts, and leaves were held in plastic mesh envelopes (5 mm pore size). All substrata were attached to the surface of the stream bed in permanently flowing riffles. On each sampling date, five replicate substrata of each kind were returned to the laboratory where replicate samples were assayed for chlorophyll a, ^{14}C-glucose incorporation, and endocellulase activity (see below); others were examined with SEM.

Nutrient-addition assays

Nutrient diffusing substrata were used to determine the effect of NO_3-N and PO_4-P additions on the development of epixylic biofilms. The substrata were 60 ml plastic cups filled with 2% agar, to some of which we added nutrients (see Winterbourn 1990). The agar surface was covered with a very thin disk (12.5 cm^2) of untreated rimu (Dacrydium cupressinum Lamb.) veneer, which acted as the diffusion surface on which the biofilm

developed. Three treatments were used: no-nutrient controls, N additions (0.66*M* $NaNO_3$), and N+P additions (0.66*M* $NaNO_3$ + 0.06*M* KH_2PO_4). Four replicate cups representing each treatment were glued to a plastic tray with Silaflex, placed in the stream on 21 August 1993 anchored to the bed of a shaded pool, and recovered after 30 days.

In a subsequent trial, untreated cellulose cloth (Wettex) and nylon plankton netting were substituted for wood veneer as the diffusion surfaces. Identical control and N+P treatments were used but the experiment was run at an unshaded, downstream site in tussock grassland as well as at the shaded forest site of Middle Bush Stream. The trial ran for 26 days.

Incorporation of ^{14}C-glucose was measured on all substrata in both trials, whereas chlorophyll analyses, endocellulase assays, and invertebrate counts were made in the second trial.

Laboratory assays

The incorporation of ^{14}C-glucose was used as a comparative measure of heterotrophic activity (Sawyer and King 1993; Chappell & Goulder 1994), and endocellulase activity provided an index of the cellulose-degrading potential of the biofilm micro-organisms. Chlorophyll a concentration was used as a measure of algal biomass. Visual comparisons of intact biofilms were made with scanning electron microscopy (SEM). In order to simulate natural environmental conditions as closely as possible, all assays were

incubated at 5°C (close to prevailing stream water temperatures), using water from Middle Bush Stream and/or pH 7 buffer.

Photosynthetic pigments were extracted from substrates of known area in 90% acetone at 5°C (24 h). Concentrations of chlorophyll a (including phaeopigments) were determined spectrophotometrically (Moss 1967a,1967b).

Uptake of ^{14}C-glucose by intact biofilms was determined by incubating substrata 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. After 5 h, substrata were rinsed with distilled water, biofilm samples (5 cm^2) were digested with 2 ml hyamine hydroxide for 20 h at 60°C. We assumed that abiotic uptake of ^{14}C-glucose was minimal and similar for all substrates. After cooling, one drop of H_2O_2 and 0.5 ml of acetic acid were added to each vial (Fox 1976); 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 (as DPM) on a Beckman LS 2800 scintillation counter. Quench was determined using the relationship between 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 phosphate buffer (pH 7) to which 2 ml of 1% carboxymethylcellulose (CMC) was added. After thorough mixing, but no grinding or maceration, vials were incubated on a shaker table at 5°C for 12 h and centrifuged. The viscosity of two subsamples of supernatant per vial was measured by determining their fall velocities in a 0.1 ml pipette (Almin &

Eriksson 1967). Enzyme activity was calculated as the difference in fall velocities of samples and "no-biofilm" controls run simultaneously (units $cm^{-2}\ h^{-1}$).

Intact biofilm samples 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 0.1*M* Na cacodylate 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.

Statistical analyses

Statistical analyses (SAS 1991) were carried out using log transformed data. Analysis of variance (ANOVA) was performed to determine differences between substrate types, incubation times, and nested ANOVA was used to compare nutrient treatments in shaded and unshaded environments on cellulose and nylon mesh substrates. Least squares means (LSM) were used to discriminate among means following a significant ($P < 0.05$) ANOVA. Microbial activity on segments of dowel buried at different depths was compared with paired *t*-tests.

RESULTS

Biofilms on sticks, leaves, natural twigs, and stones

The three organic substrata (sticks, natural twigs, and leaves) were colonised primarily by filamentous micro-organisms including fungal hyphae and actinomycetes (Fig. 1). Some

wide-diameter ("terrestrial") hyphae were found on twigs, but these had probably colonised before placement in the stream. Leaf surfaces were generally less colonised than wood surfaces, and the main micro-organisms seen were very fine, actinomycete-like filaments and bacterial rods (Fig. 1). In contrast to the organic substrates, diatoms (predominantly Achnanthes and Gomphonema spp.) were the dominant colonists of stones (Fig. 1). Bacteria were the main heterotrophs visible on stones and no fungal hyphae were seen.

Chlorophyll a concentration was very low (<0.25 ug cm^{-2}) on sticks and leaves after 60 and 90 days, although some higher-concentration patches occurred on natural twigs after Day 60 (Fig. 2A). After 90 days in the stream, chlorophyll concentration on stones was significantly higher than on the three other organic substrates (one-way ANOVA, $P = 0.0001$).

After only 60 days in the stream there were no significant differences in incorporation of ^{14}C by surface biofilms among substrates, but by Day 90, ^{14}C incorporation was significantly lower on leaves (one-way ANOVA, $P < 0.05$). The large increase in ^{14}C incorporation recorded on stones between Days 60 and 90 coincided with an increase in the abundance of diatoms, actinomycete-like filaments, and bacterial rods as indicated by SEM (Fig. 2B).

Endocellulase activity increased 2-4 fold on sticks and twigs between Days 60 and 90, and an even greater proportional increase occurred on leaves (Fig. 2C)(two-way ANOVA, $P < 0.05$). The increase presumably indicated the availability of more substrate

digestible by endocellulases, and/or a parallel functional shift in the biofilm community towards bacterial and fungal species with such activity. Compared with the three organic substrata, negligible endocellulase activity was shown by stone-surface communities on either date pointing to a fundamental difference between epilithic biofilms and biofilms colonising organic subtrata.

Stick colonisation and breakdown

As in the substrate comparison experiment, silver beech sticks were colonised by bacterial rods, fungal hyphae, and fine actinomycete-like filaments. All three groups were present on surface-incubated and buried sticks on Day 30. On Day 91 some thick patches of hyphae were found associated with 1-2 mm thick layers of mucilage. Few algal cells were seen, even on the upper surfaces of surface-incubated sticks, and their paucity was confirmed by the very low chlorophyll *a* values obtained (maximum 0.11 $\mu g\ cm^{-2}$ on Day 93).

Weight loss of buried sticks was slow over the first 238 days (15%), but a further 24% loss of dry weight occurred between Days 238 and 348 (Fig. 3). The condition of buried sticks and the amount of debris adhering to them changed noticeably over time. Sticks remained quite clean for the first 90 days, but by Day 189 considerable sand and detritus entangled with mucilage was adhering to them. On Day 238 blackening of stick surfaces was seen for the first time, and the amount of adherent debris was even greater. On Day 348 the wood surface was strongly blackened, pitted, slimy, and soft.

Biofilms that developed on buried and surface-incubated sticks incorporated very similar amounts of ^{14}C on Day 31 (Fig. 4A). Counts increased on surface-incubated sticks on Days 60 and 93 indicating a continuing increase in microbial biomass and/or activity, but buried sticks showed little change over the 189 day incubation period. Biofilms on surface-incubated sticks incorporated significantly more ^{14}C than buried sticks after day 31 (two-way ANOVA, $P < 0.05$).

Endocellulase activity varied more over the total incubation period than did ^{14}C incorporation. However, neither time nor stick placement (buried vs. surface) explained differences in enzyme activity (two-way ANOVA, $P > 0.05$). The very high mean and large standard error recorded from buried sticks on Day 60 (Fig. 4B) indicated that active, cellulose-degrading micro-organisms were abundant but patchily distributed.

Vertically buried dowels

Dowels driven vertically into the bed of Middle Bush Stream were retrieved after 189 days. Mean endocellulase activity (± 1 SE) on wood-surface biofilms 3-9 cm below the bed surface was 5.1 ± 3.6 units cm^{-2} h^{-1} and did not differ significantly from those at 19-25 cm depth (mean 1.1 ± 0.5 units cm^{-2} h^{-1})(paired *t*-tests, $P = 0.16$). Mean ^{14}C incorporation by wood-surface biofilms was 2659 ± 351 DPM h^{-1} at 3-9 cm below the bed surface did not differ significantly from those at 19-25 cm depth (mean 2013 ± 1104 DPM h^{-1})(paired *t*-tests, $P = 0.30$). Therefore, our results indicated that depth of burial in

the top 25 cm of relatively uncompacted stream bed does not affect biofilm development or activity.

Invertebrate colonisation of sticks

Mean densities (± 1 SE) of invertebrates on buried sticks increased over time from 2.5 ± 0.6 individuals per stick on Day 30 to 38.2 ± 6.3 individuals per stick after 50 weeks (Fig. 5). The main colonising taxa were tube-dwelling larval Chironomidae (principally Paucispinigera approximata Freeman), two oligochaetes, Telmatodrilus multiprostatus Brinkhurst (Tubificidae) and Pristina sp. (Naididae), and unidentified harpacticoid copepods. Densities of Chironomidae showed little change between Days 91 and 348, but numbers of oligochaetes increased over time (Fig. 5). Their greater prominence on later dates was associated with the presence of larger amounts of mucilage, sand, and fine detritus adhering to the sticks.

Mean densities of invertebrates recorded on surface-incubated sticks on Days 30 and 93 were about six and three times higher than on buried sticks collected at the same time. Chironomid larvae with tubes attached to both the upper and lower surfaces of sticks made up 64% of the colonists found on the two days.

Responses to nutrient additions

In the first trial, measurements of ^{14}C-glucose incorporation on rimu veneer disks after 30 days in the stream provided no evidence of a response to the presence of added nutrients

(one-way ANOVA, $P = 0.62$). However, in the second trial, where the effect of nutrient addition was compared in open and shaded habitats on cellulose cloth and nylon mesh substrates, ^{14}C counts were significantly higher on nylon mesh substrates within each habitat regardless of nutrient treatment (Fig. 6A)(nested ANOVA, least-squares means, $P < 0.05$).

Nutrient addition stimulated endocellulase activity (Fig. 6B) and chlorophyll a concentration (Fig. 6C)(nested ANOVA, least-squares means, $P < 0.05$). Endocellulase activity was very low on nylon mesh and was highest on cellulose cloth at the shaded site. In contrast, chlorophyll a concentration was significantly greater on cellulose cloth at the unshaded site than in the other three treatments ($P < 0.05$). In fact, endocellulase activity of cellulose cloth communities with nutrients added was greater than the maximum recorded on sticks buried in the stream (Day 180) at both the unshaded and shaded sites (Fig. 6B).

Chironomid larvae were the most abundant invertebrates on diffusion substrata after 26 days (Fig. 6D). Their numbers were greatest on nutrient-enriched substrata (nested ANOVA, least-squares means, $P < 0.05$), but no significant difference was found between shaded and unshaded sites.

The large difference in numbers of larvae on the two types of surface in the N+P treatment indicates that our results were not a simple response to nutrient additions. The thicker-textured cellulose cloth appeared to provide a particularly favourable substrate into which the larvae were able to burrow. Our SEMs showed that it also supported large

numbers of bacteria (both sites) and algae (unshaded site), and therefore an abundant food resource. Higher numbers of chironomid larvae on cellulose cloth surfaces may therefore reflect both an increase in habitat and an increase in food availability.

DISCUSSION

Sticks (tongue depressors made from untreated silver beech) buried in a beech forest stream lost about 39% of their dry weight in the 11-12 months of this study. Weight loss was slow for the first 7 months, but then increased as the wood became blackened and soft. Wood was colonised by filamentous micro-organisms, unicellular bacteria, diatoms, and invertebrates. Visually similar microbial assemblages developed on buried and surface-incubated substrata and on leaves and twigs of mountain beech collected from the forest floor. In contrast, stone surfaces were colonised by diatoms and unicellular bacteria, and few filamentous micro-organisms were seen.

In contrast to woody substrata, little endocellulase activity was recorded from stone biofilm samples after 60 and 90 days in the stream. Similarly, very low levels of endocellulase activity were found on inorganic nylon mesh substrata, whereas activity greater than the maximum recorded on sticks was obtained from cellulose cloth after 26 days in the stream. These findings indicate that at least some species involved in wood decomposition are habitat specialists that occur only on organic substrata, or that endocellulase activity is induced by the presence of an appropriate substratum (Morrison et al. 1990; Li & Heath 1993).

Endocellulase activity on sticks in Middle Bush Stream increased substantially after 1 month's incubation and, one instance excepted, exceeded that measured on identical sticks in two low pH (brown-water) South Island streams (Tank & Winterbourn 1995). The exception occured where a dense fungal population embedded in a 1-2 mm thick glycocalyx was present on the wood surface, and endocellulase activity was >10 000 units cm^{-2} h^{-1}. The high values recorded on wood in Middle Bush Stream on Day 189 were also associated with small patches of mucilage-associated mycelia. As in our study, Sinsabaugh et al. (1994) found that endocellulase activity associated with coarse plant detritus including wood fragments generally increased over time (113 days).

Our nutrient enhancement experiment, in which cellulose cloth was used as the diffusion/colonisation surface, also showed that endocellulase activity was stimulated when nutrient availability increased. SEMs of cellulose cloth surfaces showed that the addition of N+P stimulated the development of bacteria, which we infer to be the source of the extracellular enzymes. The large numbers of chironomid larvae present on the nutrient-added subtrates would have been provided with an enhanced food resource of bacteria, algae (at the unshaded site), and decomposing cellulose fibres. Why ^{14}C incorporation did not increase as well when nutrients were added is unclear, and it may be that the ^{14}C-glucose assay was not an adequate indicator of microbial activity where alternative carbon sources were available. In general, however, our findings are consistent with those of Howarth & Fisher (1976) who found that enrichment with N+P accelerated loss of weight of sugar maple leaves, and increased "leaf" respiration; and of Aumen et

al. (1983) who concluded that decomposition of wood in streams of the Pacific Northwest may be limited by nitrogen availability.

Over time, increasing amounts of mucilage, fine detritus, and sand were found adhering to wooden substrata buried in Middle Bush Stream. The presence of this material influenced the composition of the associated invertebrate community, although tube-dwelling chironomid larvae, oligochaetes, and harpacticoid copepods were the principal colonists at all times, as they were on identical substrata in four West Coast streams (Tank & Winterbourn 1995).

Unlike Chironomidae, small oligochaetes increased in numbers on sticks as the amount of adherent material increased. Mucilaginous exopolymer secretions present on the surfaces of particles can adsorb dissolved organic matter (DOM) from the surrounding water and make it available to consumers (Decho & Lopez 1993). Polysaccharide-rich fractions can be absorbed with high efficiencies (>80%) by some aquatic invertebrates, and the occurrence of an exopolymer coating on sediments more than doubled the availability of adsorbed DOM to a deposit-feeding marine polychaete (Decho & Lopez 1993). This scenario corroborates the proposal of Winterbourn (1976) that leaf litter in forested New Zealand streams is a source of DOM that is adsorbed onto leaf (or wood) surfaces where many stream invertebrates feed. The increase over time in the numbers of oligochaetes on buried wood in Middle Bush Stream is consistent with this proposal and suggests that at least in part oligochaetes are responding to the presence of a rich, mucilage-based food source. In contrast, no invertebrates were found

burrowing into sticks, a finding that is consistent with Anderson's (1982) observations that few of the invertebrates that colonise wood in New Zealand streams appear to ingest the wood itself.

Many South Island forest streams are physically harsh environments characterised by fluctuating flows, high sediment loads, and unstable beds (Winterbourn 1995). Retention and storage of course particulate organic matter (CPOM) in such streams is commonly low (Winterbourn et al. 1988, Evans et al. 1993), and invertebrates that rely on CPOM for food are frequently absent or rare (Winterbourn et al. 1981). Instead, most non-predatory benthic invertebrates are thought to feed on FPOM and stone surface biofilms that capture DOM derived from leaf litter and other sources, making it available to consumers (Winterbourn 1976, Rounick & Winterbourn 1983). Our present study has shown that wood can promote extensive biofilm development as both a carbon source and as a surface for microbial colonisation. Because of its inherent stability, woody debris would therefore appear to have a potentially significant role in supporting stream community metabolism, especially where retention of other detrital material is poor.

ACKNOWLEDGMENTS

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Figure Legends

Fig. 1. Scanning electron micrographs of the microbial biofilm colonizing A, silver beech tongue depressor (Bar: 40 μm); B, mountain beech leaf (Bar: 40 μm); C, mountain beech twig (Bar: 40 μm); and D, greywacke sandstone (Bar: 40 μm) after 90 days in Middle Bush Stream.

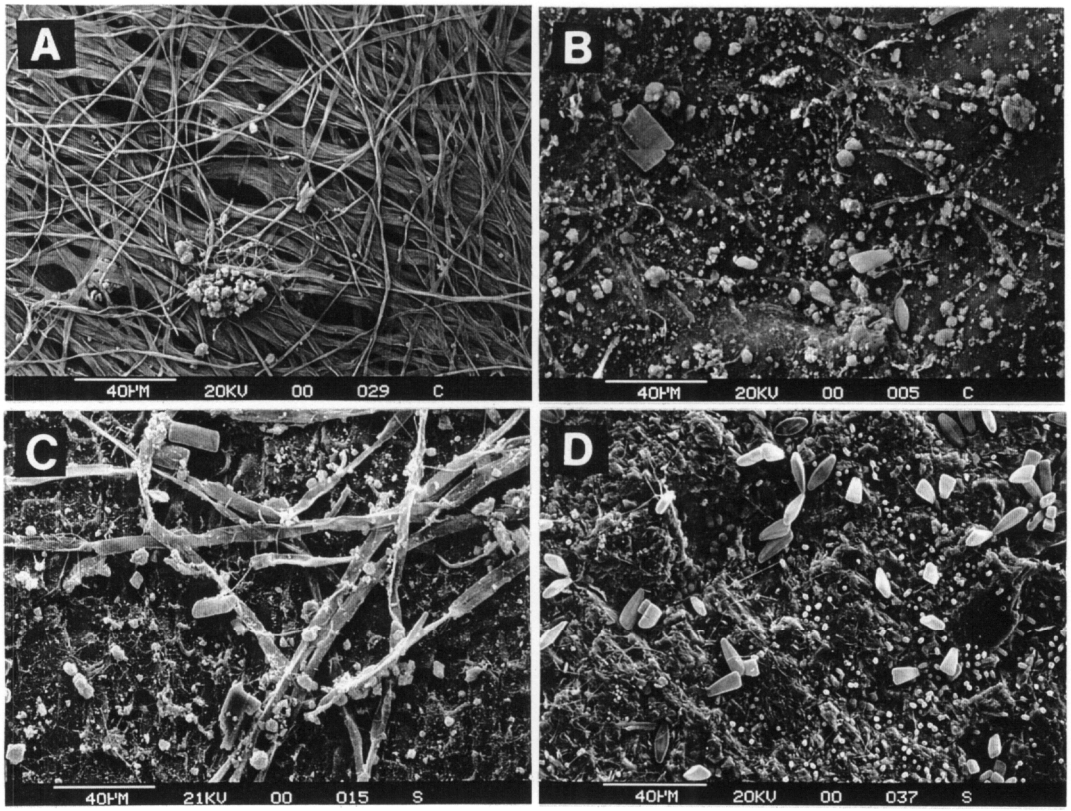
Fig. 2. A, Chlorophyll a concentration; B, ^{14}C incorporation; and C, endocellulase activity on sticks, twigs, leaves and stones taken from Middle Bush Stream after 60 and 90 days. Values are means ± 1 SE.

Fig. 3. Mean percentage dry weight loss of sticks buried in Middle Bush Stream, estimated on six occasions.

Fig. 4. A, Incorporation of ^{14}C; and B, endocellulase activity on surface-incubated and buried sticks in Middle Bush Stream over time. Values are means ± 1 SE.

Fig. 5. Numbers of Total invertebrates, Chironomidae, and Oligochaeta on buried sticks over time. Values are means ± 1 SE.

Fig. 6. A, Incorporation of ^{14}C; B, endocellulase activity; C, Chlorophyll a concentration; and D, number of Chironomidae on nutrient-diffusion substrata after 26 days in Middle Bush Stream. Results are shown for cellulose cloth (Wettex) and nylon mesh surfaces at a shaded (forested) and unshaded site.



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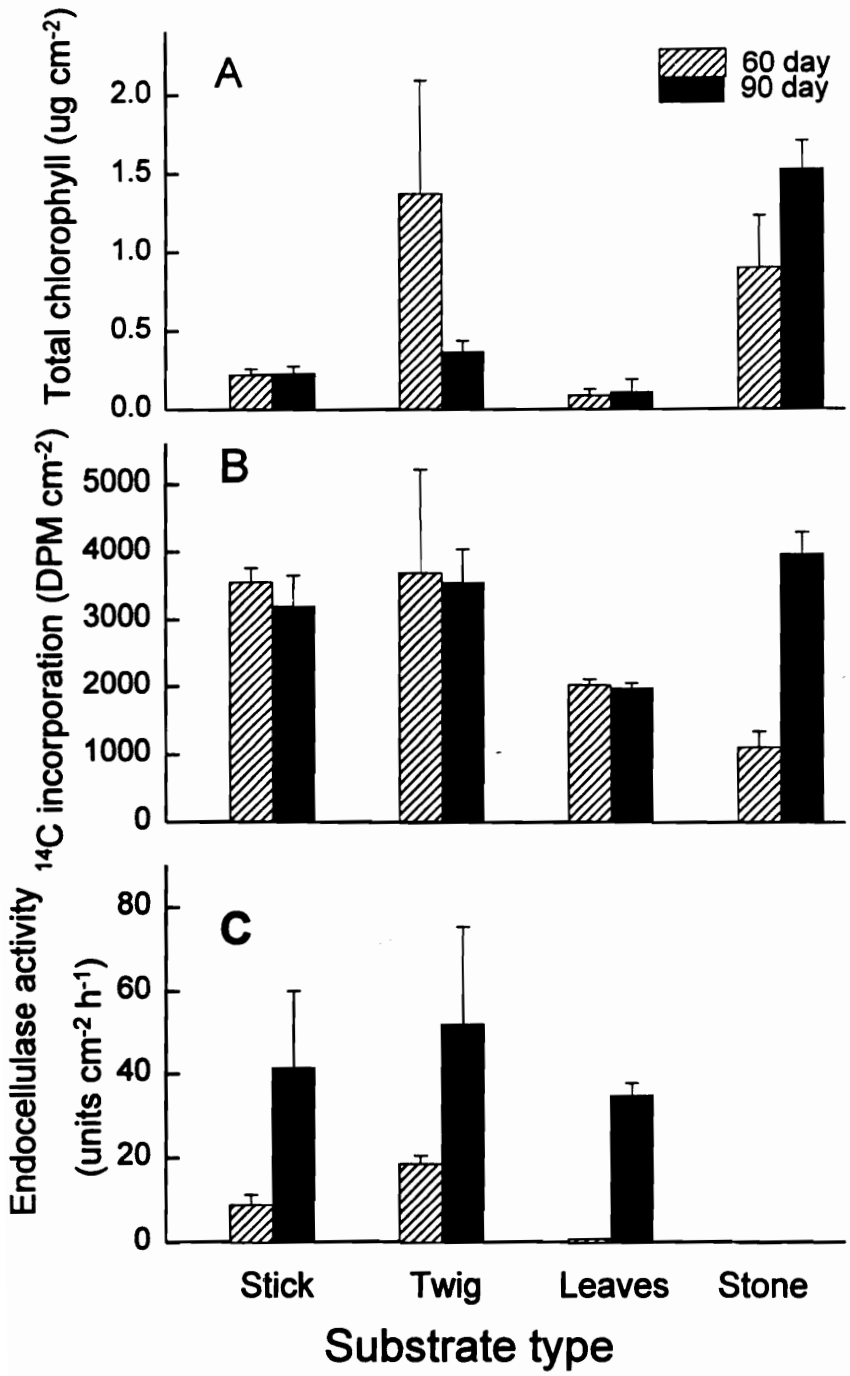
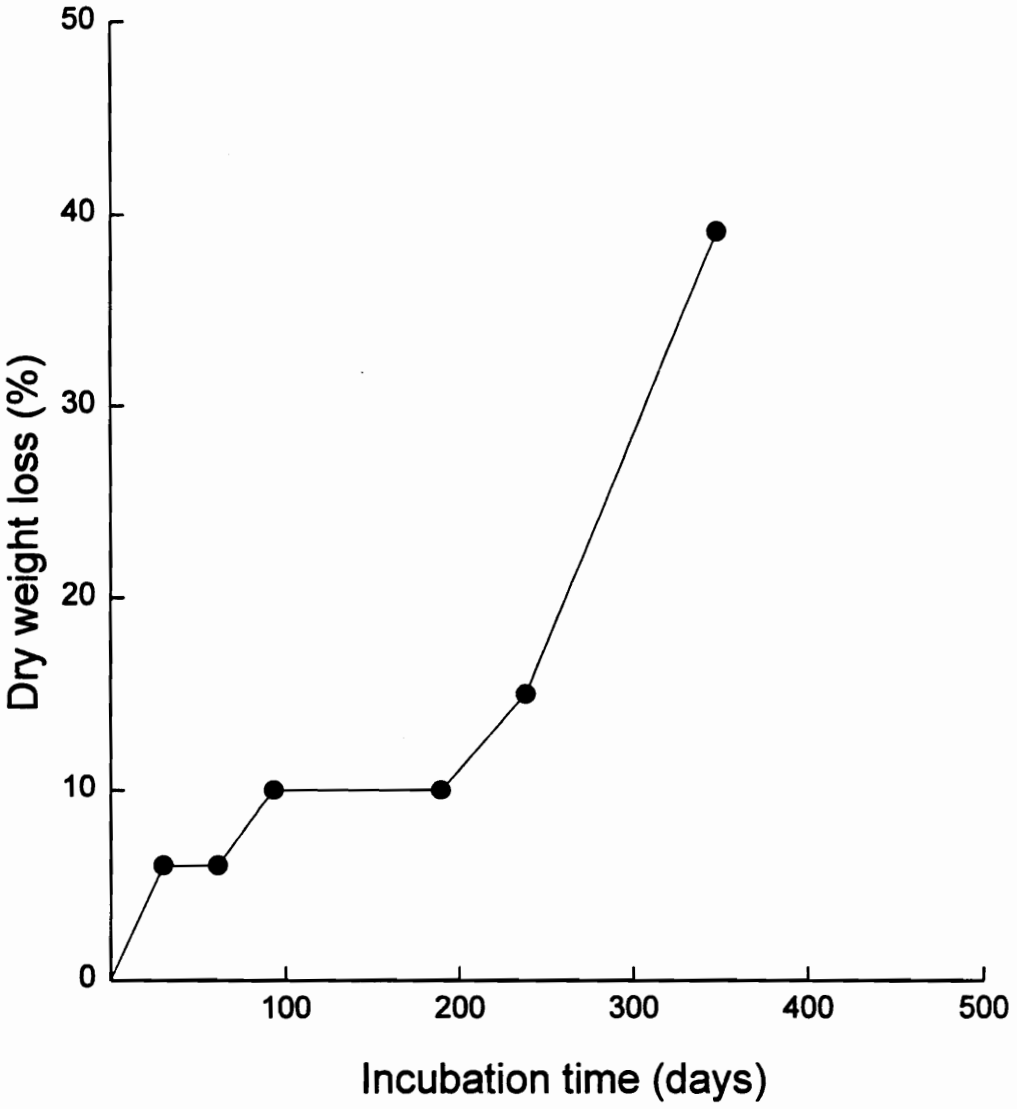


Fig. 2. A, Chlorophyll a concentration; B, 14C incorporation; and C, endocellulase activity on sticks, twigs, leaves, and stones taken from Middle Bush Stream after 60 and 90 days. Values are means +/- 1 SE.



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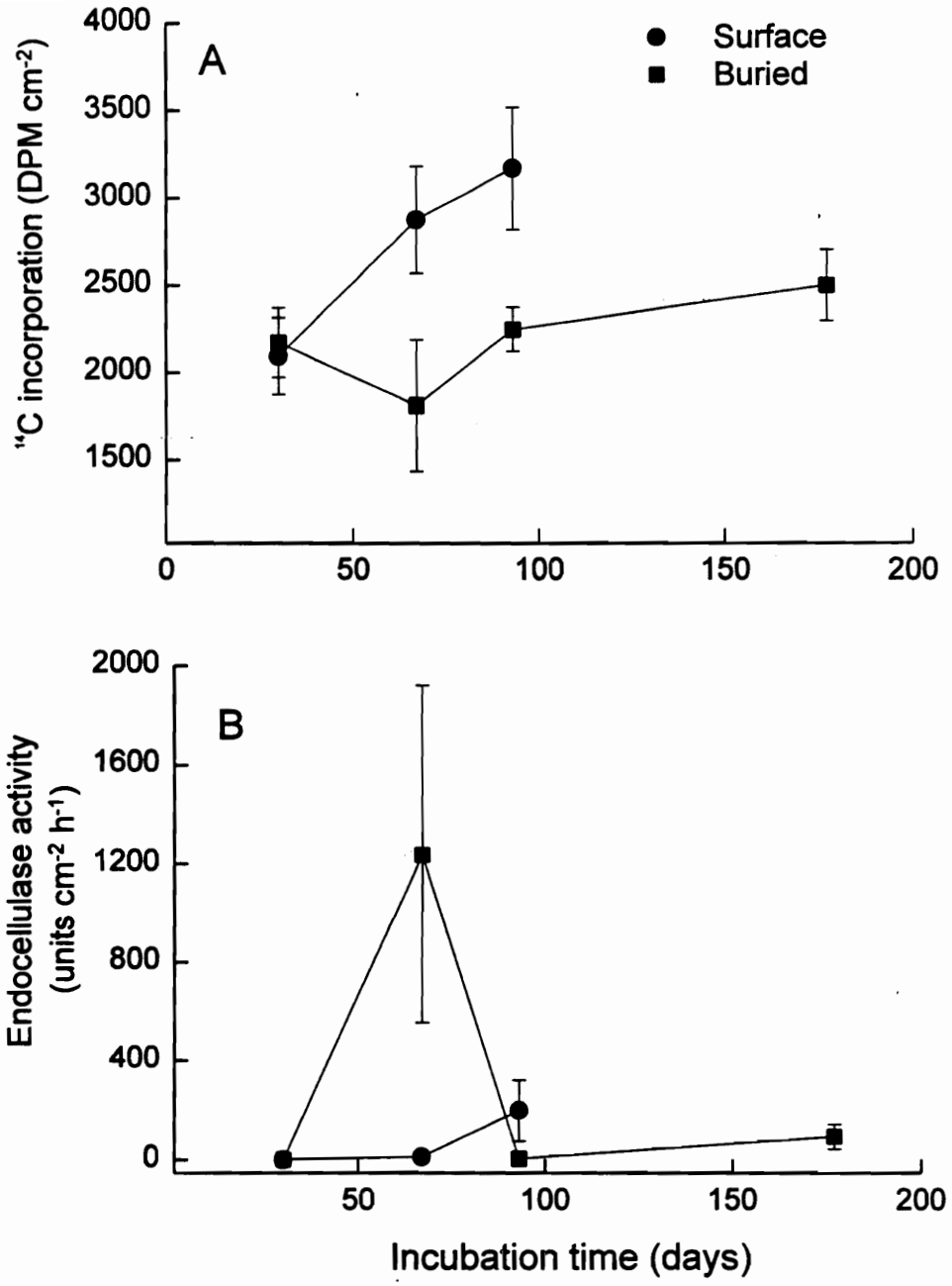

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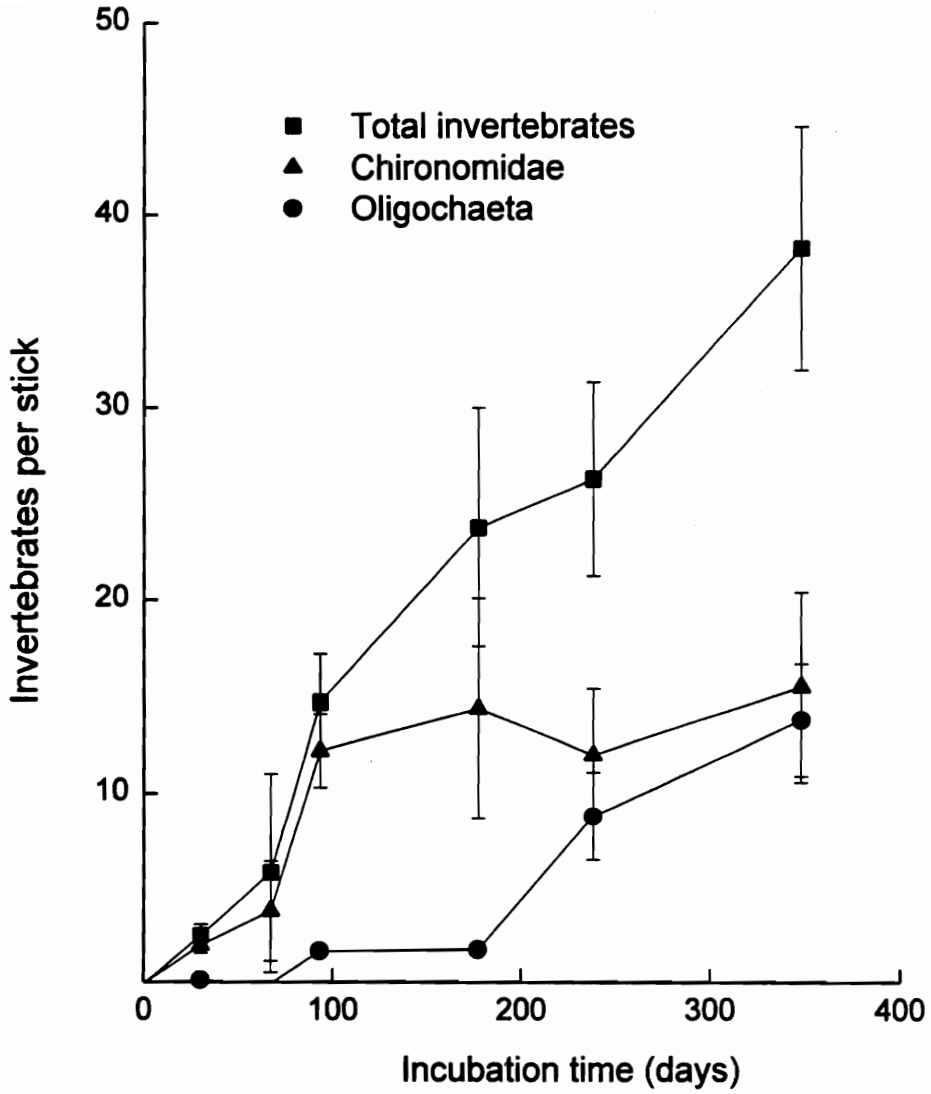

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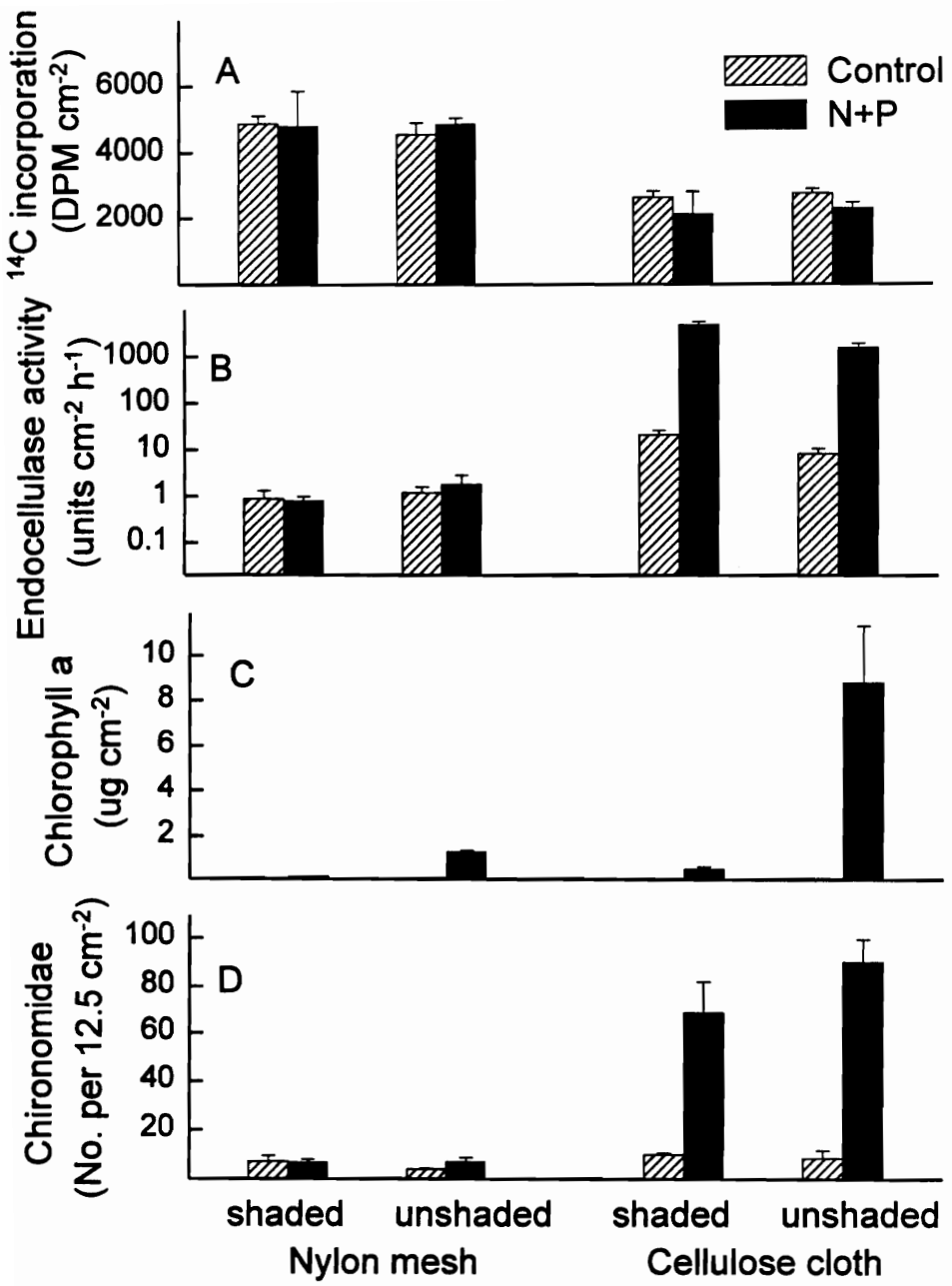

Fig. 6. A, Incorporation of 14C; B, endocellulase activity; C, Chlorophyll a concentration; and D, no. of Chironomidae on nutrient-diffusion substrata after 26 d in Middle Bush Stream. Results are shown for cellulose cloth and nylon mesh surfaces as a shaded (forested) and unshaded site.

Chapter 2:

Biofilm development and invertebrate colonization of wood in four New Zealand streams of contrasting pH[1]

[1] Tank, J.L. and M.J. Winterbourn. 1995. Freshwater Biology. 34:303-315.

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 three 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 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 sylvatica 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 sub-catchments 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 approximately 0.45 μm), 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 x 16 x 1mm), 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 oriented parallel to the flow. Sets of surface and buried sticks were placed where physical conditions were as similar as possible.

Sets of six sticks (1 surface and 1 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. 1 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 containing 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 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 five hours at 5°C in 250 ml of filtered stream water to which 25 ul of ^{14}C-glucose (10.8 GBq mmol^{-1}, 1.85 MB ml^{-1}) was added. Our incubation time was slightly shorter than that used by Sawyer & King (1993) (6 hours), but longer than the 3 hours used by Chappell and 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 hours 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 hours, and then centrifuged. The viscosity of two subsamples of supernatant per vial was measured by determining their fall-velocity in a 0.1 ml glass pipette. Enzyme activity (units/cm^2/h) 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 X. 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 hours (5^{o}C) before being transferred to new jars without food for a further 12 hours. 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 characterised 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 & Herrman, 1990). Water temperatures recorded at the four sites over the 6 month period (autumn-winter) ranged from 4 to 11°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 (~1 μm) 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 circum-neutral beech-forest stream in another part of the South

Island, indicating that these fungal mats were not restricted to acidic brown-water streams (Tank & Winterbourn, 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 μg cm^{-2} in all four streams (range 0.01- 0.24 μg 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 three months in all four streams, but no further increases were found in the three streams sampled another three 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 provided by scanning electron microscopy 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 cm 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 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 agilus (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 mucilagenous 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 with actinomycetes, bacteria, amorphous detrital aggregates and trapped silt particles. However, the methods we used did not enable identification of hyphomycete species or other fungal mycelia (Suberkropp, 1992). 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-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; Scholtz & 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^{o}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 stream water 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 microorganisms, 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 microorganisms 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 (Winterbourn & Tank, unpublished). 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 to 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. Further, nutrient-poor conditions and high levels of physical disturbance, such as were found at all four of our sites, can be expected to minimise differences in microbial community biomass and production among chemically diverse streams.

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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)

Variable	Sites			
	A1	A2	N1	N2
Altitude (m)	700	300	450	250
Temperature (^{o}C)	6.0 (4-8)	7.6 (4-10)	7.8 (6-9)	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^{o}C	19 (17-21)	31 (28-35)	64 (60-71)	165 (131-224)
Alkalinity (mg l^{-1} Ca $C0_3$)	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)	.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

Table 2: Invertebrate taxa found on sticks taken from the 4 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)

Taxa	Sites			
	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		
Naocladius 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	x	x		
Brinkhurst			x	x
Slavina sp.				
Gastropoda				
Potamopyrgus antipodarum (Gray)				x
Number of taxa	9	9	8	11
Individuals per stick (mean & 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)

Figure Legends

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

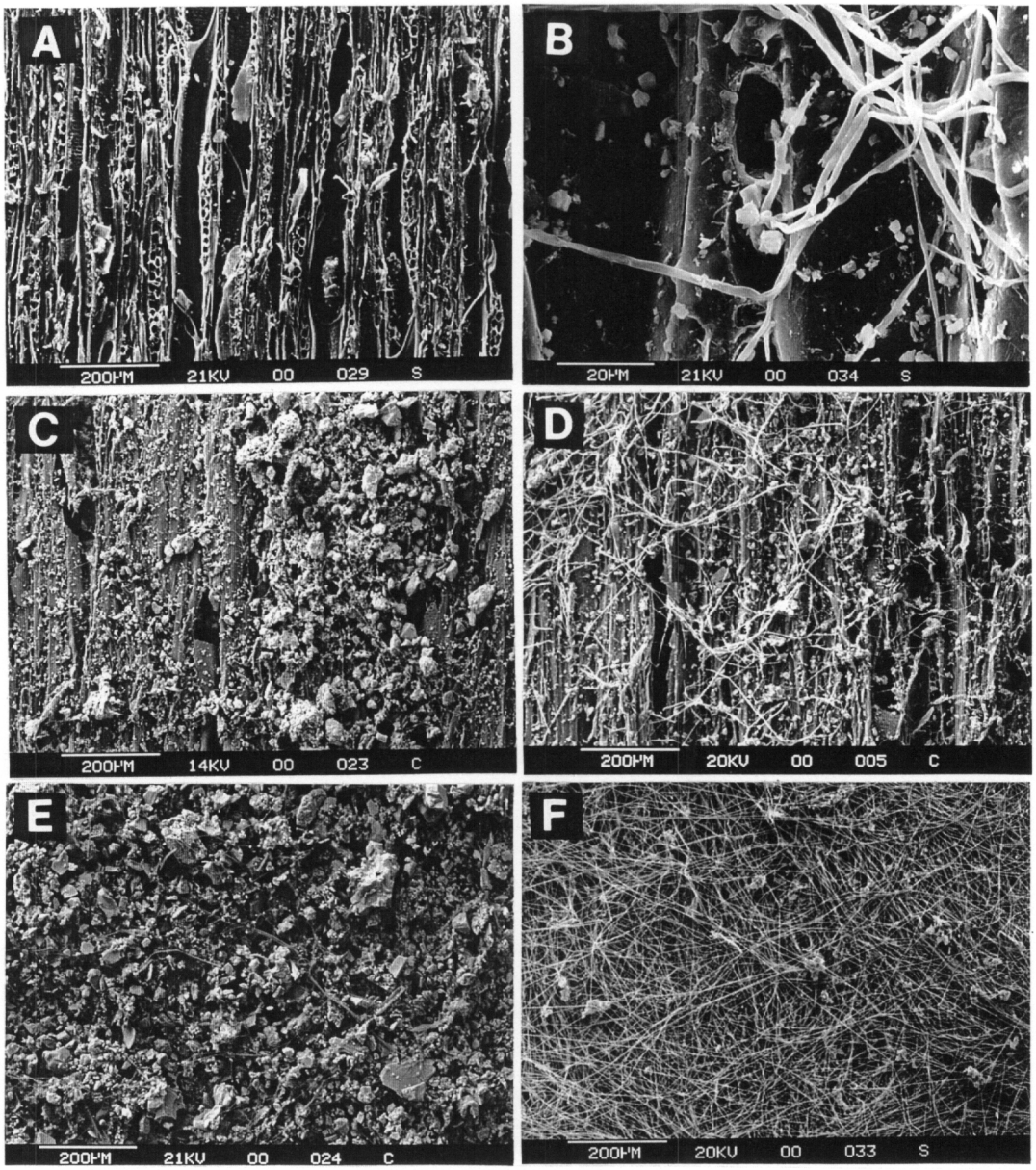
Fig. 2. Incorporation of ^{14}C by biofilms on sticks held in the four streams for up to 177 days. Mean DPM ± standard errors (SE) are plotted. No data were obtained for A2 on Day 177.

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.

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.

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



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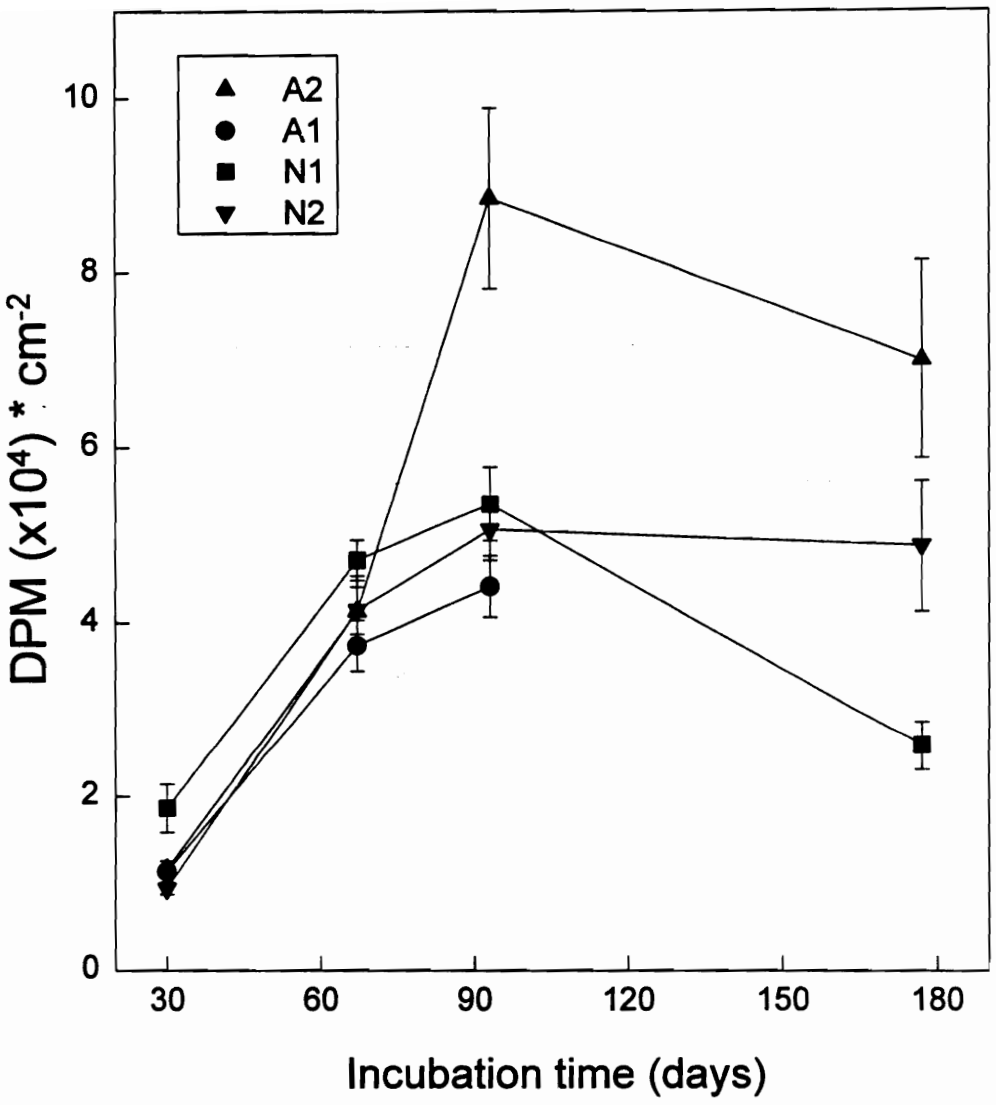


Fig. 2. Incorporation of (a) 14C by biofilms on sticks held in the four streams for up to 177 days. Mean DPM +/- SE are plotted. No data were obtained for A2 on Day 177.

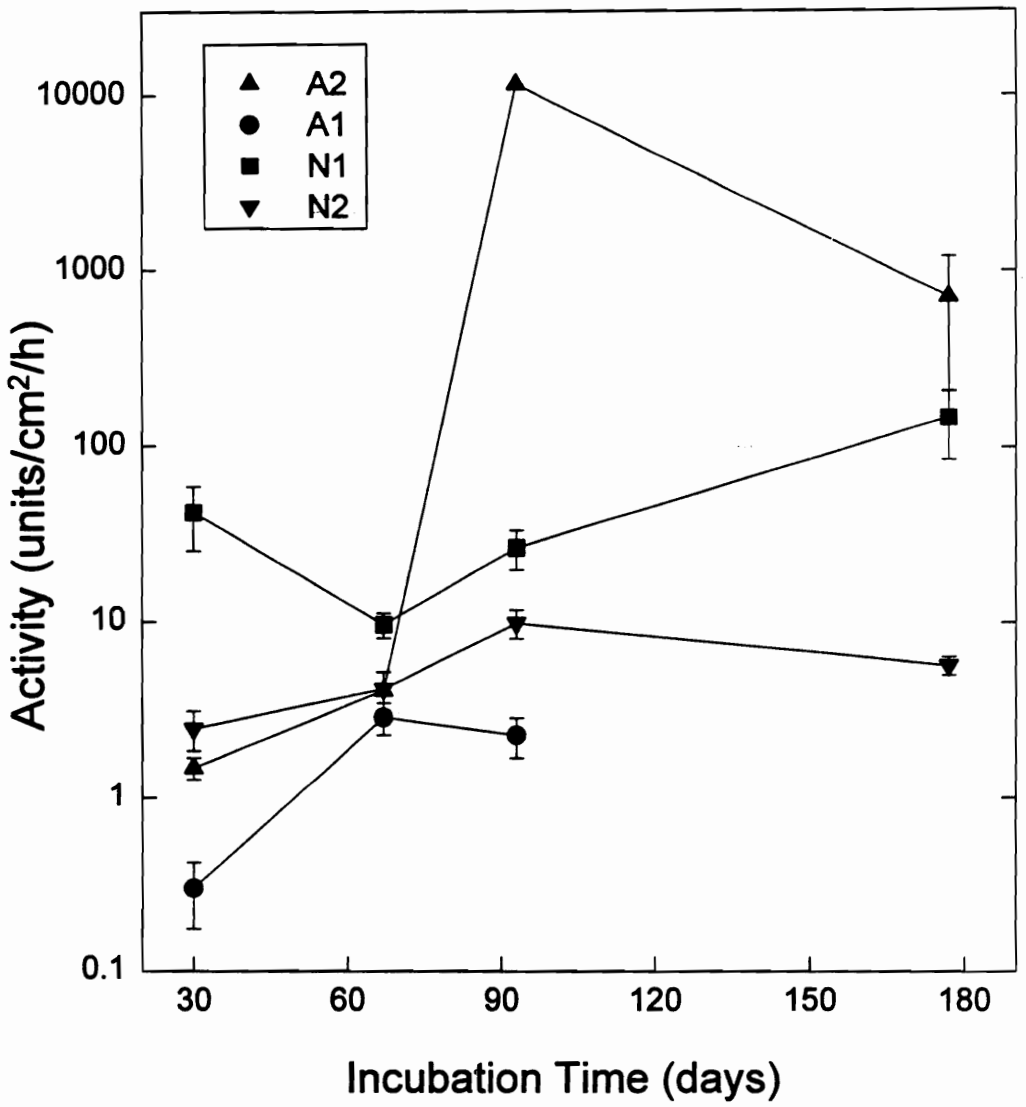
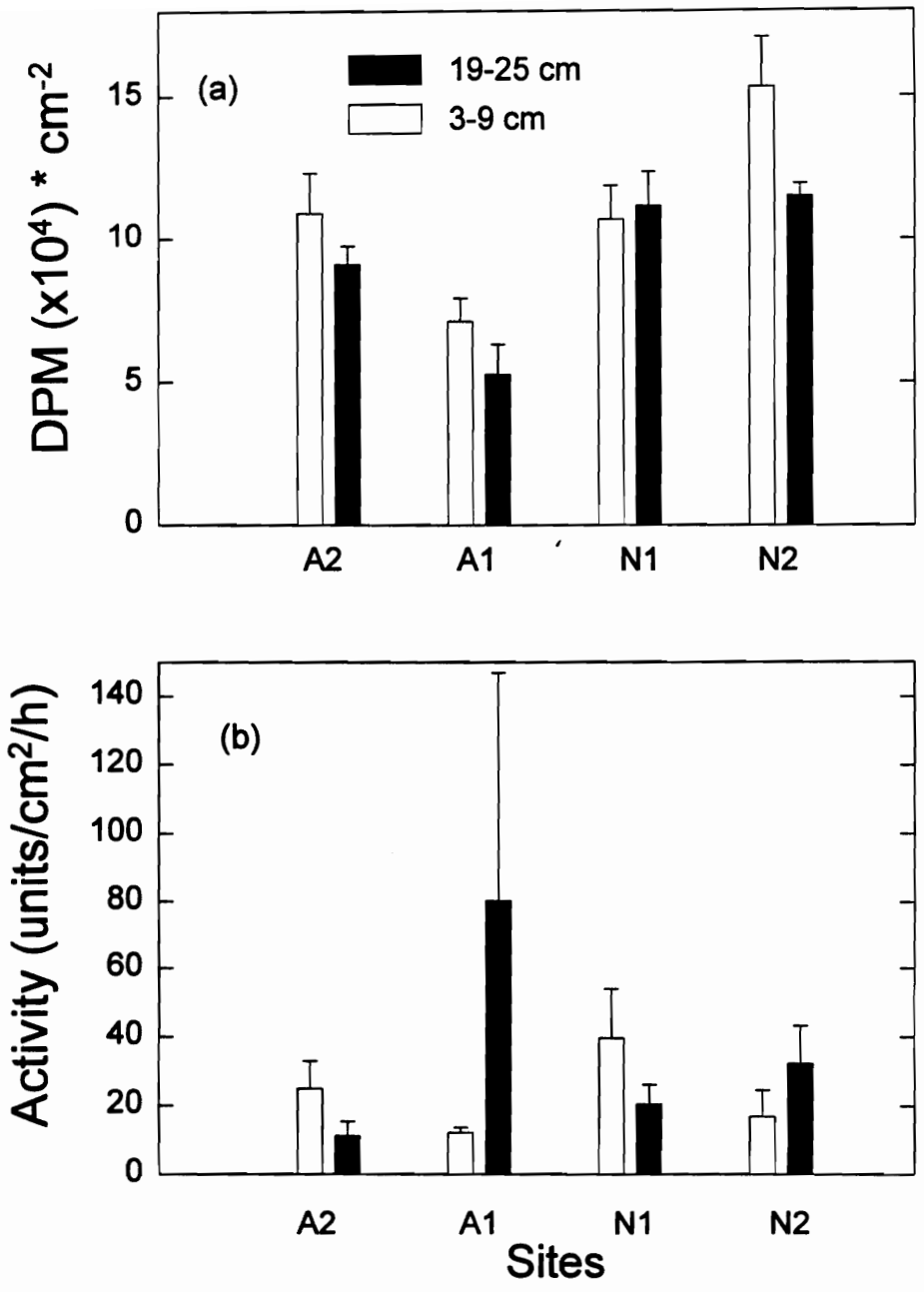


Fig. 3. Endocellulase activity of biofilm samples scraped from sticks held in the four streams for up to 177 days. Mean activities +/- SE are plotted.



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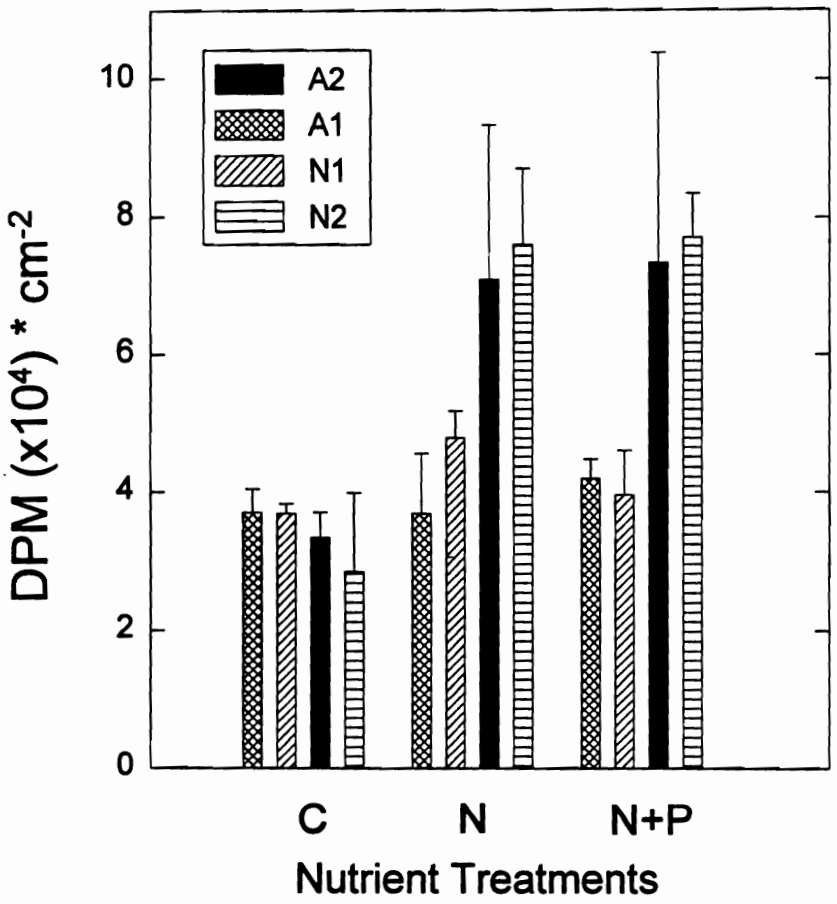

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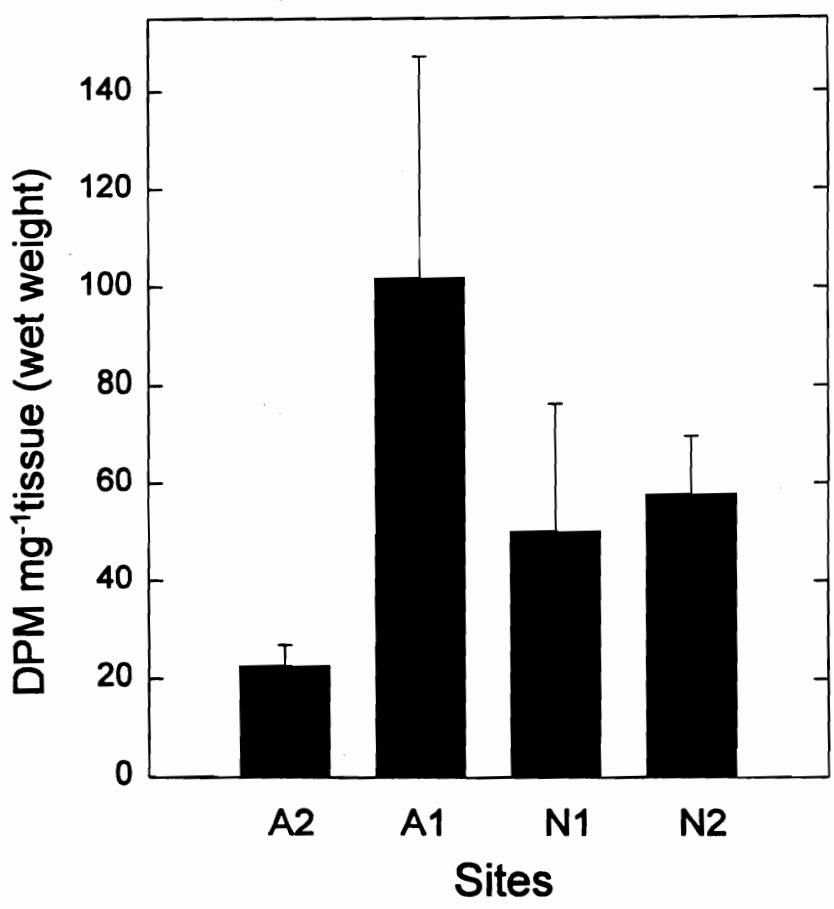

Fig. 6. Incorporation of ^{14}C by amphipods (Paraleptamphopus sp.) allowed to graze on labelled biofilms. Mean DPM +/- SE are plotted.

Chapter 3:

The influence of leaf litter and nutrients on microbial biofilm development on wood in streams.

I. Introduction

Allochthonous organic matter inputs, leaves and wood, are the energy base for stream processes in heavily shaded mountain streams (e.g., Fisher and Likens 1973, Minshall et al. 1983). Inputs of leaf litter have been excluded from a headwater stream draining a forested watershed at Coweeta Hydrologic Lab, North Carolina, since August 1993 to evaluate how elimination of the resource base (leaf litter) affects a headwater stream. Changes in organic matter and nutrient dynamics in this stream represent a first-level response to resource depression, and changes in these processes are expected to affect higher trophic levels within the stream ecosystem. While many ecologists have studied the results of an increased resource base (e.g., Elwood et al. 1981, Peterson et al. 1985), results from the depression of the energy base have not been widely studied in streams.

Most allochthonous organic matter enters small streams as leaves and wood and are exported as small particles (e.g., Fisher and Likens 1973, Webster et al. 1990, Wallace et al. 1991). A combination of factors contribute to the breakdown of organic material, including microbial colonization, physical fragmentation resulting from streamflow, and invertebrate feeding. While fragmentation results in greater loss of organic matter from a stream reach, microbial biofilm development on organic matter increases the nutritional quality for invertebrate feeding and thereby increases the efficiency with which allochthonous inputs are used within a stream reach (Cummins 1974, Bärlocher 1985).

Microbial biofilms occur on most surfaces in streams including stones, leaves, and wood. Varying proportions of bacteria, fungi, algae, detrital particles, and exoenzymes are incorporated into a gelatinous matrix to form the biofilm (Rounick & Winterbourn 1983, Lock et al. 1984). The biological components of biofilms on organic substrates (wood, leaves, and FPOM) are involved in the decomposition of their respective substrate and differ fundamentally from organic layers colonizing stone surfaces. Previous research has shown that autotrophic biomass is significantly lower while carbon degrading potential is higher on wood than on stones or leaves in New Zealand streams (Tank and Winterbourn 1996). Sinsabaugh et al. (1991) also demonstrated that epilithic and epixylic biofilms in a 4th-order boreal river were different from each other and that most of the microbial biomass and metabolic parameters they measured were greater on wood. Microbial biofilm development also differed among organic matter types. Microbial respiration rates were up to 10 times greater on decaying sticks in a forest stream than leaves (Tank et al. 1993). Golladay and Sinsabaugh (1991) suggested that more extensive biofilms can develop on wood than on leaves because of greater physical stability and persistence of wood in streams.

Most studies of organic matter processing in streams have focused on the role of leaves rather than wood because leaves normally comprise the bulk of organic matter inputs to small forested streams. Leaves disappear within 4-8 months in most temperate streams (Webster and Benfield 1986) and therefore leaves can be considered a seasonal resource in these streams. At some times of the year, wood standing stocks in small

headwater streams at Coweeta can be over 50% of all organic matter (Tank et al. 1993). Surface area estimates for small woody debris (<5 cm diameter) per unit area of streambed in the study area ranged from 0.6-0.8 m^2 of wood/m^2 (J.B. Wallace, UGA, unpublished data). While leaves enter streams primarily in autumn, wood enters streams throughout the year and often provides a substantial accumulation of energy, carbon, and nutrients (Harmon et al. 1986). For example, microbial colonization and lignocellulose degradation on large logs occurs primarily on the wood surface (Aumen et al. 1983). In contrast to leaves, wood is a long lasting organic matter resource and microbial colonization by bacteria and fungi plays the major role in the decomposition of wood, which may take years (e.g. Harmon et al. 1986).

Stream invertebrates may use wood directly or indirectly as a source of food. Wood can be used by filter feeders as a net-spinning site, while grazers can feed on surface biofilms, and gougers ingest the decomposing wood itself (Anderson et al. 1984, Benke et al. 1984). Because most invertebrates cannot digest wood directly, it has been inferred that wood does not play an important role as a food resource for invertebrates (Anderson 1982). Microbial colonization of wood, as with leaves, can increase palatability and contributes to the transfer of energy from the wood substrate to higher trophic levels (i.e., stream macroinvertebrates), but unlike leaves, the biofilm colonizing wood is available to stream macroinvertebrates year round.

Ecosystem processes and community structure are affected strongly by the resource base in streams. By eliminating leaf litter inputs to a headwater stream in 1993,

wood has become the major source of large particulate organic matter in the stream. Most studies have examined wood as a structural component of streams or as invertebrate habitat (e.g., Triska and Cromack 1980) but to fully understand energy flow in streams the microbial component of woody debris should be considered. The goals of this study were to determine the effect of leaf removal on the activity of wood biofilms and relate any changes to stream ecosystem processes.

II. Methods

A. Site Description

I studied 2 first-order streams draining Catchments 53 and 55 at Coweeta Hydrologic Laboratory , Macon County, North Carolina, USA. Coweeta is a 2270-ha experimental forest of the U.S. Forest Service located in the southern Appalachians. The forest canopy was dominated by tulip poplar (*Liriodendron tulipifera*), white oak (*Quercus alba*), red oak (*Quercus rubra*), and dogwood (*Cornus florida*). There was also a dense understory of rhododendron (*Rhododendron maxima*), which resulted in year-round shading and low rates of primary productivity (Webster et al. 1983) in the study streams.

Both study streams had low dissolved nutrients, similar water chemistry, pH between 6.7-6.8, southern aspect, and similar thermal regimes (Table 1)(J.B. Wallace, unpublished data). Both first-order streams were groundwater-fed and therefore were cool in the summer and relatively warm in the winter. Stream-bed characteristics were similar,

consisting of mixed cobble-pebble with sand-gravel, and some bedrock outcrops (14-27%)(J.B. Wallace, unpublished data).

Small woody debris (<5 cm) and leaf litter made up most of the standing crop of benthic organic matter (60-65% and 20-24% respectively). Surface area of small woody debris made up a considerable proportion of colonizable organic substrate in WS53 and was the only large organic substrate left for microbial colonization in WS55 (Table 2). Leaf litter inputs have been excluded along the length of stream (180 m) draining Catchment 55 since August 1993 by placing a mesh canopy (1.5 cm mesh) over the stream. The canopy was positioned underneath the rhododendron understory so as to maintain the normal heavily shaded light regime and yet continue to exclude rhododendron leaves (27% of total leaf litter input)(Cuffney et al. 1990).

B. Sample arrays

Untreated wood strips constructed from wood veneer (2.5 cm x 15 cm x 1 mm) of yellow poplar (*Liriodendron tulipifera*), and white oak (*Quercus alba*) were used as substrates for microbial colonization in the study streams. These two wood types were used because both species are present in the riparian forest, and natural woody debris of both wood types enters the two streams. Using wood strips made from veneer allowed for accurate estimation of surface area.

Veneer strips (hereafter referred to as sticks) were attached with cable-ties to plastic mesh holders with 5 replicates attached to each holder. The mesh holders were

staked firmly to the stream bed. Long axes of sticks were oriented parallel to the direction of flow.

In Experiment 1, six sets of oak and poplar sticks (5 sticks/set) were placed in each stream on 24 Oct. 94. Five replicate sticks per treatment were collected from each stream after 29, 51, 91,119,161, and 238 days and returned to the laboratory in containers of cold stream water. Sticks from WS55 were mostly decomposed after 238 days and therefore nothing could be collected. Microbial respiration rates at ambient stream temperature and at 20°C, and fungal biomass was determined for all sticks (5 replicates/treatment).

In Experiment 2, four sets of poplar sticks (5 sticks/set) and 4 mesh bags of rhododendron leaves, collected at abscission and oven dried, were placed in each stream on 24 Apr. 95. Five replicate sticks and one bag of rhododendron leaves were collected from each stream after 30, 61, 89, and 126 days and returned to the laboratory in containers of cold stream water. Microbial respiration rates at ambient stream temperature and at 20°C, fungal biomass, and cellobiohydrolase activity were determined for sticks and leaves (5 replicates /treatment).

C. Microbial respiration

Respiration rates, as microbial oxygen uptake, were measured on sticks and leaves using a Gilson differential respirometer. Five subsamples of each substrate from both streams were incubated after each collection for a total of 20 samples/ collection date. Samples were placed in 15-ml Gilson flasks containing 5 ml of filtered stream water from

their respective streams. Filters soaked with potassium hydroxide were placed in the center wells of flasks to absorb evolved CO_2. Each set of replicate samples was run once at ambient stream temperature and once at a constant reference temperature (20°C) after an appropriate acclimation period. Flasks containing uncolonized wood served as controls and confirmed that no abiotic uptake of oxygen was occurring.

Samples were incubated 2.5-5 hours, depending on temperature, in order to obtain sufficient oxygen change for accurate readings. After each incubation, samples were oven dried at 50°C, weighed, ashed for 45 minutes at 550°C, rewetted to restore water of hydration, dried, and re-weighed to obtain ash free dry mass (AFDM) for each sample. These samples were also used to calculate breakdown rates.

Respiration rates were expressed on a surface area or gAFDM basis and calculated using the formula described below (Umbreit et al. 1964);

$$X = (\Delta V_g)\frac{P - P_w}{P_1}\frac{T_1}{T}$$

where

X= oxygen uptake (μL)
ΔV_g= change in volume of respirometer (μL)
P= total gas pressure w/in respirometer (mm Hg)
P_w= vapor pressure of water at temperature (T) in mm Hg
P_1= standard pressure = 760 mm Hg
T_1= standard temperature = 273.15 °K
T= temperature of water bath (°K)

D. Fungal biomass

Fungal biomass of biofilms on sticks was estimated by ergosterol content and was measured using methods outlined by Newell et al. (1988) and modified by R.L.

Sinsabaugh (personal communication). Subsamples of known surface area (2.5 cm x 3 cm) were placed in 15-ml Falcon tubes with 5 ml of methanol and refluxed in a dry block heater for 2 h at 65°C. Tubes were removed from heater, cooled, saponified by adding 1ml of 4% KOH in methanol, and refluxed for another 0.5 h at 65°C. Samples were cooled, centrifuged, and the supernatants were decanted into clean 15-ml Falcon tubes. The pellets were resuspended in 2 ml of HPLC-grade methanol, centrifuged again, and the rinse methanol was added to the tubes containing the extracts along with 1 ml water. Sample were then extracted 2 times with 2 ml pentane. Pentane extracts were combined and evaporated in a fume hood. The residue was redissolved with 1 ml of HPLC-grade methanol and filtered through a 0.45-μm syringe filter into clean 5-ml polypropylene tubes. Ergosterol was quantified using a reverse-phase HPLC system configured as follows: solvent = methanol; flow rate = 2ml/min; column = Nova-Pak ODS C18 3.9 mm x 75 mm; absorbence detector = 282 nm with a range of 0.500; quantification = Waters integrator with attenuation at 256; ergosterol retention time = 1.6 min; replication = five per sample; standards = 5, 10, 25, and 50 μg/ml solution of ergosterol and methanol. Ergosterol content was converted to fungal biomass using a general conversion factor of 6 mg ergosterol/g fungal biomass (Newell et al. 1988).

E. Cellulose degradation potential

Biofilms scraped from substrates of known surface area were assayed for the activity of cellobiohydrolase (CBH, EC 3.2.1.91), an extracellular enzyme involved in the degradation of lignocellulose as outlined by Sinsabaugh et al. (1994). Five (2.5 cm x 3.0

cm) subsamples were cut from wood and leaf substrates from each stream on each collection date. Biofilm was scraped from each subsample with a razor blade into separate test tubes containing 2 ml of 50 mM acetate buffer (pH = 5). Two milliliters of 2 mM p-nitrophenyl-cellobioside in 50 mM acetate buffer (pH = 5.0) were added, and tubes were capped and incubated on a platelet mixer at 3 rpm at 20°C for 4 h. Controls were wood biofilm scrapings suspended in 4 ml of acetate buffer without enzyme. After incubation, samples were centrifuged, and 2 ml of supernatant were added to test tubes containing 0.2ml of 1 N NaOH. After adding 8 ml of water, each tube was vortexed, and absorbence at 410 nm was measured on a spectrophotometer. Activity was expressed as μmol of substrate hydrolyzed per h per cm^2 substrate.

F. Nutrient-addition assays

Experiment 3 involved using nutrient diffusing substrates to determine the effect of NO_3-N and PO_4-P additions on the development of epixylic biofilms. The substrates were 60-ml plastic cups filled with 2% agar, to some of which we added nutrients (e.g., Winterbourn 1990). The agar surface was covered with a disk (12.5 cm^2) of untreated poplar veneer, which acted as the diffusion surface on which the biofilm developed. Four treatments were used: controls, N addition (0.66*M* $NaNO_3$), P addition (0.51*M* KH_2PO_4) and N+P additions (0.66*M* $NaNO_3$ + 0.06*M* KH_2PO_4). Five replicate cups of each treatment were glued to a plastic tray, two trays were anchored to stream beds of each stream on 26 August 1995, and one tray from each stream was recovered after 29 and 63

days. Microbial respiration and fungal biomass on the wood disks were determined for 5 replicate samples of each treatment from each stream at each collection date as described above.

G. Groundwater inflow identification in study streams

Sodium chloride (NaCl) was used as a conservative tracer of water flow to identify areas of groundwater inflow in the litter exclusion and reference streams (Triska et al. 1989, Stream Solute Workshop 1990, Webster and Ehrman 1996). A solution of NaCl was injected at a constant rate into the streams with a Mariotte bottle to raise stream chloride concentration to approximately 20 mg/L using general procedures outlined by Triska et al. (1989). After the injection had been running for sufficient time to reach a plateau in concentration at the sampling station located 30 m downstream (about 1 hr), chloride was measured at 5 m intervals in the stream using a chloride-specific probe. A steady decrease in concentration was expected due to an increase in flow over the stream reach, but distinct drops in chloride concentration indicated areas of high groundwater inflow.

H. Statistical Analyses

All data were log transformed for statistical analysis. Nested analysis of variance (ANOVA) was used to compare respiration rates, ergosterol values, and CBH activities between streams for different substrate types (oak vs. poplar wood, or poplar wood vs. rhododendron leaves) on different sampling dates. Nested ANOVA was also used to compare nutrient treatments in the two streams on different collection dates. Least

squares means (LSM) were used to differentiate between means when significance was found in the ANOVA ($p<0.05$).

Breakdown rates (k) of wood and rhododendron leaves were calculated by regressing the natural log (ln) of mean percentage AFDM remaining of 2.5 cm x 3.0 cm substrates used for respiration analysis on exposure time in days. The negative slope of the regression line is equal to the breakdown rate (k) of the substrate (e.g., Petersen and Cummins 1974).

III. Results

A. Experiment 1: poplar vs. oak wood

Microbial respiration on poplar and oak sticks incubated at ambient stream temperature, expressed per unit surface area, was significantly higher in the exclusion stream (C55) than in the reference stream (C53) on all collection dates (Fig.1, nested ANOVA, $p<0.0001$). Similar trends in respiration were seen when sticks were incubated at 20°C, both when respiration was expressed per unit surface area and per g AFDM. Generally, there were no significant differences in microbial respiration between oak and poplar sticks except on two dates (day 29 and day 55) in the exclusion stream when respiration was significantly higher on poplar (nested ANOVA, $p<0.05$). Overall, mean respiration at ambient temperature averaged over all collection dates was 4 times higher in the exclusion stream than in the reference stream (2.05 vs. 0.55 µl $O_2/cm^2/h$, respectively).

Fungal biomass was significantly higher in the exclusion stream than in the reference stream on all collection dates (Fig. 2, nested ANOVA, p=0.0001) but unlike respiration rates, fungal biomass dropped significantly by day 161 in the exclusion stream. Fungal biomass was low throughout the 238 day incubation period in the reference stream. There was no significant difference between fungal biomass on oak and polar sticks within streams. Overall, fungal biomass averaged 7 times higher in the exclusion than in the reference stream as compared to the reference (means, 0.37 and 0.05 mg/cm^2 respectively).

B. Experiment 2: poplar sticks vs. rhododendron leaves

In Experiment 2, microbial colonization on poplar sticks was compared to rhododendron leaves in both streams. Microbial respiration on poplar sticks, incubated at ambient stream temperature, expressed per unit surface area, was again significantly higher in the exclusion stream as compared to the reference on all collection dates (e.g. Fig. 3, nested ANOVA, p=0.0001). Similar results were obtained when sticks were incubated at 20°C, both when respiration was expressed per unit surface area or per g AFDM. Within streams, microbial respiration was always higher on poplar sticks than rhododendron leaves. Respiration rates on rhododendron leaves were low in both streams, and there were no significant differences between streams (Fig. 3). Mean respiration on poplar sticks at ambient temperature averaged over all collection dates was 3.45 µl O_2/cm^2/h in the exclusion stream vs. 2.35 µl O_2/cm^2/h in the reference stream.

Fungal biomass was more variable in Experiment 2 than in Experiment 1. By day 31, highest fungal biomass was found on rhododendron leaves in the exclusion stream, but soon decreased. Biomass on other treatments was highly variable, and no clear trends were discernible (Fig. 4). On day 89 and 126, fungal biomass was significantly higher on sticks incubated in the exclusion stream (nested ANOVA, $p<0.05$).

Cellobiohydrolase (CBH) activity was used as a measure of cellulose degrading potential. CBH activity on poplar sticks was significantly higher in the exclusion stream on days 30, 61, and 89 (nested ANOVA, $p<0.05$), but by day 126 activity on sticks had decreased in the exclusion stream and increased in the reference stream resulting in no significant differences in CBH activity between streams (Fig. 5). Generally, CBH activity on rhododendron leaves in both streams was very low. The CBH assay on rhododendron could only be completed for day 30 and day 61 due to the increasing fragility of the leaf substrate.

C. Seasonal trends in microbial colonization.

Microbial respiration (per unit surface area) on poplar sticks was compared between the incubations beginning in October (Experiment 1) and April (Experiment 2) using data from incubations made at 20°C. The respiration rates in the exclusion stream during incubations beginning in October and April were not significantly different (Fig. 6, nested ANOVA, LSMeans, $p>0.05$). In contrast, respiration rates in the reference stream were significantly higher in April than October (nested ANOVA, LSMeans,

p=0.0001). In fact, by April, respiration rates in the reference stream had increased to levels that were not significantly different from October rates in the exclusion stream.

Fungal biomass on poplar sticks in the exclusion stream during the incubation beginning in October was significantly higher than in April (Fig. 7, nested ANOVA, LSMeans, p=0.0001). Fungal biomass on poplar was very low in the reference stream during both October and April experiments, but values were still slightly higher in autumn (nested ANOVA, LSMeans, p=0.001).

D. Decomposition rates

Both oak and poplar lost weight over the study period in the exclusion stream, but neither wood type lost weight in the reference stream (Fig. 8A). Calculated decomposition rates, $-k$, were 0.00146 %/d for oak and 0.00401 %/d for poplar sticks in the exclusion stream. Decomposition rates were not calculated for the reference stream.

All substrates lost weight over time in Experiment 2 in April (Fig. 8B). Poplar sticks decomposed more quickly than rhododendron leaves, and decomposition rates for sticks in the exclusion stream ($-k$ = 0.0085 %/d) were faster than sticks in the reference stream ($-k$ = 0.00476 %/d). Poplar sticks decomposed faster during April than October.

The ratio of fungal biomass to wood strip or rhododendron leaf mass changed over time and varied with season . In Experiment 1 the ratio of fungal biomass to substrate mass was higher in the exclusion stream than the reference (Fig. 9A) through day 161. Afterward, stability of the substrate decreased dramatically and fungal biomass dropped as well. In Experiment 2, contrasts appeared immediately between substrates

(rhododendron vs. poplar sticks) instead of between streams (Fig. 9B). Although variable through time, the ratio of fungal biomass to substrate mass was higher for rhododendron leaves than for poplar wood in both streams except for poplar in the exclusion stream on day 89. As incubation time increased, fungal biomass/substrate mass ratios on poplar in the exclusion stream were higher than in the reference stream, but the ratios were still lower than those for rhododendron leaves. In general, ratios of fungal biomass to wood mass in the exclusion stream were higher in October than in April, but there were no seasonal differences in ratios in the reference stream.

E. Effect of groundwater and nutrient additions

Releases of a conservative tracer were used to identify areas of groundwater inputs. Chloride concentration was plotted against distance from the injection site for both the exclusion and reference streams (Fig. 10A, Fig. 10B). Locations where distinct drops in chloride concentration occurred were identified (4 in exclusion and 3 in reference) indicating areas of groundwater seepage. In the exclusion stream, background levels for NH_4-N, NO_3-N, and PO_4-P measured at the flume were generally low whereas water chemistry from groundwater input sites in the exclusion stream were much higher (NH_4-N = 19 ug/L vs. 3 ug/L, NO_3-N = 17 ug/L vs. 10 ug/L, and PO_4-P = 17 ug/L vs. 1 ug/L).

In Experiment 3, we tested the effect of nutrient additions on wood biofilms in both streams using nutrient releasing substrates. After 29 days there were no significant differences between sites in microbial respiration for any treatment: control (C); nitrogen

(N); phosphorus (P); and nitrogen + phosphorus (N+P). After 63 days, biofilm development had progressed, and substrates in the exclusion stream had higher respiration rates than the reference for C, N, and P treatments. However there were no significant differences between microbial respiration rates between the exclusion and reference streams for N+P (Fig. 11, nested ANOVA, $p<0.05$).

Similar effects of nutrient additions were seen in estimates of fungal biomass in both streams. Fungal biomass was higher in the exclusion stream on wood disks of the controls, N or P alone (Fig. 12). When disks were treated with N+P, fungal biomass in the reference stream increased and there were no longer differences between streams (Fig. 12) although statistical differences between treatments were not significant.

IV. Discussion

1. Effect of litter exclusion on microbial respiration and fungal biomass

The goal of this study was to determine the effect of resource depression (i.e. leaf exclusion) on the microbial biofilm colonizing the remaining organic matter source (wood). Previous research on microbial biofilms colonizing organic matter has shown that expressing respiration per unit AFDM was an inappropriate way to compare biological activity on organic matter with different surface area to volume ratios (Tank et al. 1993). When microbial respiration on wood was expressed per unit surface area, biofilms on wood had higher respiration rates than leaves in Coweeta streams. In this study, microbial respiration on wood was significantly higher in the litter exclusion

stream, whether respiration was expressed per unit surface area or per g AFDM. The surface area to volume ratio of wood strips was much higher than natural sticks due to the flat, thin nature of strips of veneer. Trends in respiration may have been different if natural cylindrical sticks had been used.

Microbial respiration was consistently higher at 20°C than at ambient temperatures, but relative differences between substrate type and stream remained the same, and respiration in the exclusion stream was always higher than in the reference stream. Temperature affects microbial respiration as well as the diffusion rate of oxygen across the water substrate interface (Hargrave 1969). Previous studies have shown a positive linear relationship between temperature and microbial respiration rate (e.g. Tank et al. 1993).

2. Seasonality in microbial respiration and fungal biomass

Respiration rates in the reference stream in October were significantly lower than in April, despite warmer stream temperatures in April. In contrast, microbial respiration rates in the litter exclusion stream were not significantly different between October and April. Essentially equal respiration rates in the exclusion stream in October and April incubations implies that something was overriding the effects of higher stream temperatures in April.

Fungal biomass on poplar sticks in both streams were lower from Experiment 1 in October to Experiment 2 in April by almost an order of magnitude. Temperature is

known to affect fungal colonization, and Suberkropp (1984) identified two major patterns in the structure and function of fungal communities: a summer fungal assemblage which grows between 5°- 10° and 30°- 40° with an optima of 25°- 30° and a winter assemblage which grows between 1° and 25°- 30° with an optima of 20°. Our study streams are spring fed headwater streams and therefore exhibit temperature regimes conducive for year round dominance of a winter assemblage. For this reason decreases in fungal biomass from October to April may not be a result of shifts in community structure (Suberkropp 1984). As an alternative explanation, fungal biomass may have declined as a result of an increased proportion of resources being allocated to conidial production at the expense of hyphal growth (sensu Baldy et al. 1995). Further study of fungal community structure and seasonal trends in fungal biomass on wood in headwater streams is needed.

3. Nutrients and wood biofilms

Microbial biofilms on organic substrates are regulated by both internal characteristics of the substrate (Gessner and Chauvet 1994) and external environmental factors such as temperature and nutrient concentrations (Suberkropp and Chauvet 1995). Previous research has focused primarily on the effect of nutrients on the decomposition of leaf litter and implied that microorganisms colonizing leaf litter obtained phosphorus from the water column (Elwood et al. 1981, Mulholland et al. 1984). More recently, it has also been shown that fungi can obtain nitrogen from streamwater passing over organic matter surfaces and higher concentrations of fungal biomass occur in streams with higher

nitrate concentrations (Suberkropp 1995). Additionally, higher nutrient concentrations result in faster rates of leaf breakdown (Suberkropp and Chauvet 1995). In two other streams at Coweeta, Meyer and Johnson (1983) attributed higher rates of fungal sporulation, leaf breakdown, and microbial biomass to high nitrate concentrations of streamwater. Nutrients may also be driving the dynamics of wood biofilms. For this study, higher nutrients (N +P) in the exclusion stream than the reference stream may have contributed to the generally higher microbial activity on wood biofilms.

Woody debris was the only large particulate organic matter source for colonization in the exclusion stream. In the reference stream, leaves were present in abundance in October, along with wood, yet leaves were virtually absent by April as a result of decomposition and invertebrate feeding. Therefore, during October and April, there were two very different regimes for nutrient allocation. Previous research has attributed the presence of leaf litter in October and November to decreases in both N and P concentrations in stream water as a result of microbial uptake (Mulholland and Rosemond 1992). By April, the only large particulate organic matter source in the reference stream was wood, hence more nutrients were available for microbial uptake per unit surface area of wood due to the absence of leaves. This scenario was similar to the one we saw year-round in the litter exclusion stream.

The release of a conservative tracer into each of the study streams indicated multiple areas of groundwater input into both of our study streams. Water samples from these designated areas showed higher concentrations of nitrogen and phosphorus relative

to nutrient concentrations obtained from samples taken in the thalweg. In Fig. 13, nutrient upwelling locations are noted as shaded oval areas. In a stream without leaves (the reference stream in April or the litter exclusion stream year-round), nutrients are available over a larger streambed area due to a reduction in the surface area of microbially colonized organic matter. In contrast, when leaves and wood are present (e.g., reference stream in October), zones of higher nutrient availability do not extend as far away from their upwelling point because there is an increased surface area of microbially colonized organic matter and dissolved nutrients entering from groundwater inputs are very rapidly immobilized. Therefore, the increased respiration rates that were seen in the exclusion stream in October and April, and in the reference stream in April, may represent the augmentation of microbial activity due to an increases in nutrient availability.

I tested this hypothesis experimentally using nutrient releasing substrates to augment available nutrients to the biofilm on poplar disks. Results indicated that the addition of nitrogen and phosphorus together resulted in an increase in microbial respiration rates and fungal biomass in the reference stream to levels found in the exclusion stream. In contrast, nutrient addition in the exclusion stream did not significantly increase respiration rates indicating that the substrates in the exclusion stream were not nutrient limited at that time.

4. Comparison of the biofilm on poplar vs. oak

There was no significant difference in microbial respiration and fungal biomass between oak and poplar sticks. These results imply that the two wood types used in this

study provide similar colonization substrates for microbes. In the only other study to compare microbial colonization on different wood types, Shearer and Von Bodman (1983) monitored fungal colonization on twigs from cherry, oak, silver maple, and sycamore trees that were placed in an Illinois stream for 30 months. Of the 33 species of Ascomycetes collected, 4 species were dominant colonizers, and there were no differences in colonization between wood type. I found no previously published studies comparing respiration rates or microbial biomass indicators between wood species in streams. Colonization by particular fungal species appears to depend on the sequence of colonization by species and it is difficult to predict particular species assemblages (Shearer and Von Bodman 1983). Preliminary identification of dominant colonizers on wood from this study showed that one major type of hyphae was responsible for most of the biomass in both streams. I was unable to culture this species in the laboratory and therefore could not identify it. If there is no specificity in species composition by wood type, perhaps functional properties such as respiration rates and fungal biomass would also remain non-specific. Although overall microbial activity may be similar between wood types, initial rates of microbial colonization seems to be initially sensitive to differences between oak and poplar sticks. On the first two collection dates (days 29 and 55) poplar had significantly higher respiration rates. After day 55 there were no differences between wood types.

In contrast to the lack of difference in respiration rates on different wood types, there were significant differences in breakdown rates in the exclusion stream and poplar

sticks decomposed faster than oak (Fig. 8A). Melillo et al. (1983) found that chemically labile wood types (i.e., low % lignin, low lignin/nitrogen ratio) had faster decomposition rates. The poplar wood in this study has a lower lignin/nitrogen ratio than oak and therefore was expected to decompose faster. Petersen and Cummins (1974) described a processing continuum with a hierarchy of leaf species ranked in order of their decomposition rates and the same concept might apply to wood.

Overall, our calculated breakdown rates for wood were much faster than those previously published. Decomposition of wood is strongly influenced by surface area: volume ratios which in turn influences oxygen diffusion rates and sediment accumulation (Triska and Cromack 1980, Aumen et al. 1983, Golladay and Webster 1988). Wood strips used in this study were very thin (ca.1 mm) and therefore had very high surface area: volume ratios and breakdown rates were faster (0.0015-0.0085/d) than those reported by Golladay and Sinsabaugh (1991) for birch ice cream sticks (0.0016-0.0019/d) and Melillo et al. (1983) for wood chips from a variety of species (0.0007-0.0033/d).

In summary, despite decomposition rates varying by wood species, we found no differences in respiration rates of the biofilms colonizing oak and poplar. Microbial colonization of wood has been shown to be primarily a surface phenomena as a result of poor oxygen diffusion into compacted woody tissue (Aumen et al. 1983). Wood, regardless of species, can provide a stable microbial substrate for microbial biofilms, which in turn may be a consistent food resource for invertebrates, especially in spring and summer months when few leaves are available.

5. Comparison of the biofilm on wood vs. rhododendron

Wood had higher respiration rates and fungal biomass than rhododendron leaves. Softening and fragmentation of leaf surfaces curtails biofilm development (Golladay and Sinsabaugh 1991) whereas wood can remain stable for years thereby providing a long-lasting substrate for microbial biofilm development (Aumen et al. 1983, Golladay and Sinsabaugh 1991). These results concur with previous studies by Golladay and Sinsabaugh (1991) and Tank et al. (1993) confirming that wood has higher microbial activity than leaves (per unit surface area) and wood provides a more temporally constant substratum than leaves for microbial colonization (Shearer and Webster 1991).

6. Microbial assays as ecosystem indicators

Three aspects of microbial colonization were used to estimate the impact of litter exclusion on microbial colonization of wood: two were activity based measures (microbial respiration and CBH activity) and one was a biomass indicator (ergosterol). Decomposition rates were also estimated through measure of mass loss of substrates over time. The biomass measure used in this study estimated only the fungal component of the microbial community and bacterial biomass is not addressed. Scanning electron micrographs of wood biofilms have indicated that microbial colonization on wood is primarily fungal (Tank & Winterbourn 1995,1996). Studies on leaves have indicated that fungal biomass may exceed bacterial biomass by an order of magnitude, and fungi accounted for over 90% of total microbial biomass on leaves (Findlay and Arsuffi 1989, Baldy et al. 1995). There are no studies comparing bacterial and fungal biomass on wood

in streams. Preliminary bacterial counts using acridine orange were conducted in this study and very few bacterial cells were seen.

In general, the effect of litter exclusion on the microbial biofilm colonizing wood was mirrored in the three microbial assays. Golladay and Sinsabaugh (1991) found a similar positive correlation between estimates of fungal biomass and a suite of extracellular enzyme assays. Because the two activity measures, CBH activity and microbial respiration, showed similar trends, either of these assays were sensitive enough to detect biofilm differences between the study streams. The CBH assay combined with other extracellular enzyme assays can provide a useful characterization of the carbon degrading potential of the microbial biofilm (e.g. Sinsabaugh et al. 1991) as an alternative to the general microbial activity approach of the respiration assay.

7. Summary

Results from this study have shown that the microbial biofilm on wood in the litter excluded stream is enhanced in relation to the reference stream. Fungal biomass on wood in the exclusion stream was seven times as high as in the reference stream. Higher respiration and fungal biomass in the exclusion stream may be a result of additional nutrient availability from groundwater inputs that are less rapidly immobilized due to the presence of fewer microbially colonized substrates (i.e., no leaves in the stream). In contrast, nutrient inputs from groundwater are rapidly immobilized in the reference stream due to increased organic substrate surface area. Long-term data from nutrient releases conducted in the study streams have shown that nitrogen (as NH_4) and

phosphorus (as SRP) uptake lengths are significantly shorter in the reference stream indicating greater nutrient limitation in streams with leaves (Webster et al., unpublished data). Forested headwater streams at Coweeta have detritus based food webs, but results from this study imply that nutrients are also a governing force in this ecosystem (Polis and Strong 1996).

Previous research has shown that higher stream nutrient concentrations can translate into higher growth rates of invertebrate shredders (O'Hop et al. 1984). Results from my study have shown that increases in stream nutrient concentrations resulted in increased biofilm activity on wood. Wood biofilms may be used as a food resource by traditional leaf shredding invertebrates exhibiting omnivory when leaf resources are scarce in late spring and summer. Since wood enters streams year-round and is a long-lasting organic substrate for microbial colonization, wood may be looked at as an important bridge between pulses of high quality leaf litter that enters streams only in autumn. Wood biofilms may be critical in stabilizing the stream ecosystem to variability in the resource base. In this study, bottom up nutrient effects, via microbial biofilms, may play a regulatory role in detrital food chains in streams.

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Table 1. Physical and chemical characteristics of study streams. Values are annual means since 1985. (J.B. Wallace, unpublished data)

VARIABLE	WATERSHED 53	WATERSHED 55
Temperature	12.3	12.2
Elevation (m a.s.l.) at flume	820	810
Watershed area (ha)	5.2	7.5
Average discharge (L/s)	1.06	1.72
Maximum discharge (L/s)	30.3	46.9
pH	6.8	6.7
NO_3-N (μg/L)	8	10
NH_4-N (μg/L)	2	3
TKN (μg/L)	30	28
SRP (μg/L)	3	1
Cl (μg/L)	0.538	0.656
K (μg/L)	0.441	0.397
Na (μg/L)	1.060	0.777
Ca (μg/L)	0.599	0.468
Mg (μg/L)	0.423	0.371
SO_4 (mg/L)	0.357	0.399
SiO_2 (mg/L)	8.015	6.812
HCO_3 (mg/L as $CaCO_3$)	4.79	3.92

Table 2. Wood standing stocks in wetted perimeter of study streams for two years. Wood surface area is expressed as m^2 of wood per m^2 of streambed whereas wood volumes are presented as kg of wood per m^2 of streambed. Values are annual means and standard errors are presented in parentheses. (J.B. Wallace, unpublished data)

YEAR	REFERENCE STREAM	EXCLUSION STREAM
1994		
Wood surface area (m^2/m^2)	0.864 (0.208)	1.212 (0.156)
Wood volume (kg/m^2)	7.4 (2.6)	11.5 (3.7)
1995		
Wood surface area (m^2/m^2)	1.098 (0.257)	0.967 (0.175)
Wood volume (kg/m^2)	13.8 (5.5)	8.9 (2.4)

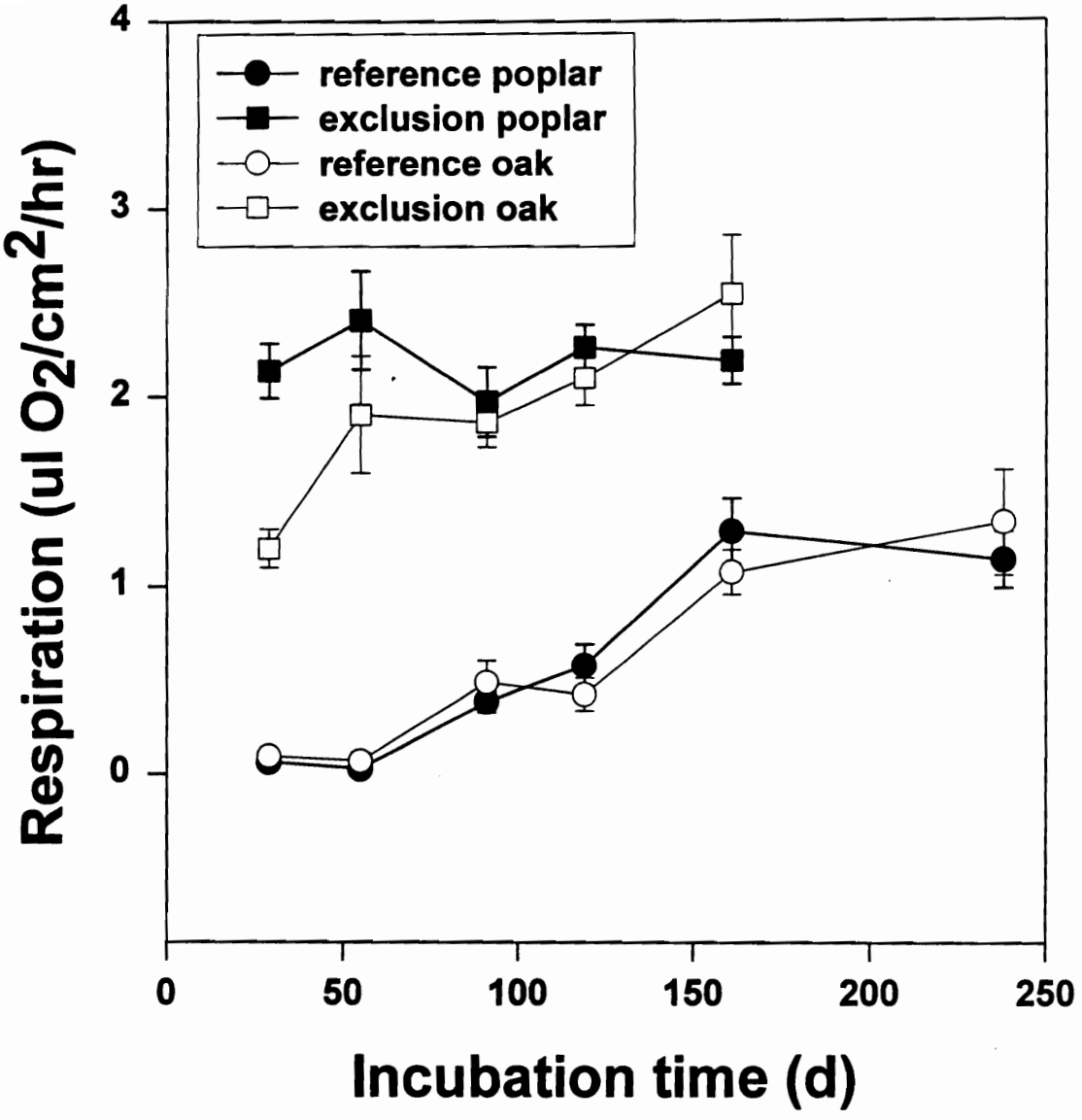

Fig. 1. Experiment 1: microbial respiration in streams with and without leaves. Begun 24 Oct 94.

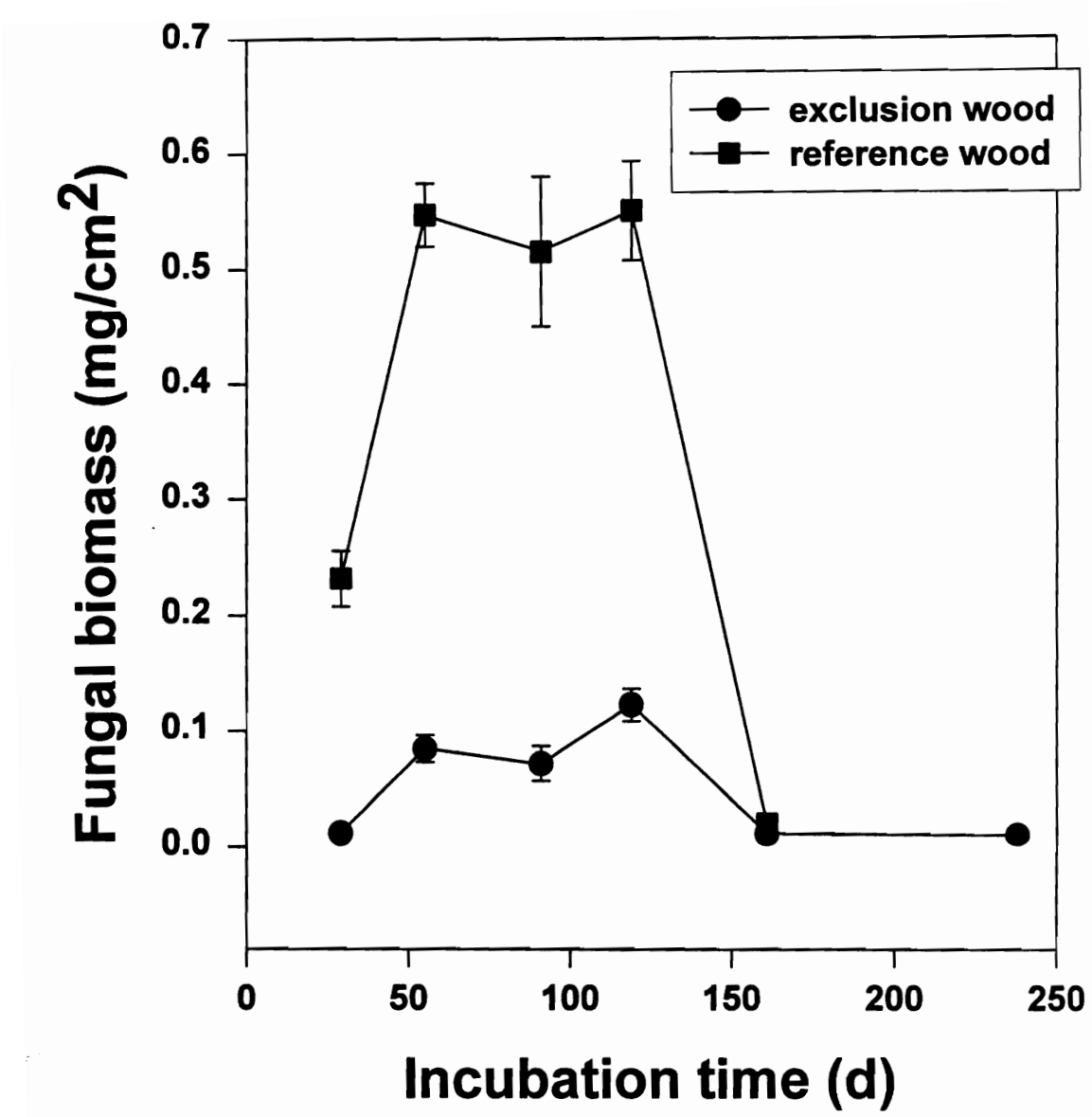

Fig. 2. Experiment 1: fungal biomass on wood in streams with and without leaves. Begun 24 Oct 94.

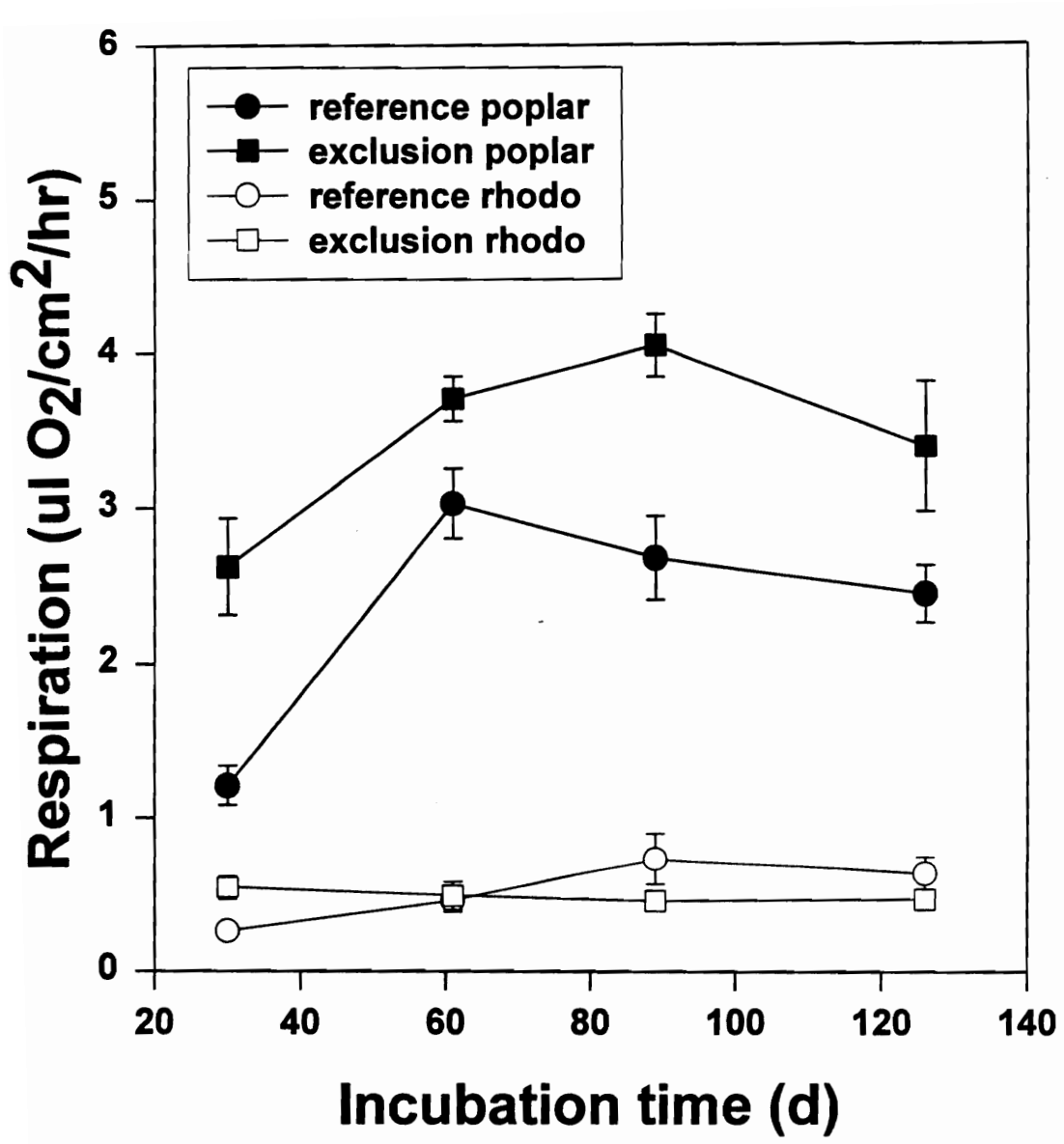

Fig. 3. Experiment 2: microbial respiration in streams with and without leaves. Begun 24 Apr 95.

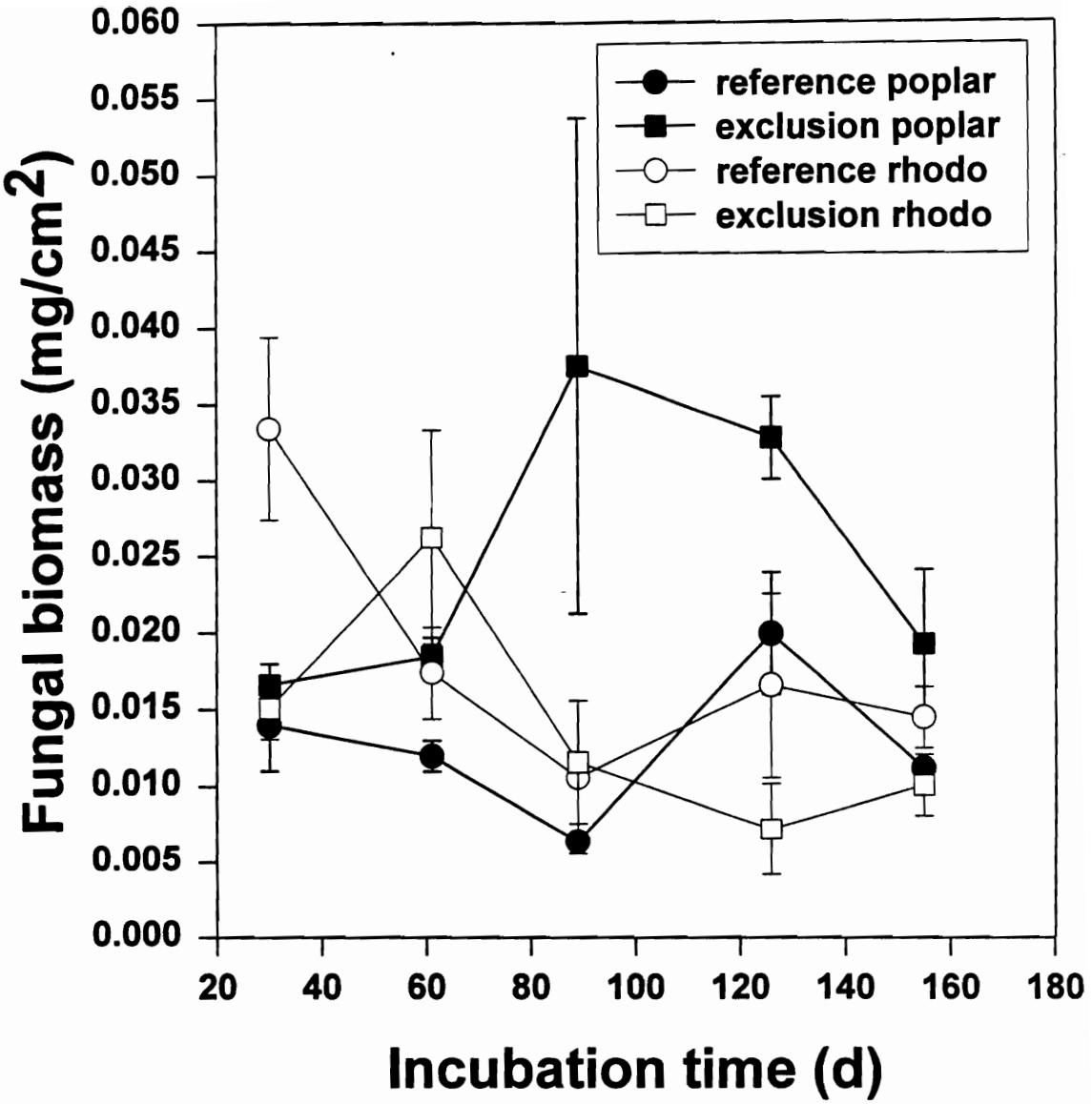

Fig. 4. Experiment 2: fungal biomass in streams with and without leaves. Begun 24 Apr 95.

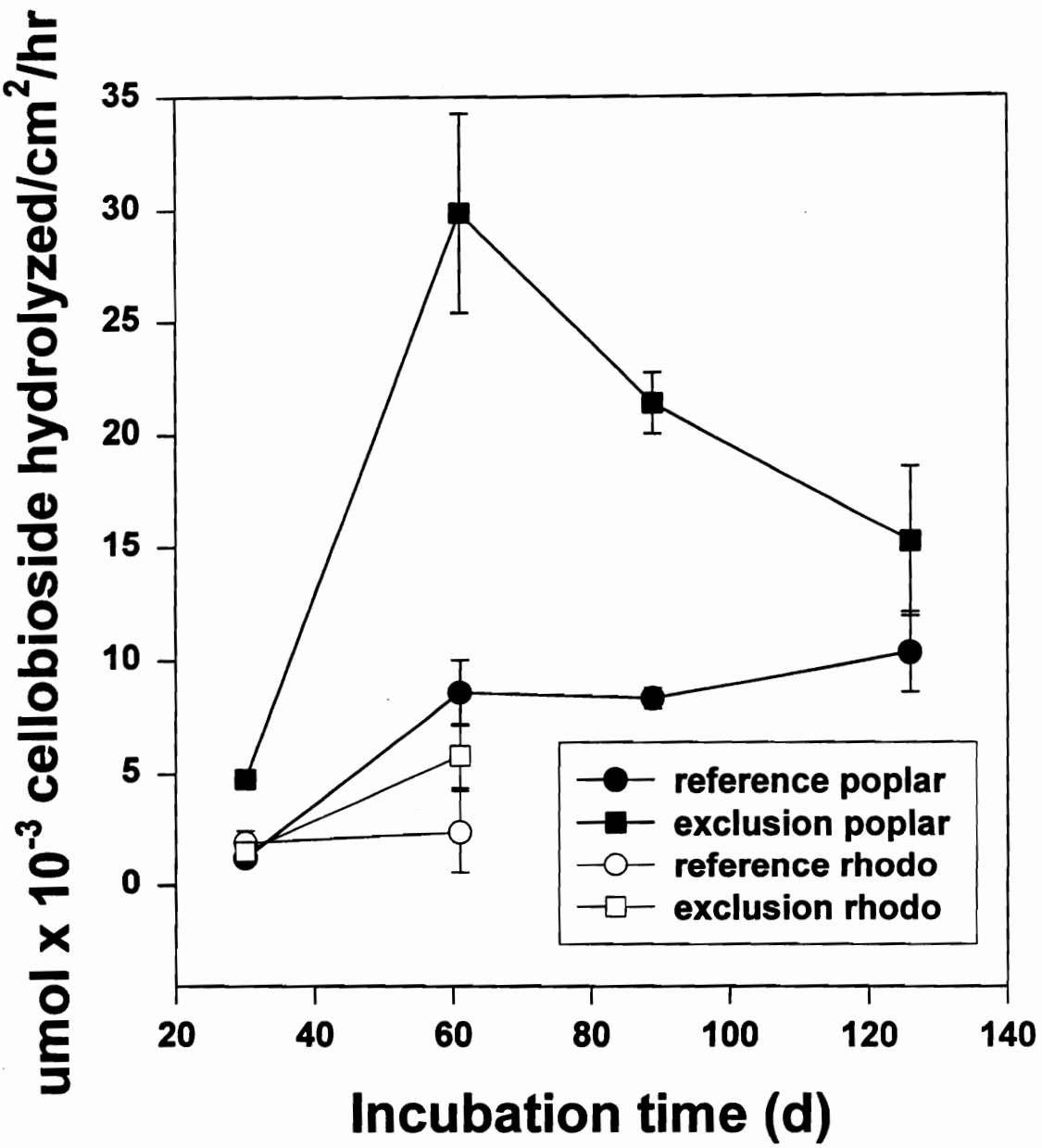

Fig. 5. Experiment 2: cellulose degrading potential in streams with and without leaves. Begun 24 Apr 95.

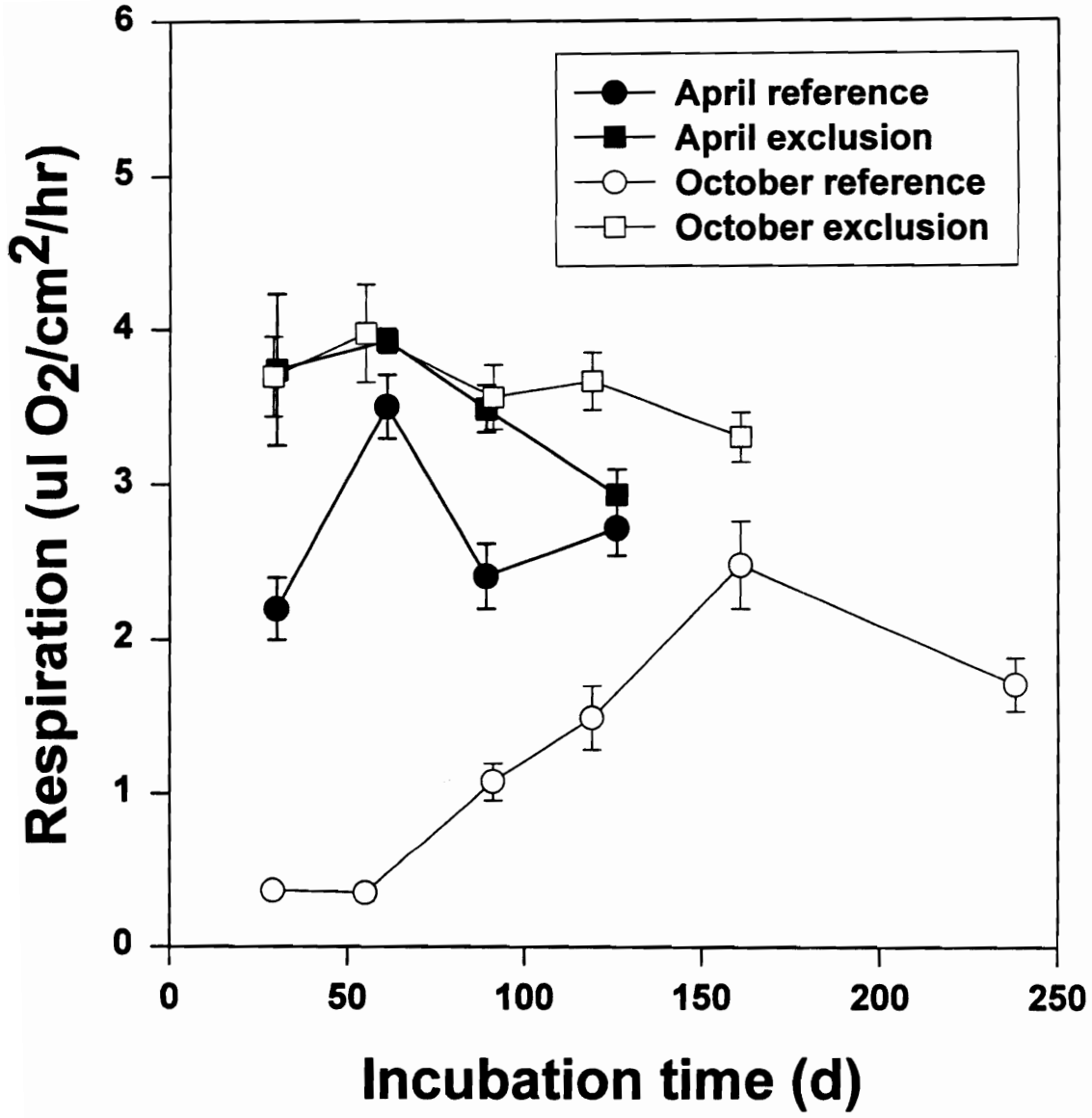

Fig. 6. Seasonal trends: microbial respiration on poplar veneers in streams with and without leaves.

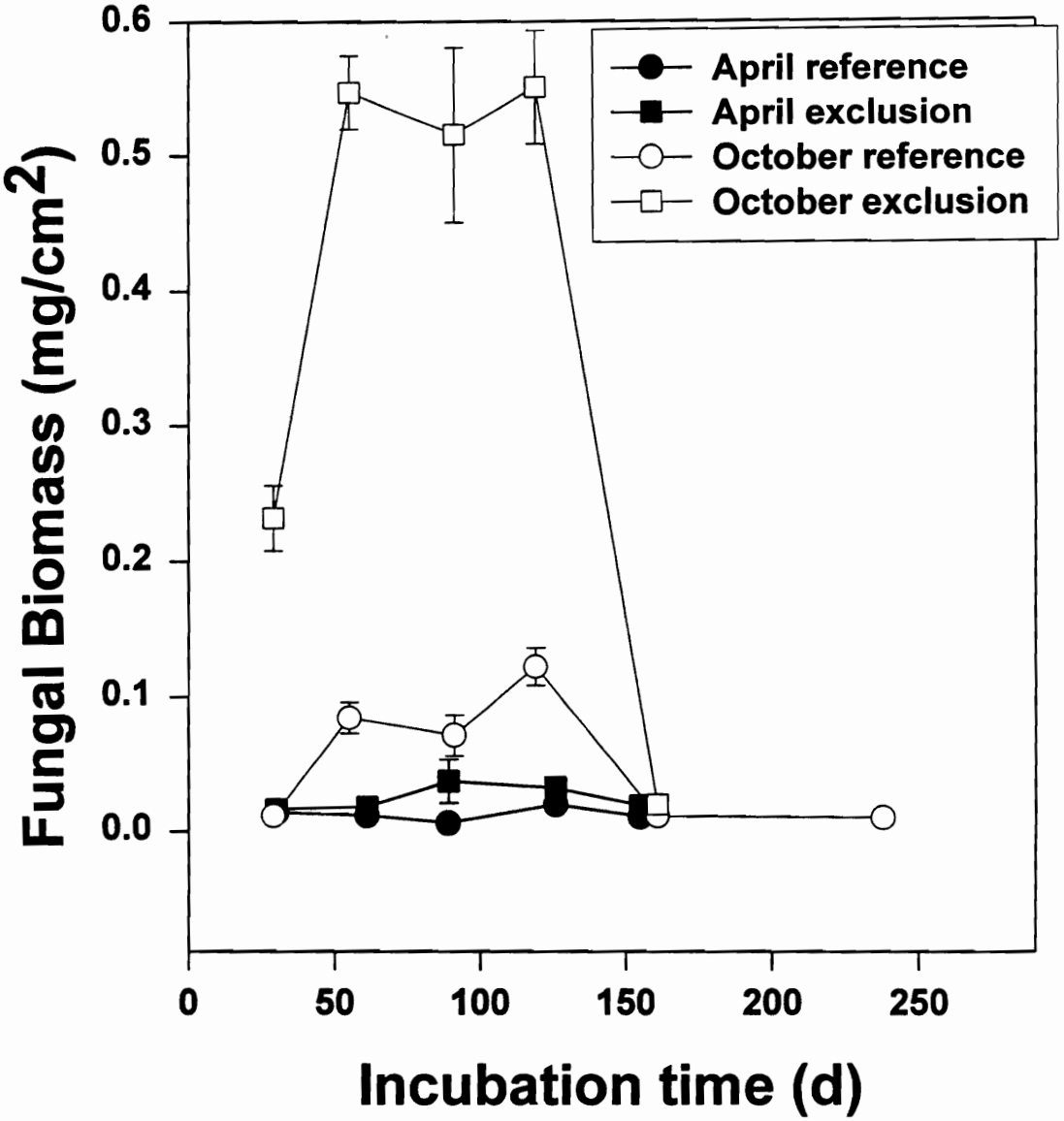

Fig. 7. Seasonal trends: fungal biomass on poplar veneers in streams with and without leaves.

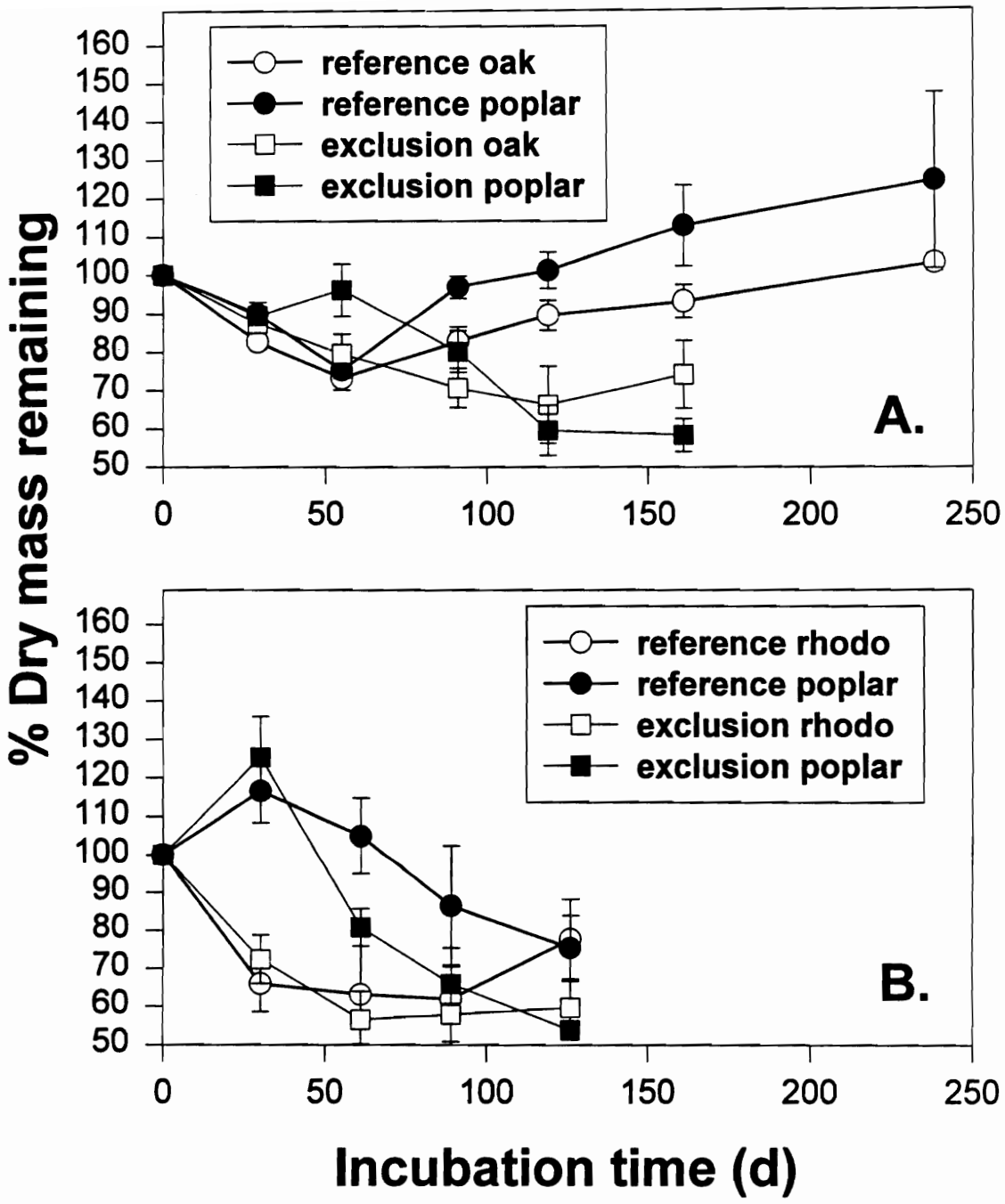

Fig. 8. Substrate mass loss for A. experiment 1 (begun 24 Oct 94) and B. experiment 2 (begun 24 Apr 95) in streams with and without leaves.

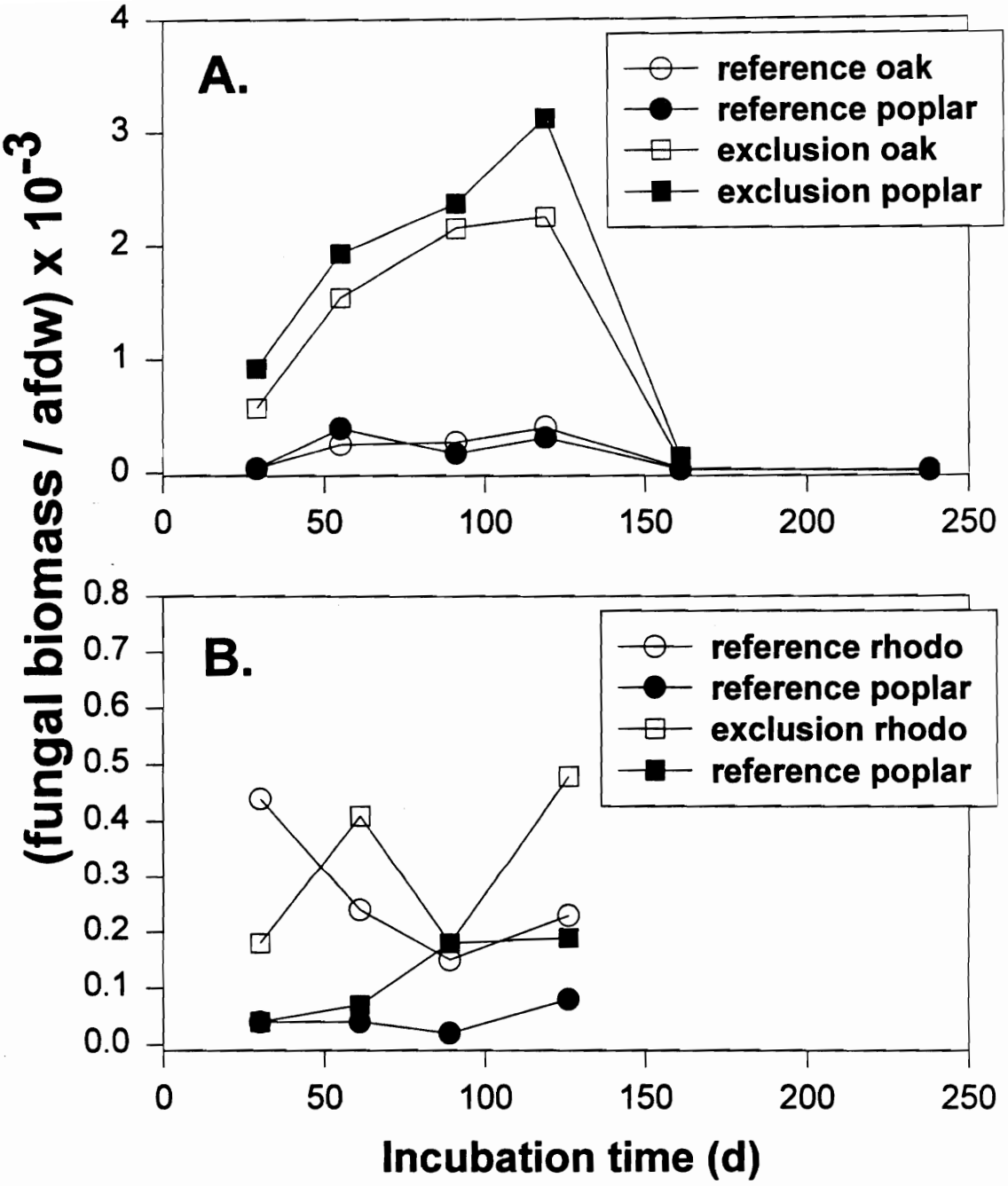

Fig. 9. Ratio of fungal biomass to afdw for A. experiment 1 (begun 24 Oct 94) and B. experiment 2 (begun 24 Apr 95) in streams with and without leaves.

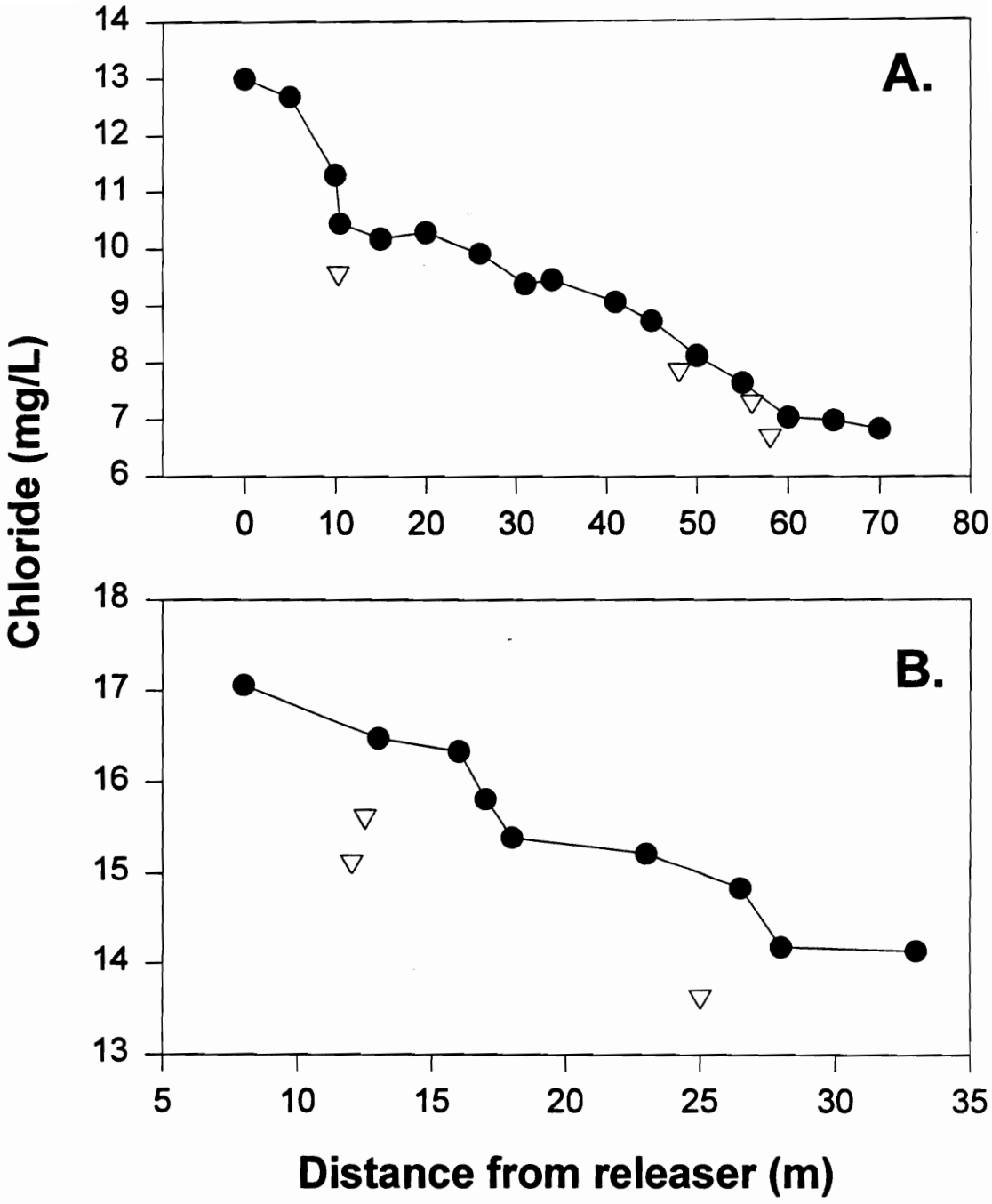

Fig. 10. Groundwater inflow for A. exclusion stream and B. reference stream. Filled circles represent samples from thalweg and open triangles represent grounwater input samples.

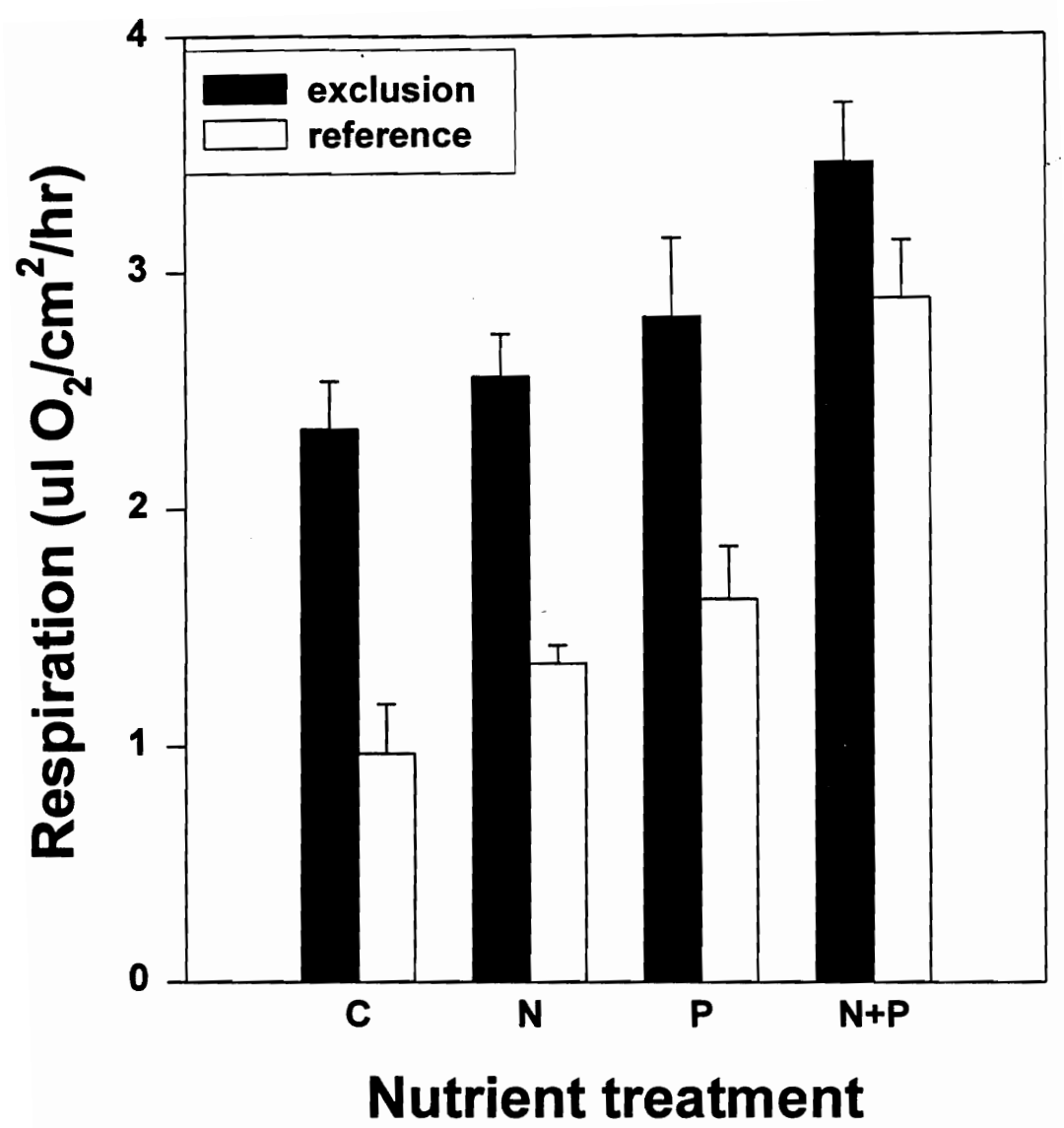

Fig. 11. Effect of nutrient addition on respiration on poplar disks in streams with and without leaves. C= control, N= NO_3 added, P= PO_4 added, and N+P= NO_3 and PO_4 added.

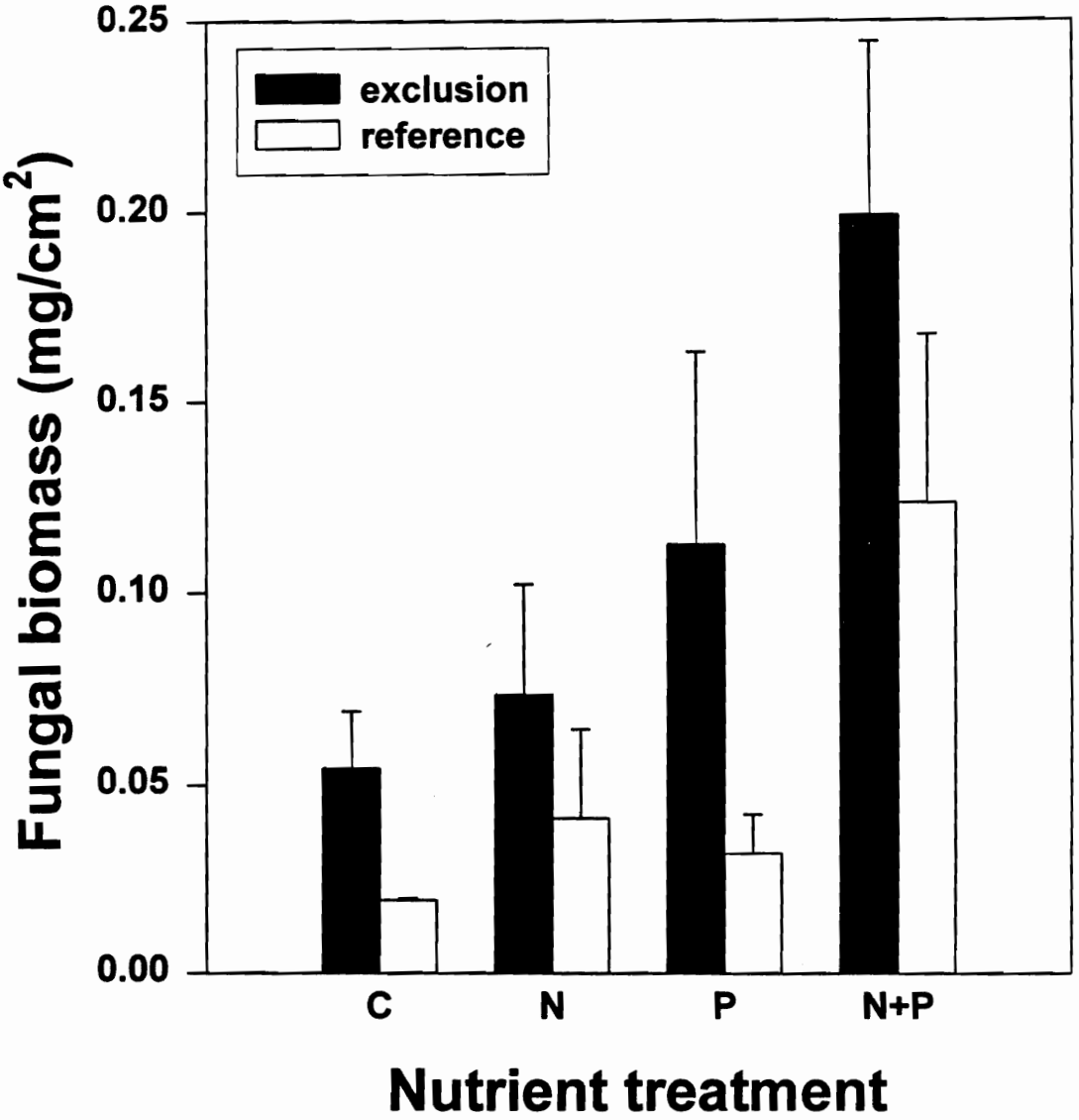

Fig. 12. Effect of nutrient addition on fungal biomass on poplar disks in streams with and without leaves. C= control, N= NO_3 added, P= PO_4 added, and N+P= NO_3 and PO_4 added.

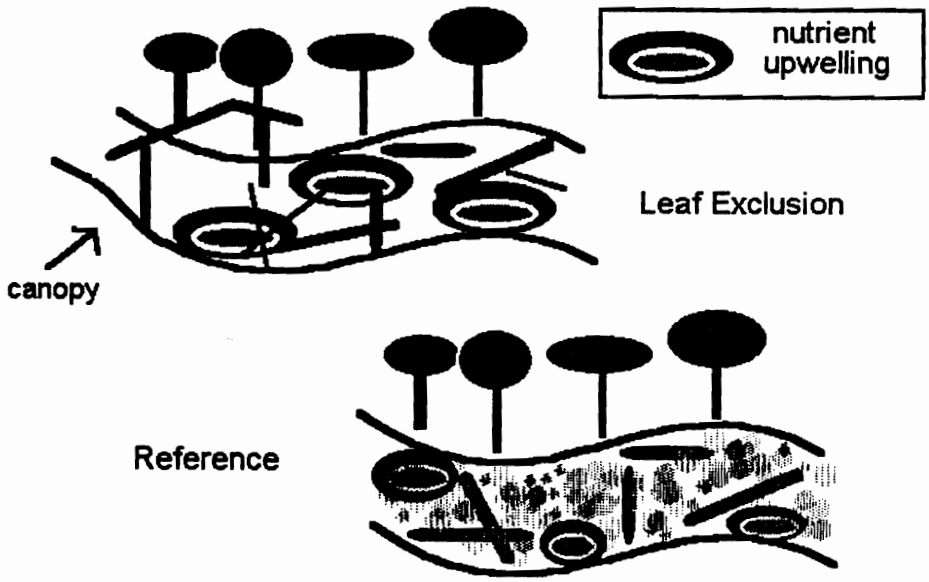

Fig. 13. Conceptual diagram of areas of high-nutrient groundwater upwelling sites in the leaf exclusion and reference streams. Nutrients are more rapidly immobilized in the reference stream where both leaves and wood were present.

Chapter 4:

Effect of leaf litter exclusion on microbial enzyme activity associated with wood biofilms in streams.

I. Introduction

Wood has long been considered an important structural component in streams (Harmon et al. 1986). Recent research has also demonstrated its importance as a microbial substrate for biofilm development (Golladay and Sinsabaugh 1991, Sinsabaugh et al. 1991, Tank and Winterbourn 1995, 1996). Wood surfaces may support higher microbial biomass, primarily fungi, than leaf substrates (Golladay and Sinsabaugh 1991, Tank et al. 1993, Tank et al. in prep). Since there are few freshwater organisms that consume wood directly, microbial colonization of wood provides a vector for carbon transfer to higher trophic levels in stream food webs. Unlike leaves, wood offers physical stability allowing for extensive microbial colonization and exposure of new substrate for colonization throughout the decomposition process. Leaves are a pulsed, short-term input of carbon to a stream system, while wood represents a long-lasting organic matter resource.

Leaf litter inputs have been excluded from a first-order stream (C55) at Coweeta Hydrologic Lab, Macon Co., North Carolina, since August 1993 in an attempt to determine how removal of the majority of the resource base will affect stream structure and function. Since leaf exclusion, woody debris in the exclusion stream has become a primary organic matter source.

Microbial degradation of large particulate organic matter is mediated by enzymatic activity occurring outside the cell (e.g., Sinsabaugh et al. 1991). The enzymes

involved in lignocellulose degradation and those involved in nitrogen and phosphorus cycling are most critical to degradation of organic matter (Sinsabaugh and Moorhead 1996). There is a strong correlation between microbial decomposition of particulate organic matter and extracellular lignocellulose activity (Sinsabaugh et al. 1992, Sinsabaugh and Linkins 1993, Sinsabaugh et al. 1994b). Models derived from the relationships between extracellular enzymes and decomposition predict that temperature, pH, and nutrient availability affect microbial activity through regulation of enzyme activity (Sinsabaugh et al. 1994b).

Previous studies have shown that activities of a few selected enzymes can be used as indices to clarify the complex hydrolytic and oxidative reactions that drive microbial decomposition (Sinsabaugh et al. 1994b). Because wood decomposes slowly, monitoring extracellular enzymes seems a more useful approach to estimate microbial activity related to wood decomposition than weight loss. Despite slow decomposition, wood has been shown to be a locally active site for microbial development and thereby a food source for higher trophic levels. Enzymatic indicators of microbial activity offer the benefits of high spatial and temporal resolution unlike gravimetric methods used to estimate decomposition rates (Sinsabaugh et al. 1994a).

The objective of this study was to determine the effect of leaf litter exclusion on the biofilm colonizing wood in a small headwater stream. I monitored the activity of a suite of 6 extracellular enzymes involved in decomposition of wood over 3 months to

obtain "enzymatic signatures" in time and space for wood biofilms in the leaf excluded and reference streams.

II. Methods

A. Site Description

This study was located in 2 first-order streams draining Catchments 53 and 55 respectively, at Coweeta Hydrologic Laboratory , Macon County, North Carolina, USA. Coweeta is a 2270-ha experimental forest of the U.S. Forest Service located in Nahantahala National Forest in the eastern part of the southern Appalachians. The forest canopy is dominated by tulip poplar (*Liriodendron tulipifera*), white oak (*Quercus alba*), red oak (*Quercus rubra*), and dogwood (*Cornus florida*). There is also a dense understory of rhododendron (*Rhododendron maxima*) which results in year-round shading and low rates of primary productivity (Webster et al. 1983).

The two streams are low-nutrient, soft water streams with similar water chemistry characteristics, pH between 6.7-6.8, similar thermal regimes, and run in a southern aspect (Table 1). Both first-order streams are groundwater-fed and therefore cool in summer and relatively warm in winter. Stream-bed substrates are similar consisting of mixed cobble-pebble with sand-gravel, and some bedrock outcrops.

Leaf-litter inputs have been excluded along the length of stream (180m) draining Catchment 55 since August 1993 by placing a mesh canopy (1.5 cm mesh) over the stream. The canopy was positioned beneath the rhododendron understory so as to

maintain normal shade and yet exclude rhododendron leaves (normally 27% of total leaf litter input, J.B. Wallace, unpublished data). Before leaf exclusion in Catchment 55 and in the reference stream, Catchment 53, small woody debris (<5 cm) and leaf litter made up most of the standing crop of benthic organic matter (60-65% and 20-24%, respectively, J.B. Wallace, unpublished data). Surface area of small woody debris represented a considerable proportion of colonizable substrate in C53 and was the only large substrate left for microbial colonization in C55 since the leaf exclusion.

B. Sample arrays

Untreated wood veneer strips (2.5cm x 15 cm x 1 mm) of yellow poplar were used as substrates for microbial colonization in the 2 streams. Veneer strips (hereafter referred to as sticks) were attached with cable-ties to plastic mesh holders (6 sticks per holder). The mesh holders were staked to the stream-bed such that the long axes of the sticks were oriented parallel to stream flow. Twelve sticks were collected from each stream after 28, 58, and 86 days and returned to the laboratory in containers of cold stream water.

C. Enzyme assays

Twelve sticks were randomly divided into 4 equal samples of 3 sticks each for each collection date in each stream. Samples were suspended in 100 ml acetate buffer (pH = 5) and homogenized with a Brinkmann polytron. The suspensions (4 samples per stream) were assayed for activity of 6 extracellular enzymes. The enzymes assayed (and their respective substrates in parentheses) were as follows: cellobiohydrolase (p-nitrophenyl (pNP)-cellobioside); B-1,4-glucosidase (pNP-B-D-glucopyranoside); B-

xylosidase (pNP-B-xylopyranoside); phosphatase (pNP-phosphate); phenol oxidase (L-3,4-dihydroxyphenylalanine(L-DOPA)); and peroxidase (L-DOPA with 0.2 ml of 0.3% hydrogen peroxide solution). Final peroxidase activity was calculated as the difference between phenol oxidase activity and total peroxidase activity. All assays were conducted at 20 °C in pH 5 acetate buffer with 4 analytical replicates and 2 controls per sample (Sinsabaugh et al. 1994b). Five 2-ml subsamples from each suspension were placed in pre-weighed aluminum pans, weighed, dried (55 °C, 24 h), ashed (550 °C, 1 h), and reweighed to determine g AFDM per analytical sample. Enzyme activity was then expressed per g AFDM.

D. Fungal Biomass

Fungal biomass was estimated by ergosterol content of biofilms colonizing wood using methods outlined by Newell et al. (1988) and modified by R.L. Sinsabaugh (personal communication). Subsamples of known surface area (2.5 cm x 3 cm x 1 mm) were placed in 15-ml Falcon tubes with 5 ml of methanol and refluxed in a dry block heater for 2 h at 65°C. Tubes were cooled, saponified by adding 1 ml of 4% KOH in methanol, and refluxed for another 0.5 h at 65 °C. Samples were cooled, centrifuged, and the supernatants were decanted into clean 15-ml Falcon tubes. The pellets were resuspended in 2 mls of HPLC-grade methanol, centrifuged again, and the methanol used to rinse the pellets was added to the tubes containing the extracts along with 1 ml water. Each sample was extracted 2 times with 2 ml pentane. Pentane extracts were combined and evaporated in a fume hood. The residue was redissolved with 1 ml of HPLC-grade

methanol and filtered through a 0.45 μm syringe filter into clean 5-ml polypropylene tubes. Ergosterol was quantified using a reverse-phase HPLC system configured as follows: solvent = methanol; flow rate = 2ml/min; column = Nova-Pak ODS C18 3.9 mm x 75 mm; absorbence detector = 282 nm with a range of 0.500; quantification = Waters integrator with attenuation at 256; ergosterol retention time = 1.6 min; replication = five per sample; standards = 5, 10, 25, and 50 μg/ml solution of ergosterol and methanol. The general conversion factor of 6 mg ergosterol/g was used to estimate fungal biomass (Newell et. al. 1988).

E. Data Analyses

All data were log transformed due to non-normal distributions and statistical analyses were carried out using log transformed data. Two-way analysis of variance (ANOVA by date and stream) was used to compare enzyme activity and ergosterol values between streams on different sampling dates. Least squares means (LSM) were used to differentiate between means when significance was found in the ANOVA ($p<0.05$).

III. Results

Overall, wood substrates in the leaf exclusion stream had significantly higher enzyme activity than in the reference stream for 5 of 6 enzymes (Fig. 1, ANOVA, LSMeans, $p< 0.0001$ for all enzymes except peroxidase). Peroxidase activity showed the reverse trend and the reference stream had significantly higher activity than the exclusion stream (ANOVA, LSMeans, $p< 0.0001$).

In the reference stream, activity for all enzymes except peroxidase remained fairly constant throughout the 3-mo incubation period. Peroxidase activity in the reference stream increased through time, and activities on days 58 and 86 were significantly higher than on day 28 (ANOVA, LSMeans, $p= 0.0001$). In contrast, enzyme activity in the leaf exclusion stream steadily increased through time in all enzymes except peroxidase. Enzyme activity was always significantly higher on day 86 than on day 28 for B-glucosidase, cellobiohydrolase, xylosidase, phosphatase, and phenol oxidase (ANOVA, LSMeans, $p< 0.05$ for all).

On days 28 and 58, total organic matter content of the biofilms were not significantly different between the two streams and ranged between 65 and 80 % organic matter (Fig. 2). However by day 86, biofilm organic matter content in the leaf exclusion stream remained high, while percent organic matter in the reference stream decreased to 33%. This reduction in percent organic matter coincided with a visible accumulation of sand-like sediment on sticks in the reference stream.

Fungal biomass (mg/cm^2) on sticks in the leaf exclusion stream was significantly higher than in the reference stream on all three collection dates (ANOVA, LSMeans, $p< 0.05$) and generally increased over time (Fig. 3). Fungal biomass on day 86 was significantly higher than on day 28 (ANOVA, LSMeans, $p< 0.05$). Generally, there was a positive relationship between fungal biomass and enzyme activity for each of the extracellular enzymes in the leaf exclusion stream, excluding peroxidase (Fig. 4).

Phosphatase and total oxidative activity (phenol oxidase + peroxidase) per unit fungal biomass in each stream can be used to estimate relative differences in energy expenditure of the microbial biofilm toward nutrient acquisition (Sinsabaugh and Moorhead 1994). Average phosphatase activity (representative of phosphorus acquisition) was over 20 times greater in the reference stream than in the leaf exclusion stream (45.02 vs. 2.14 average activity/h/mg fungal biomass, respectively). Average total oxidative activity (representative of nitrogen acquisition) was over 40 times greater in the reference stream than in the leaf exclusion stream (92.71 vs. 2.23 average activity/h/mg fungal biomass, respectively).

The ratios of the activities of cellulolytic enzymes compared to the activity of nutrient acquiring enzymes were used to estimate relative nutrient availability in each stream (Sinsabaugh et al. 1993). These ratios were calculated for each stream as:

$$\text{Relative P availability} = \frac{Ec}{Ep} = \frac{BG/P + CBH/P + X/P}{3}$$

$$\text{Relative N availability} = \frac{Ec}{En} = \frac{BG/TO + CBH/TO + X/TO}{3}$$

where:

Ec = B-glucosidase (BG), cellobiohydrolase (CBH), and xylosidase(X)
Ep = phosphatase
En = total oxidative activity = (phenol oxidase + peroxidase)

Activity data for each enzyme were transformed into unitless values (0-1) by dividing each sample by the maximum activity for that enzyme in the data set thereby eliminating the weighting effect caused by comparing different enzymes (Sinsabaugh and Moorhead

1994). Relative phosphorus availability (Ec/Ep) was twice as high in the leaf exclusion stream when compared to the reference stream (1 vs. 0.54). Relative nitrogen availability (Ec/En) was 5 times as high in the leaf exclusion stream as compared to the reference stream (1 vs. 0.22).

IV. Discussion

Effect of leaf exclusion on extracellular enzyme activities

Microbial colonization of wood is primarily a surface phenomenon (Aumen et al. 1983, Golladay and Sinsabaugh 1991, Sinsabaugh et al. 1992, Tank and Winterbourn 1995). Wood biofilms consist primarily of fungi and bacteria located in a mucilagenous matrix (Lock et al. 1984, Golladay and Sinsabaugh 1991). The mucilagenous matrix can accrue extracellular enzymes, but fine particulates can be trapped in the biofilm as well. Low microbial activity is often associated with the accumulation of sediments on organic surfaces in streams (Golladay and Sinsabaugh 1991, Tank et al. 1993). Inorganic sediments cover biofilms, hinder oxygen and nutrient diffusion through the biofilm, and hence decrease microbial activity.

The exclusion of leaf litter from the experimental stream significantly altered the composition and microbial activity of the biofilm colonizing wood. Extracellular enzyme activity over the 3-mo incubation period, for all enzymes except peroxidase, was significantly higher in the leaf exclusion stream (Fig. 1). While enzyme activities in the reference stream were always low, activities in the leaf exclusion stream were within the

range of those observed in other aquatic systems (Fig. 1)(Sinsabaugh et al. 1992, Scholz and Boon 1993, Sinsabaugh et al. 1994b, Jackson et al. 1995). B-glucosidase and phenol oxidase activity in the leaf exclusion stream was at the high end of the ranges reported in other studies indicating active decomposition of ligno-cellulose components of the sticks. Peroxidase activity was very low in both streams.

Percent organic matter of wood biofilms were different between the two streams only after 86 days when sedimentation in the reference stream reduced the percent organic matter of the wood biofilm (Fig. 2). Although this may have affected biofilm activity and hence extracellular enzyme activity on day 86, it apparently was not a factor affecting activity in the reference stream on the 2 earlier collection dates.

Despite the fact that organic content of the wood biofilms from both streams was similar before the 86 day collection date, fungal biomass was always significantly higher in the exclusion stream indicating that other factors aside from sediment accumulation, such as streamwater chemistry, were influencing biofilm development both streams. Fungal biomass on sticks was always very low in the reference stream. In contrast, fungal biomass on sticks in the leaf exclusion stream was within the range of values found in other studies (Golladay and Sinsabaugh 1991, Newell and Fell 1992, Tank et al. in prep.) and almost an order of magnitude higher than fungal biomass on leaves (Golladay and Sinsabaugh 1991). By day 86 fungal biomass was at the high end of published estimates for first to fourth-order streams (Golladay and Sinsabaugh 1991).

Effect of nutrients on enzyme activities

Because wood substrates placed in both streams for colonization were identical, differences in microbial biofilm activity must be regulated by factors other than substrate quality. Nutrient concentrations of streamwater may be responsible for differences in enzyme activities between the exclusion and reference streams. Nitrogen has long been considered a potentially limiting nutrient in the decomposition of litter (e.g., Harmon et al. 1986, Webster and Benfield 1986, Fog 1988). Nitrogen content, in the form of C:N ratios, is often used as an indicator of organic matter quality. Both nitrogen and phosphorus content are very low in wood and therefore nitrogen and phosphorus requirements for decomposition of woody substrates must be supplied by exogenous factors (Sinsabaugh et al. 1993).

Fog (1988), reviewing over 60 nitrogen amendment studies, found that supplying inorganic or simple organic exogenous nitrogen speeds the decomposition of labile organic matter while slowing degradation of refractory organic matter components (e.g., lignin) by repressing phenol oxidase activity. In this study, phenol oxidase activity was higher per g organic matter in the exclusion stream (Fig. 1), but lower per unit fungal biomass (2.23 vs. 92.71 average activity/h/mg fungal biomass, respectively). These results support the contention that nitrogen was more limiting in the reference than exclusion stream.

Phosphorus has also been linked to decomposition of leaves in streams (Elwood et al. 1981). In addition to the nitrogen limitation described above, results from this study

also indicated that extracellular activity involved in phosphorus acquisition was higher per unit fungal biomass in the reference stream than in the exclusion stream (45.02 vs. 2.14 average activity/h/mg fungal biomass, respectively). Previous studies have shown that there is an inverse relationship between phosphatase activity and environmental availability of phosphorus (Mulholland and Rosemond 1992, Sinsabaugh et al. 1993), and phosphatase activity has been used as an indicator of phosphorus limitation in aquatic systems (e.g., Wetzel 1981). Although Sinsabaugh et al. (1993) did not find phosphorus to be limiting in a previous study of wood decomposition in a lotic environment, the Coweeta streams used in this study have very low background levels of nitrogen and phosphorus and could be co-limited by these nutrients.

Comparative enzyme activity assays used in conjunction with nutrient concentration measurements may be an accurate indicator of nutrient limitation in streams (Sinsabaugh et al. 1993). A model has been developed by Sinsabaugh and Moorhead (1996) for the Microbial Allocation of Resources among Community Indicator Enzymes (MARCIE). The proposed model connects the availability of nitrogen and phosphorus to litter decomposition rates based on the allocation of energetic resources to extracellular enzyme production by microbes (Sinsabaugh et. al 1993, Sinsabaugh et al. 1994b, Jackson et al. 1995, Sinsabaugh and Moorhead 1996). The model predicts that mass loss rates are directly related to cellulolytic activity. Hence for my study, the model predicted that mass loss rates for wood would be higher in the exclusion stream than in the reference stream. But what is the underlying cause for higher cellulolytic activity in

the exclusion stream? The model also predicts that microbial communities will behave as a unit and maximize production by optimizing allocation of resources among macronutrients thereby creating a tradeoff between carbon, nitrogen, and phosphorus acquisition (Sinsabaugh et al. 1993). Enzymes involved in carbon acquisition have been linked to litter quality while activity of enzymes involved in acquisition of nitrogen and phosphorus are more related to environmental availability of those nutrients. When cellulolytic activity (Ec) was compared to nutrient acquisition (En and Ep), relative phosphorus availability was twice as high in the leaf exclusion stream and there was 5 times as much nitrogen available in the leaf exclusion stream than the reference stream.

Other microbial assay indicating nutrient limitation

In previous research in the study streams, I examined the effect of nitrogen and phosphorus addition on microbial respiration on wood. Results showed that when both N + P were added, microbial respiration increased in the reference stream to levels that were not significantly different from the exclusion stream (Tank et al. in prep.). Nutrient releasing substrates containing only nitrogen or phosphorus did not result in higher respiration rates thereby indicating a co-limitation of nitrogen and phosphorus in this stream. Additionally, long term data from nutrient releases conducted in the study streams indicated that uptake lengths of soluble reactive phosphorus (SRP) and ammonia (NH_4) were significantly shorter in the reference stream indicating more nutrient limitation in the stream containing leaves (Webster et al., unpublished data).

Measuring enzyme activities is a more direct estimation of the "microbial perception" of the environment than are chemical data from the water column (Sinsabaugh and Moorhead 1994). Water chemistry data from the exclusion and reference streams showed no differences between streams (Table 1), yet nutrient-acquiring enzyme activity (per unit fungal biomass) was significantly higher in the reference stream. Perhaps there was no correlation between the microbial response to nutrient limitation, as represented by enzyme allocations, and water chemistry data because chemical data cannot reflect cycling dynamics at the microbe-substrate interface. Water chemistry only represents a net effect of biotic and abiotic processes in the stream. Previous research has shown that the only significant correlation between nutrients and enzyme activity is between the microbial enzyme activity and the nutrient content within the organic substrate being colonized by the microbe (Sinsabaugh et al. 1993). Relative enzyme activities could be used as an alternative to nutrient amendment experiments thereby precluding the need for manipulative experiments and instead relying on in situ variables to determine the effect of nutrients on microbial communities in headwater streams.

Summary

In summary, extracellular enzyme activity and fungal biomass were higher on wood in the stream where leaf litter had been excluded. Measurement of relative activities of selected extracellular enzymes comparing carbon and nutrient acquisition suggested

nutrient limitation in the reference stream. These results concur with estimates of nutrient uptake lengths, which have shown that the exclusion stream has longer uptake lengths for both phosphorus and nitrogen (as NH_4). In addition, results from a previous study using nutrient releasing substrates indicated that the reference stream is nutrient limited by both nitrogen and phosphorus. These results indicated that the microbial activity of wood biofilms may be nutrient limited in Coweeta streams. The presence of leaves in forested headwater streams mediates microbial activity on other organic substrates (e.g. wood) through the immobilization of a limited supply of nutrients from streamwater. The presence of leaves in streams may slow the decomposition of other types of course particulate organic matter such as wood. It is unknown whether proportions of different leaf species may alter rates of nutrient immobilization and therefore wood decomposition. In contrast, perhaps the degree of nutrient limitation is strictly a result of the amount of colonizable leaf surface area as compared to wood, regardless of species. Despite these unanswered questions, results from this study indicated that stream nutrient concentrations may be considered a controlling factor in the cycling of carbon through stream ecosystems.

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Table 1. Physical and chemical characteristics of study streams. Values are annual means since 1985. (J.B. Wallace, unpublished data)

VARIABLE	WATERSHED 53	WATERSHED 55
Temperature	12.3	12.2
Elevation (m a.s.l.) at flume	820	810
Watershed area (ha)	5.2	7.5
Average discharge (L/s)	1.06	1.72
Maximum discharge (L/s)	30.3	46.9
pH	6.8	6.7
NO_3-N (μg/L)	8	10
NH_4-N (μg/L)	2	3
TKN (μg/L)	30	28
SRP (μg/L)	3	1
Cl (μg/L)	0.538	0.656
K (μg/L)	0.441	0.397
Na (μg/L)	1.060	0.777
Ca (μg/L)	0.599	0.468
Mg (μg/L)	0.423	0.371
SO_4 (mg/L)	0.357	0.399
SiO_2 (mg/L)	8.015	6.812
HCO_3 (mg/L as $CaCO_3$)	4.79	3.92

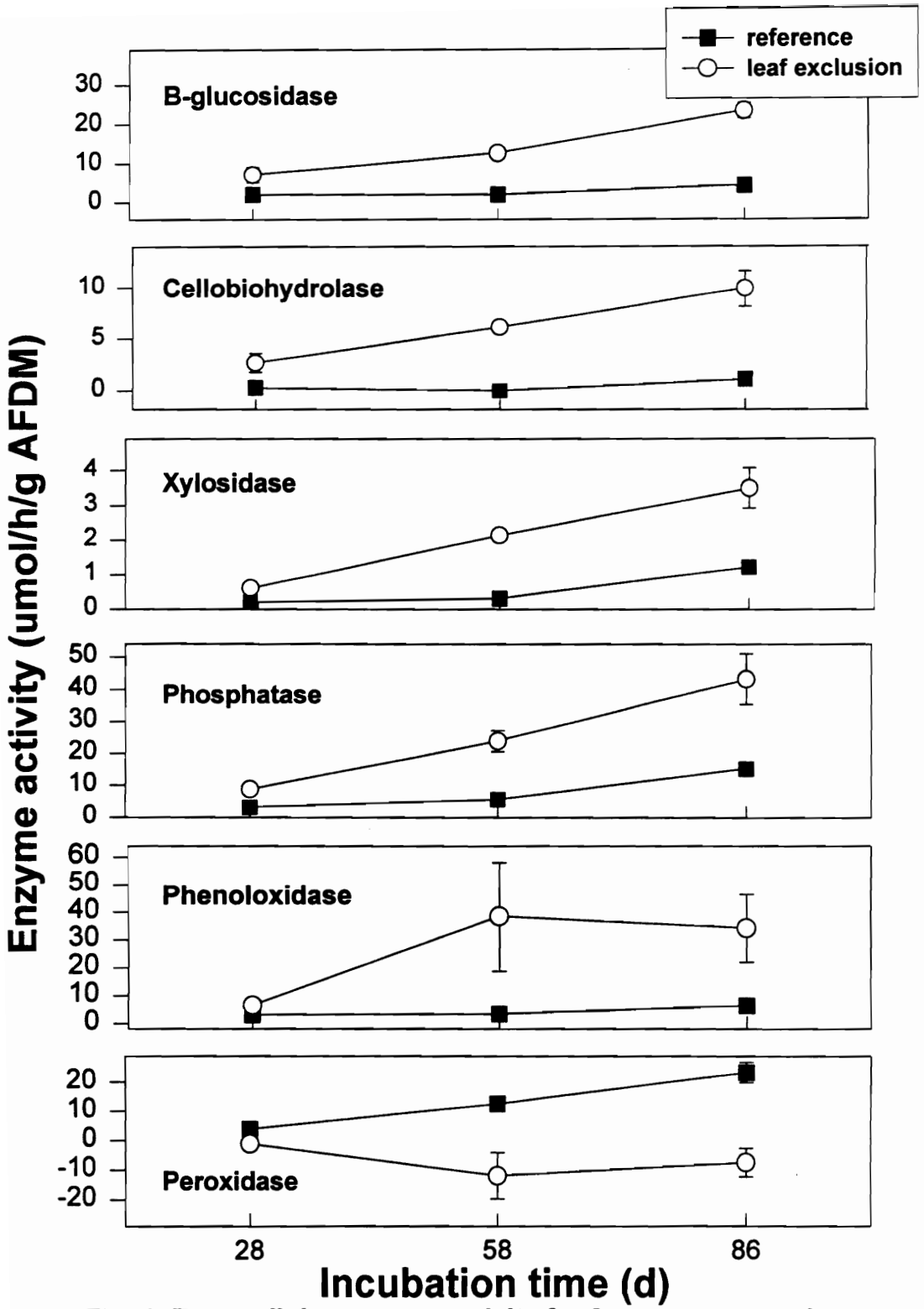

Fig. 1. Extracellular enzyme activity for 6 enzymes over time in leaf exclusion and reference streams.

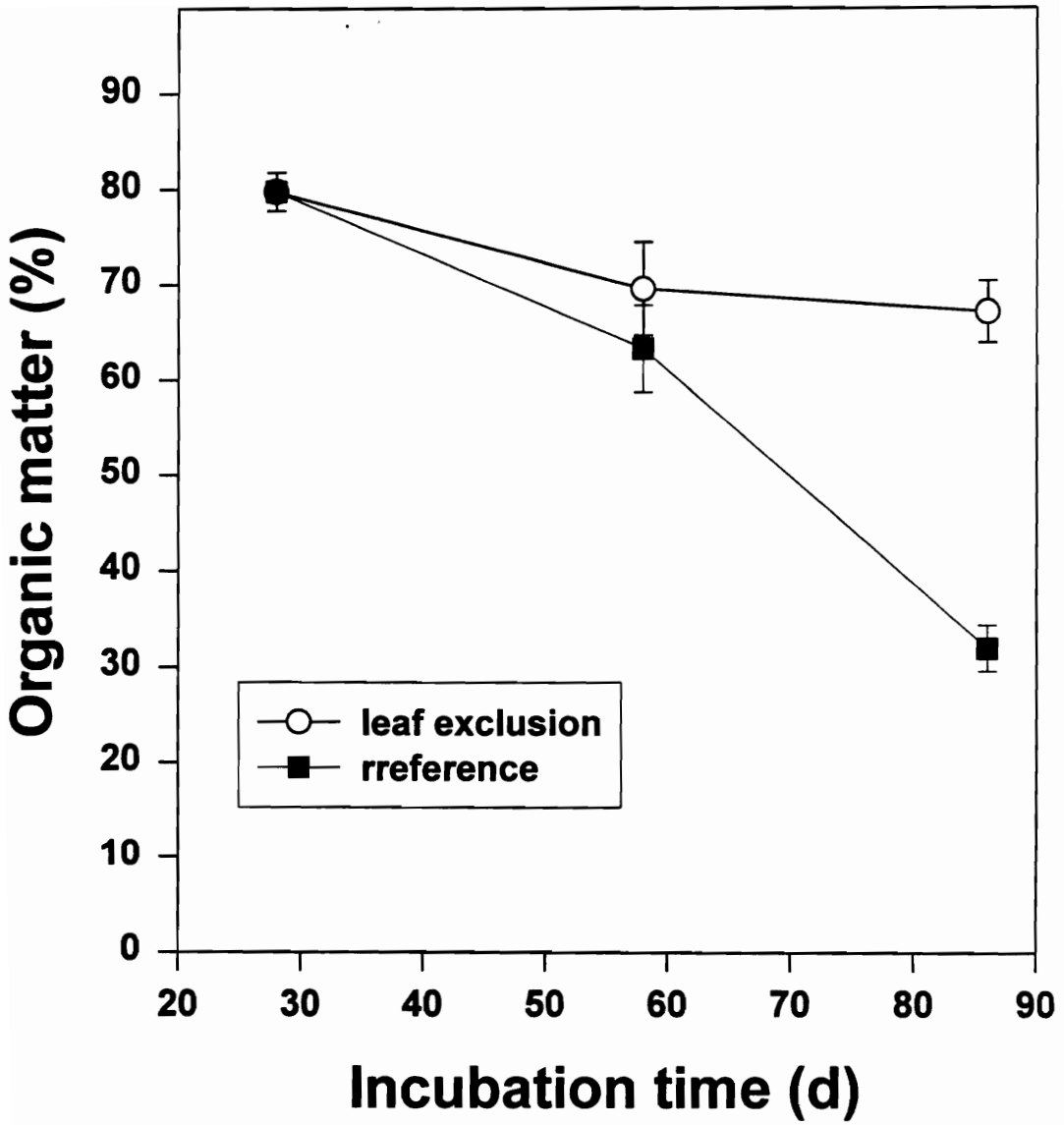

Fig. 2. Percent organic matter through time in leaf exclusion and reference streams. Begun 8 Sept 95.

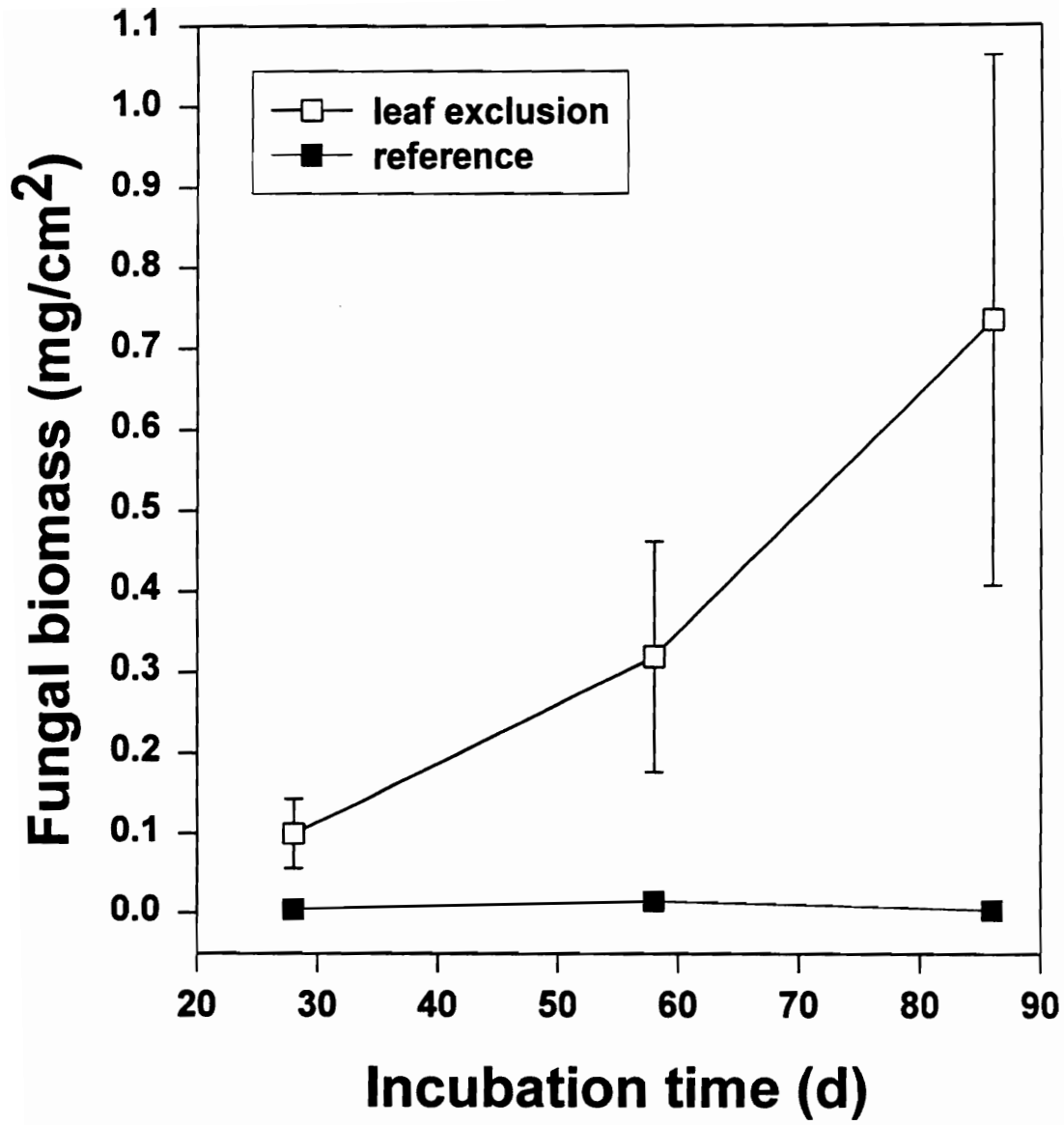

Fig. 3. Fungal biomass on wood through time in leaf exclusion and reference streams. Begun 8 Sept 95.

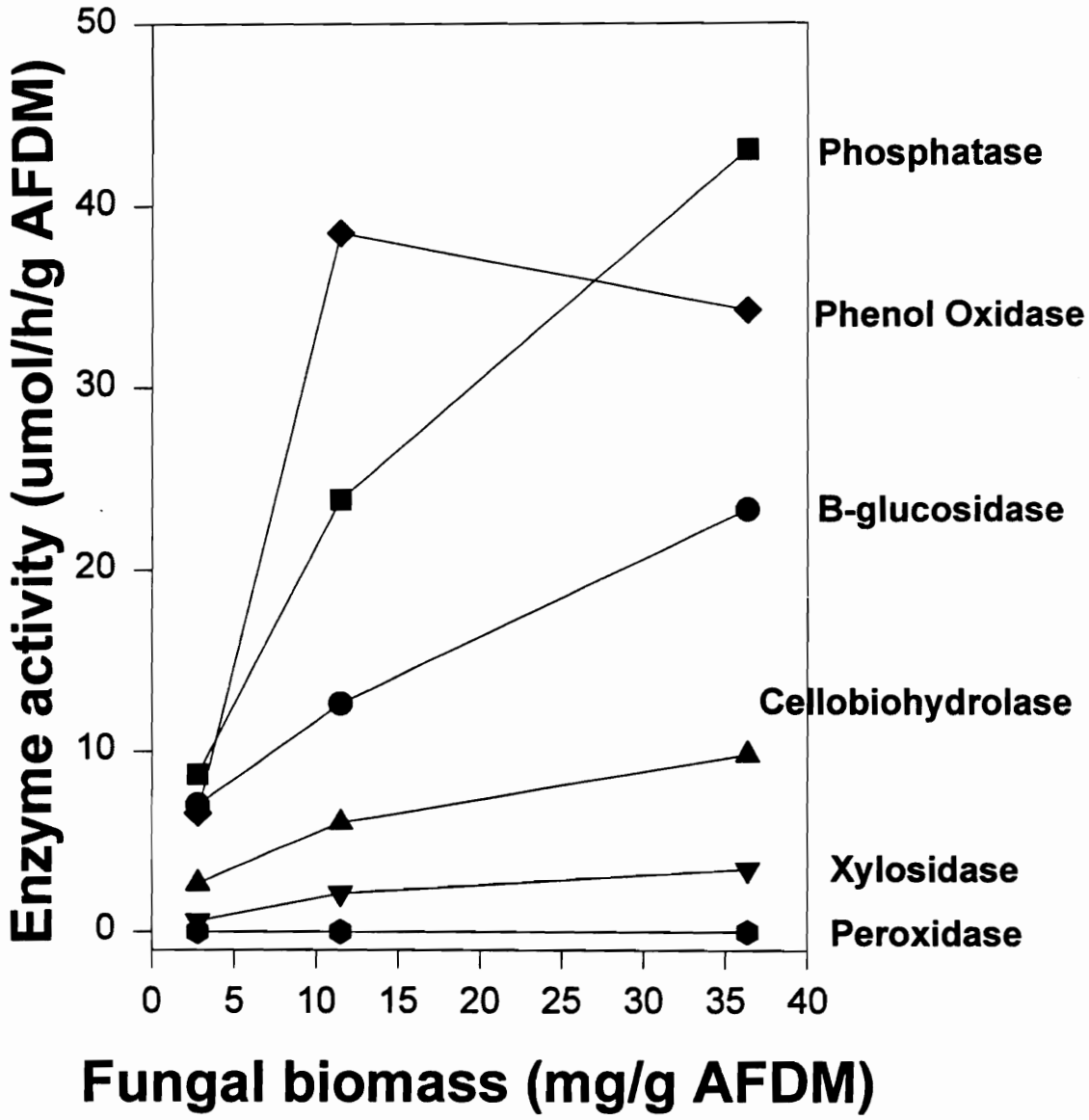

Fig. 4. Fungal biomass vs. enzyme activity for 6 extracellular enzymes in the leaf exclusion stream.

Chapter 5:

Growth of a shredding stonefly (*Tallaperla* sp.) feeding on wood colonized by microbial biofilm.

Abstract

Two 3-week laboratory feeding studies were conducted with stonefly nymphs (*Tallaperla* sp.) to see if they would grow when fed solely on wood and associated microbial biofilm. Pieces of oak wood were incubated for 1 month (study 1) and 2 months (study 2) in 4 streams at Coweeta Hydrologic Laboratory, Macon Co., North Carolina to allow microbial biofilms to develop. Ten randomly selected nymphs were weighed and placed in each chamber along with 6 pieces of "microbially conditioned" wood. There were 10 chambers containing conditioned wood, 2 chambers with conditioned oak leaves, 2 chambers with unincubated wood, and 1 chamber with no organic material for each of the 4 streams. *Tallaperla* grew equally well on wood incubated for 1 month or 2 months. There were no differences between *Tallaperla* growth on wood vs. leaves in either study. Nymphs lost weight when no organic material was provided. Fungal biomass was higher on wood than on leaves in both studies. Fungal biomass estimates on wood from feeding chambers at the end of each feeding study were not significantly different from biomass at the beginning indicating that *Tallaperla* were not food limited. Results from this study indicate that *Tallaperla* can survive and grow while feeding solely on the microbial biofilm colonizing wood. This study demonstrates that, in the absence of leaf litter, a common shredder can use wood biofilms as a food resource.

Introduction

In streams draining forested catchments, course particulate organic matter inputs from the riparian zone are made up of both leaf litter and woody debris. Studies of decomposition and subsequent feeding by invertebrates have focused on leaf litter as the primary food resource in shaded streams (e.g. Anderson and Sedell 1979). The biomass of fauna associated with wood is generally lower than that associated with leaf litter (Anderson 1982, Anderson et al. 1978), and wood is generally considered a poor food resource due to a high C:N ratio (Dudley and Anderson 1982). In fact, in a review on the importance of diets on aquatic insect growth, Anderson and Cummins (1979) ranked wood as one of the lowest quality food resources.

Wood contributes to stream ecosystems in a number of ways, but studies of wood in streams have focused primarily on its role as a structural component (Harmon et al. 1986). Fallen logs and branches define channel morphology as well as forming debris dams, which accumulate additional organic matter. Invertebrates may use wood directly or indirectly as a food source. Some filter feeders use wood as a net-spinning site (e.g., Benke et al 1985), while grazers may feed on surface biofilm, and gougers ingest decomposing wood itself. Hax and Golladay (1993) found positive correlation between macroinvertebrate density on wood and the degree of microbial biofilm development. In contrast, other studies have found that invertebrates colonize wood solely as habitat but not as a food resource (Dudley and Anderson 1982). Studies in lowland streams have

shown that wood snags enhance habitat complexity and therefore increase stream species diversity (Benke et al. 1985, O'Connor 1991).

Wood decomposes slowly and therefore provides a long lasting organic substrate for microbial colonization. This colonization results in a biofilm, which may contain varying proportions of bacteria, fungi, algae, detrital particles, and exoenzymes incorporated into a gelatinous matrix (Lock et al. 1984). Because most invertebrates cannot digest wood directly, it has been inferred that wood is less important as a food resource (Anderson and Sedell 1979), but studies at Coweeta have shown higher fungal biomass, carbon degrading potential, and microbial respiration on wood than on rhododendron leaves per unit surface area (Tank et al. in prep). This evidence suggests that wood may have been underestimated as a food resource in shaded streams.

Nymphs of the stonefly *Tallaperla* spp. are categorized as shredders (Merritt and Cummins 1984) and are found in high densities (up to 500/m^2) in headwater streams in the southern Appalachians (Wallace et al. 1970, Elwood and Cushman 1975). *Tallaperla* is found typically on decomposing leaf litter in autumn and winter (O'Hop et al. 1984), but in late spring and summer, when leaf litter is scarce in streams, I have often found these shredders on decomposing wood.

The objectives of this study were to determine if *Tallaperla* could grow on a diet of wood colonized by a microbial biofilm and to compare growth on wood to leaves. Wood and leaves were incubated in 4 different streams for 1 and 2 month periods and used in laboratory feeding trials to compare the effect of microbial biofilm development

among streams on invertebrate growth. Growth of *Tallaperla* nymphs that were fed solely on wood and its associated biofilm was determined and compared to growth of nymphs fed decomposing leaf litter.

Site Description

Wood and leaf substrates were incubated in 2 first-order study streams drain Catchments 53 and 55 and 2 second-order streams draining Catchments 7 and 14 at Coweeta Hydrologic Laboratory , Macon County, North Carolina, USA. Coweeta is a 2270-ha experimental forest of the U.S. Forest Service located in Nahantahala National Forest in the eastern part of the southern Appalachians. The forest canopy is dominated by tulip poplar (*Liriodendron tulipifera*), white oak (*Quercus alba*), red oak (*Quercus rubra*), and dogwood (*Cornus florida*). There is also a dense understory of rhododendron (*Rhododendron maxima*) which results in year-round shading and low rates of primary productivity (Webster et al. 1983).

All of the study streams have relatively low nutrient concentrations, similar water chemistry characteristics, circumneutral pH, and similar thermal regimes. Catchment 53 and C14 have been used as reference catchments for a number of ecosystem studies at Coweeta. Catchment 7 was clear-cut 19 years ago and is regrowing. All streams are groundwater fed and are therefore cool in the summer and relatively warm in the winter. Stream-bed characteristics are similar consisting of mixed cobble-pebble with sand-gravel, and some bedrock outcrops. Small woody debris (<5 cm) and leaf litter make up most of the standing crop of benthic organic matter. Surface area of small woody debris

makes up a considerable proportion of colonizable substrate in C53, C7, and C14, and at the time of this study was the only large substrate left for microbial colonization in C55 as leaves had been excluded from C55 for 3 years (Wallace et al. unpublished data).

Methods

I incubated white oak (*Quercus alba*) wood strips cut from veneer (2.5 cm x 7.5 cm x 1mm) and white oak leaf packs in streams draining the 4 watersheds for 1 month and 2. *Tallaperla* nymphs (0.5 - 1 cm long), conditioned wood and leaves, and filtered water from each stream were collected at the end of each incubation period. Ten chambers (15.5 x 4 cm, circular) containing 6 conditioned wood strips, 2 chambers with 11 g of conditioned leaves, and 1 chamber with only water were prepared for each of the 4 streams. Ten *Tallaperla* were placed in each chamber along with water from the respective stream. All chambers were placed in an environmental chamber with a constant temperature of 15 °C, aeration from airstones, and a 12-h light, 12-h dark day/night cycle. Nymphs were kept in the feeding chambers for 3 weeks and water was exchanged weekly with filtered fresh water from each respective stream. Chambers were checked daily to remove and weigh exuviae and dead *Tallaperla*.

For each of the 2 feeding trials, pre-feeding and post-feeding wet weights (nearest 0.1 mg) were taken for each group of 10 *Tallaperla* as well as for the substrate in each chamber using a standard method of blotting excess water from material before weighing. Wet weights were converted to dry weights using pre-determined conversion factors.

Relative growth rates (RGR)(mg/g/d)(Waldbauer 1968) and % growth per day were calculated for *Tallaperla* in each chamber using the formulas:

$$RGR = \frac{(\text{final weight} - \text{initial weight})/\text{average weight}}{\text{incubation time}}$$

$$\frac{\%\ \text{growth}}{\text{day}} = \frac{(\text{final weight} - \text{initial weight}) * 100/\text{initial weight}}{\text{incubation time}}$$

Fungal biomass samples were collected before and after both the feeding periods on leaf and wood substrates. Samples were randomly selected from 3 of 10 wood chambers and 1 of 2 leaf chambers from each stream represented. Additionally, organic material left in the 4 streams during the 3-week laboratory feeding studies was collected and analyzed for fungal biomass to compare to post-feeding fungal biomass. Values were reported as means ± standard errors (SE).

Fungal biomass was estimated as ergosterol content of biofilms colonizing sample substrates and was measured using methods outlined by Newell et al. (1988) and modified by R.L. Sinsabaugh (personal communication). Subsamples of known surface area (2.5 cm x 3 cm x 1 mm) were placed in 15-ml Falcon tubes with 5 ml of methanol and refluxed in a dry block heater for 2 h at 65 °C. Tubes were removed from the heater, cooled, saponified by adding 1 ml of 4% KOH in methanol, and refluxed for another 0.5 h at 65 °C. Samples were cooled, centrifuged, and the supernatants were decanted into clean 15-ml Falcon tubes. The pellets were resuspended in 2 ml of HPLC-grade methanol, centrifuged again, and this methanol was added to the tubes containing the extracts along with 1 ml of water. Each sample was extracted 2 times with 2 ml of

pentane. Pentane extracts were evaporated in a fume hood. The residue was redissolved with 1 ml of HPLC-grade methanol and filtered through a 0.45-μm syringe filter into clean 5-ml polypropylene tubes. Ergosterol was quantified using a reverse-phase HPLC system configured as follows: solvent = methanol; flow rate = 2 ml/min; column = Nova-Pak ODS C18 3.9 mm x 75 mm; absorbence detector = 282 nm with a range of 0.500; quantification = Waters integrator with attenuation at 256; ergosterol retention time = 1.6 min; replication = 5 per sample; standards = 5, 10, 25, and 50 μg/ml solution of ergosterol and methanol. To convert ergosterol content to fungal biomass, a conversion factor of 6 mg ergosterol/g fungal biomass was used (Newell et al. 1988).

Results

Relative growth rates (RGR) for the 2 feeding studies are expressed in dry weight using ratios of wet weight to dry weight determined from nymphs collected from the same streams and are reported as means ± standard errors (SE). Relative growth rates (RGR) and % growth per day for *Tallaperla* fed on 1-month wood and leaves (Fig. 1A, B) were compared to those fed on 2-month substrates (Fig. 2A, B). RGR for *Tallaperla* fed on 1 month conditioned wood was 6.28 ± 0.98 mg/g/d (Fig. 1A) and was not significantly different than *Tallaperla* RGR for 2-month wood (9.34 ± 1.80 mg/g/d)(Fig. 2A, paired t-test, p=0.1930). RGR for *Tallaperla* fed on 1 month leaves was 10.33 ± 4.80 mg/g/d and was not significantly different from *Tallaperla* RGR for 2-month leaves (mean 13.48 ± 6.59 mg/g/d)(Paired t-test, p=0.5370). There were no significant

differences in *Tallaperla* growth rates between wood and leaves for 1 or 2-month substrates (Paired t-tests, p= 0.4019, p= 0.4780 respectively) or among streams within each study (Mann-Whitney Rank Sum, p>0.05).

Percent growth per day ranged from 0.62 to 0.98 %/d for *Tallaperla* fed on 1-month substrates (Fig. 1B) and was not significantly different from *Tallaperla* fed on 2-month substrates (range 0.71 to 1.71 %/d) (Fig. 2B, Mann-Whitney Rank Sum, p>0.05). Estimates for % growth per day were not significantly different between substrates or among streams within each study nor between studies (1-month vs. 2-month)(Mann-Whitney Rank Sum, p>0.05 for all). Also, the number of exuviae collected from each treatment was not significantly correlated with *Tallaperla* growth. *Tallaperla* survival was high for all chambers (average survival >85%) including nymphs in control chambers containing only water.

Fungal biomass on wood and leaves was used as an assay for food quality. Fungal biomass on 2-month substrates (Fig. 3B) was significantly higher than fungal biomass on 1-month substrates (Fig. 3A) (paired t-tests, wood p= 0.0209, leaves p= 0.0002) The average temperature in the streams during the 1-month incubation was 18 °C, while 2-month substrates were incubated at an average stream temperature of 14 °C. Fungal biomass on substrates at the beginning of each feeding study was not significantly different from fungal biomass after the feeding study (Fig. 3A,B) (paired t-tests, wood p = 0.8487, leaves p= 0.5142). When final fungal biomass on feeding study substrates was compared to substrates that remained in the streams at Coweeta, there were no significant

differences in either the feeding study using 1-month or 2-month substrates (Mann-Whitney Rank Sum, p=0.311, p=0.717 respectively). Overall, fungal biomass on wood (mean = 0.29 ± 0.06 mg/cm^2) was higher than on leaves (mean = 0.08 ± 0.01 mg/cm^2) for both feeding studies (paired t-tests, p = 0.0034). Linear regressions of mean fungal biomass vs. RGR for each stream were not significant in either feeding study.

At the conclusion of both feeding studies, wood and leaves from each chamber were weighed. Wet weights of wood from 1-month substrates changed little overall: gains or losses were less than 5% (Fig. 4A.) Wood strips from 2-month substrates lost weight (9-13 % wet weight)(Fig. 4B). Leaves were less stable, broke apart, and lost weight in both studies. Wood accumulated a 1-2 mm thick biofilm made up of fungal hyphae and its associated mucilagenous matrix. Many small fecal pellets clung to wood strips in chambers despite gentle shaking before weighing. *Tallaperla* were observed feeding on wood surfaces but we detected no wood gouges or holes that would indicate ingestion of wood itself and presumed that only biofilm material was being ingested. There were no fecal pellets found in the control chambers containing *Tallaperla* and water only after the first 2 days of each study.

Discussion

Growth rate for Tallaperla

Relative growth rates (RGR) and estimates of % growth per day indicated that *Tallaperla* grew equally well on wood colonized in streams for 1 month and 2 months.

Variability in growth rates was higher on leaves than on wood. Nymphs were left in empty chambers for 12 hours to allow for gut clearance but variability in final weights may have been due to food remaining in *Tallaperla* guts (Waldbauer 1968). Since differences in growth rates among streams were minimal, further studies on *Tallaperla* growth between leaves and wood should increase chamber replication for each food type.

Food resource availability in streams varies as a result of seasonal changes in inputs, or stochastic events such as streambed scouring resulting from a storm. Omnivory may be beneficial to shredders during times of reduced resources. Results from this study indicated that *Tallaperla* grew as well on wood as on leaf material (Figs. 1 and 2). Roeding and Smock (1989) demonstrated that a shredder (the caddisfly *Pycnopsyche*) can switch from leaves to wood. Webster and Waide (1982) hypothesized that slower decomposing leaves like rhododendron may support shredder diet during spring and summer when faster decaying leaves have already decomposed. Biofilms on wood also can be considered an alternate food resource, and I have frequently found *Tallaperla* on wood when leaves are scarce. There also were no differences in *Tallaperla* growth among the 4 streams in each study, despite the fact that fungal biomass was significantly higher in stream A during both studies. Additionally, fungal biomass was higher on wood incubated for 2 months than 1 month. Differences in fungal biomass among streams and between 1 and 2-month incubations did not result in differences in *Tallaperla* growth. These results imply that microbial conditioning of wood had reached a level capable of

supporting nymphal growth after only 1 month and that there was no additional growth benefit from 2 months incubation. Nymphs were not food limited in either feeding study.

Substrate quality

Food quality is a primary determinant of shredder growth in streams (reviewed by Anderson and Cummins 1979, Anderson and Sedell 1979, Anderson and Cargill 1987). Results from this study indicated that there were no differences in food quality between wood and leaves because there were no differences in *Tallaperla* growth. Quality of leaf material for consumption has been judged by measures of conditioning through microbial colonization and subsequent softening of the leaf. For wood, softening through microbial colonization is a delayed process and can take years, but studies of wood biofilms have shown high microbial activity (Tank and Winterbourn 1995) and substantial accumulation of fungal biomass within 1 month of submersion in streams (Tank et al. in prep, this study). For this reason, macroinvertebrate feeding studies using wood must incorporate actual estimates of microbial biomass to judge wood biofilm quality rather than traditional indirect measures of conditioning like softening.

In this study, fungal biomass on wood was significantly higher than on leaves during both feeding studies (Fig. 3A, B). Fungal biomass and microbial respiration expressed per unit surface area has been consistently higher on wood compared to leaves in Coweeta streams (Tank et al. 1993, Tank et al. in prep). Higher microbial biomass on leaves has been shown to improve detrital quality and macroinvertebrate detritivore

growth (Anderson and Cummins 1979). In contrast, despite higher fungal biomass on 2-month incubated substrates than 1-month substrates, growth rates of *Tallaperla* were not significantly different. Higher fungal biomass did not result in faster *Tallaperla* growth whether microbially conditioned leaves or wood were used as a food resource. Fungal biomass after 1 month conditioning may have been adequate to maintain *Tallaperla*, implying that there was a surplus of food available after only 1 month incubation. On the other hand higher fungal biomass may not equate with higher food quality if fungal species are not palatable to the consumer. Additionally, it is unknown whether the mucilagenous matrix made of polysaccharides that is secreted by some fungi may selectively consumed and more easily digested. Further research on assimilation efficiencies of wood biofilms may help to clarify these scenarios.

Although leaves and wood are both considered detritus, there may be differences in the characteristics of the "substrate/microbe complex" formed once microbial colonization has occurred. The common operational definition of detritus implies that the microbes are conceptually inseparable from the organic substrate, because the relationship is so "intimate that independence is never observed in nature" (Anderson and Sedell 1979). In another study of *Tallaperla* growth, Findlay et al. (1986) found that fungal carbon associated with leaf litter could account for only 25-45% of the carbon respired by the stonefly. Microbial exoenzymes are responsible for modifying the carbon in the leaf litter and can make substrate carbon more digestible (Arsuffi and Suberkropp 1984) thereby making the microbes and organic substrate inseparable as the food

resource. Woody debris may be considered differently in this respect. Fungal carbon associated with wood may account for a great proportion of the consumption by invertebrates because they generally just graze the wood biofilm. In this study, weight changes in the wood substrate were quite small, ranging from a 4% loss to a 5% gain for 1-month substrates and an 8-15% loss for 2-month substrates (Fig.4B). Large quantities of fecal pellets accumulated every week confirming that material was being ingested. We must assume that the material ingested was not the wood itself but the well-developed fungal biofilm and its associated mucilagenous matrix. Assimilation efficiencies for woody material are low, and little evidence exists to support the direct use of woody tissue as food by aquatic insects (Anderson and Sedell 1979). Despite its low inherent quality as food, wood provides a stable, long term substrate for microbial biofilm development. Sloughing of decomposed, softened wood reveals fresh layers for colonization (Golladay and Sinsabaugh 1991). In essence, wood provided the carbon source for fungal growth, but fungal (and perhaps algal) carbon provided the source for *Tallaperla* growth.

In this study, nymphal growth from wood (and its biofilm) represented a much smaller percentage of the total wood available in a chamber than leaves. By using an average growth rate of 15 mg/g/d, an average nymphal dry weight as 0.015 g, average dry leaf weight per chamber of 1 g, and average dry wood weight of 5 g, one can back calculate to determine what percentage of the substrate was represented by the nymphal growth. Nymphal growth on wood represented about 0.09% of the wood, while growth

on leaves represented about 0.5% of the total weight. This implies that *Tallaperla* growth when fed on conditioned wood was primarily a result of biofilm consumption rather than wood material itself.

Substrate incubation time did not have an affect on *Tallaperla* growth despite changes in fungal biomass, and fungal biomass therefore did not correlate with growth. Fungal biomass was significantly higher on wood in the 2-month substrates than 1-month substrates. Fungal biomass for both feeding studies fell within ranges previously reported for wood in other streams (Golladay and Sinsabaugh 1991, Sinsabaugh et al. 1991) as well as streams at Coweeta (Tank et al. in prep). Fungal biomass before and after each feeding study was not significantly different implying that high fungal production was able to compensate for losses due to *Tallaperla* feeding, and therefore changes in biomass were not detected. *Tallaperla* were not ingesting wood and apparently fed exclusively on wood biofilm. *Tallaperla* growth may be more accurately correlated with fungi by examining fungal production rather than fungal biomass (Suberkropp 1995).

Feeding strategy by a shredder

The typical feeding strategy for *Tallaperla* is to completely skeletonize leaves, and the presence and distribution of leaf detritus determines *Tallaperla* abundance (Wallace et al. 1970). *Tallaperla* may be evolutionarily adapted to shred leaves (Merritt and Cummins 1984), but this study has shown that when leaves are absent, *Tallaperla* can graze wood biofilms and grow. Because microbial colonization of wood in aquatic

systems is limited to wood surfaces (Aumen et al. 1983, Anderson et al. 1984), we infer that *Tallaperla* are grazing the biofilms. Any weight loss in wood would be a result of fungal breakdown rather than direct invertebrate feeding.

It appears *Tallaperla* are able to "graze" the fungal biofilm in a way similar to that of a scraper grazing periphyton. In this study, grazing did not deplete fungal biomass suggesting that *Tallaperla* were not food limited. Preliminary examination of *Tallaperla* mouthparts using scanning electron micrographs (SEM) indicated that there is a possibility for omnivory in this genus and therefore an ability to switch food types when resources indicate a need (J. Harding and J.L. Tank, unpublished data). The *Tallaperla* labrum appeared to be a scoop-like structure covered with setae which may aid in scraping of a substrate. Setae on the glossa and paraglossa may be effective in gathering the biofilm material on wood. The mandibles were not typical of shredders (Merritt and Cummins 1984) and appeared "scoop-like" rather than "shredding" further implying that a scraping mode of ingestion is possible by *Tallaperla.* Further study of the relationship between mouthpart structure and feeding is necessary to clarify the possible role of omnivory in shredder growth.

Scrapers have typically been characterized as feeding on autochthonous epilithic layers (Cummins 1974, Merritt and Cummins 1984) but it may be appropriate to modify this functional feeding group (sensu Cummins 1974) to include scraping of wood biofilms. No convincing studies have been published that demonstrate that a shredder can complete its entire life cycle on an exclusive diet of decomposing leaves (Anderson and

Sedell 1979) and most studies of gut contents reveal variable diets. Since the life cycle of *Tallaperla* lasts 2 years, with emergence occurring between May and June (Wallace et al. 1970, Elwood and Cushman 1975), larvae endure at least one season of leaf shortage during late spring through autumn. Food switching in this case could be described as seasonal omnivory occurring within the same developmental stage induced through resource depression. Preliminary gut analyses on *Tallaperla* collected from 2 of the study streams showed fungal hyphae and pycnidia (fruiting vesicles) in the upper gut of *Tallaperla*, particularly during spring months when leaves were rare in the streams. Consumption of the microbial biofilm colonizing wood offers a high quality alternative to the consumption of the refractory carbon and nitrogen in wood itself (Anderson and Cargill 1987).

In summary, results from this study have shown that a common shredder, *Tallaperla*, can grow equally well on wood biofilms and leaf litter in laboratory feeding experiments. Colonization time of wood and leaves (1 month vs. 2 month) did not change patterns in *Tallaperla* growth. Fungal biomass was higher on wood than on leaves when expressed per unit surface area and fungal biomass was not depleted as a result of *Tallaperla* feeding. *Tallaperla* were not food limited in this study. *Tallaperla* are known to be closely associated with and are efficient shredders of leaf litter (Wallace et al. 1970) but results from this study imply that in the absence of leaf litter, wood biofilms may serve as an important food resource for stream macroinvertebrates. Energy, stored as

carbon, in wood can be transferred between trophic levels in a stream food web and the microbes colonizing wood serve as the vector for that transfer.

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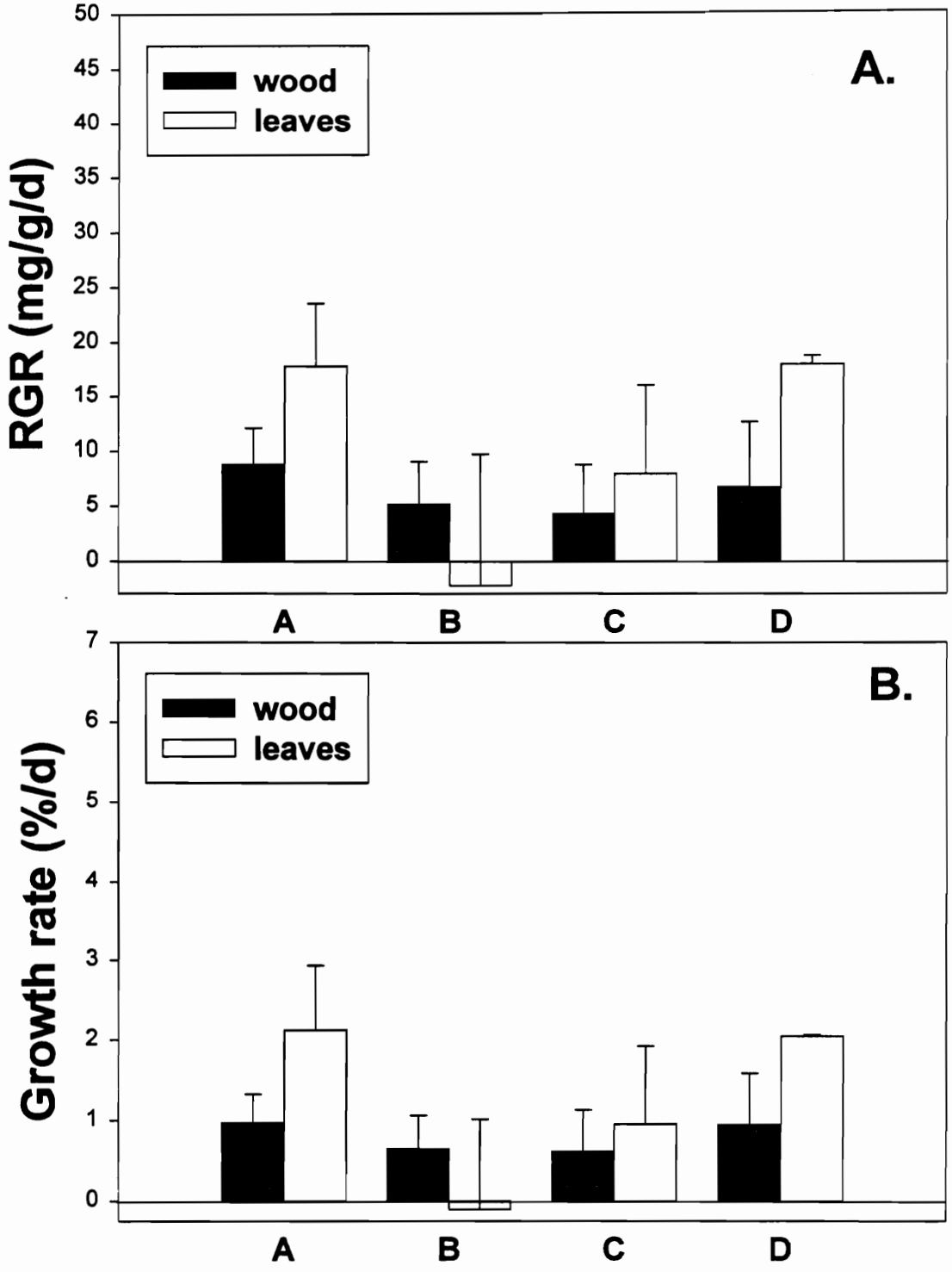

Fig. 1: Tallaperla growth on 1-month substrates. A. Relative Growth Rates (mg/g/d) and B. % Growth rate (%/d). A through D refer to substrates taken from 4 streams. (N=48)

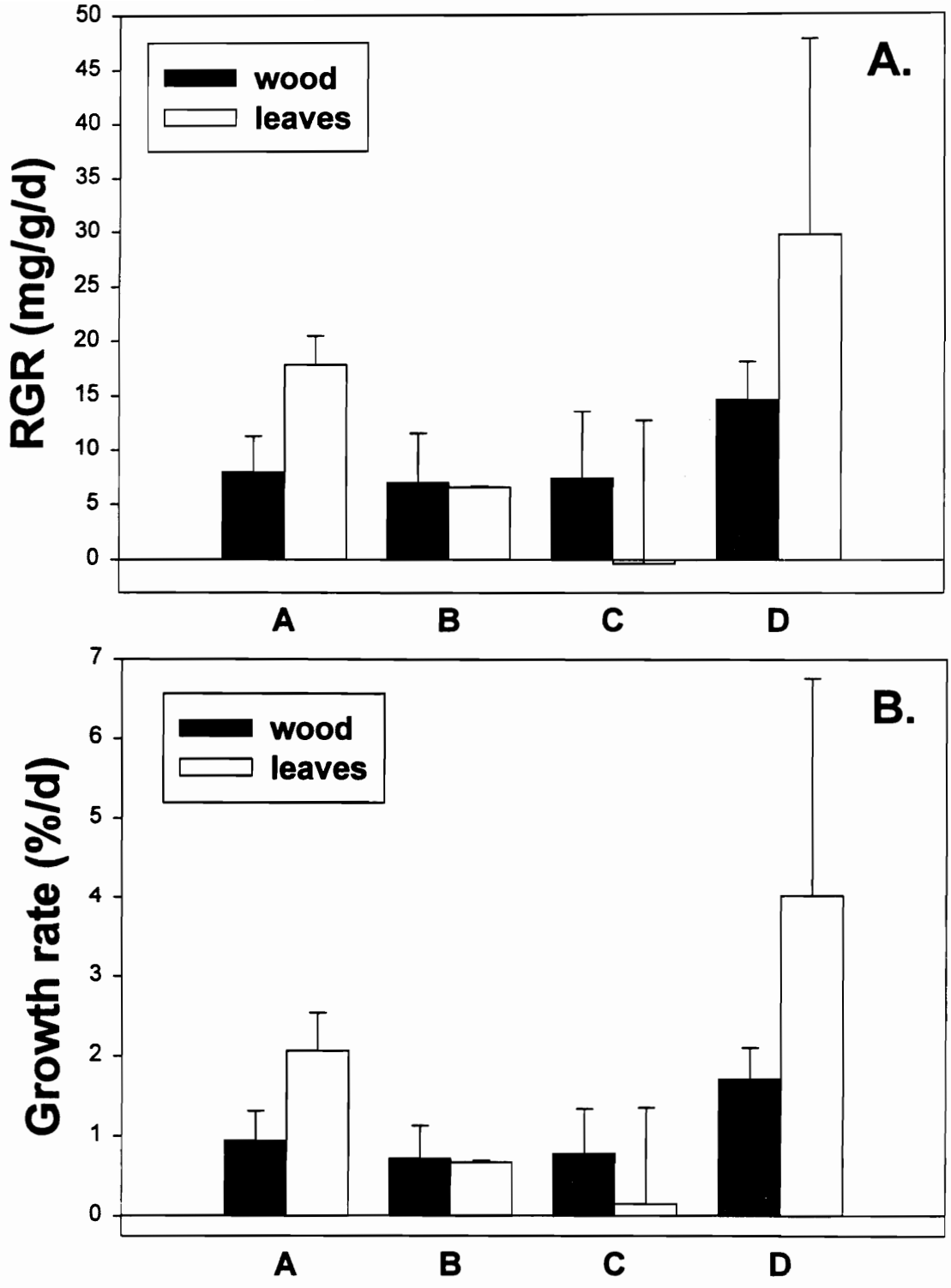

Fig. 2: Tallaperla growth on 2-month substrates. A. Relative Growth Rates (mg/g/d) and B. % Growth rate (%/d). A through D refer to substrates taken from 4 streams. (N=48)

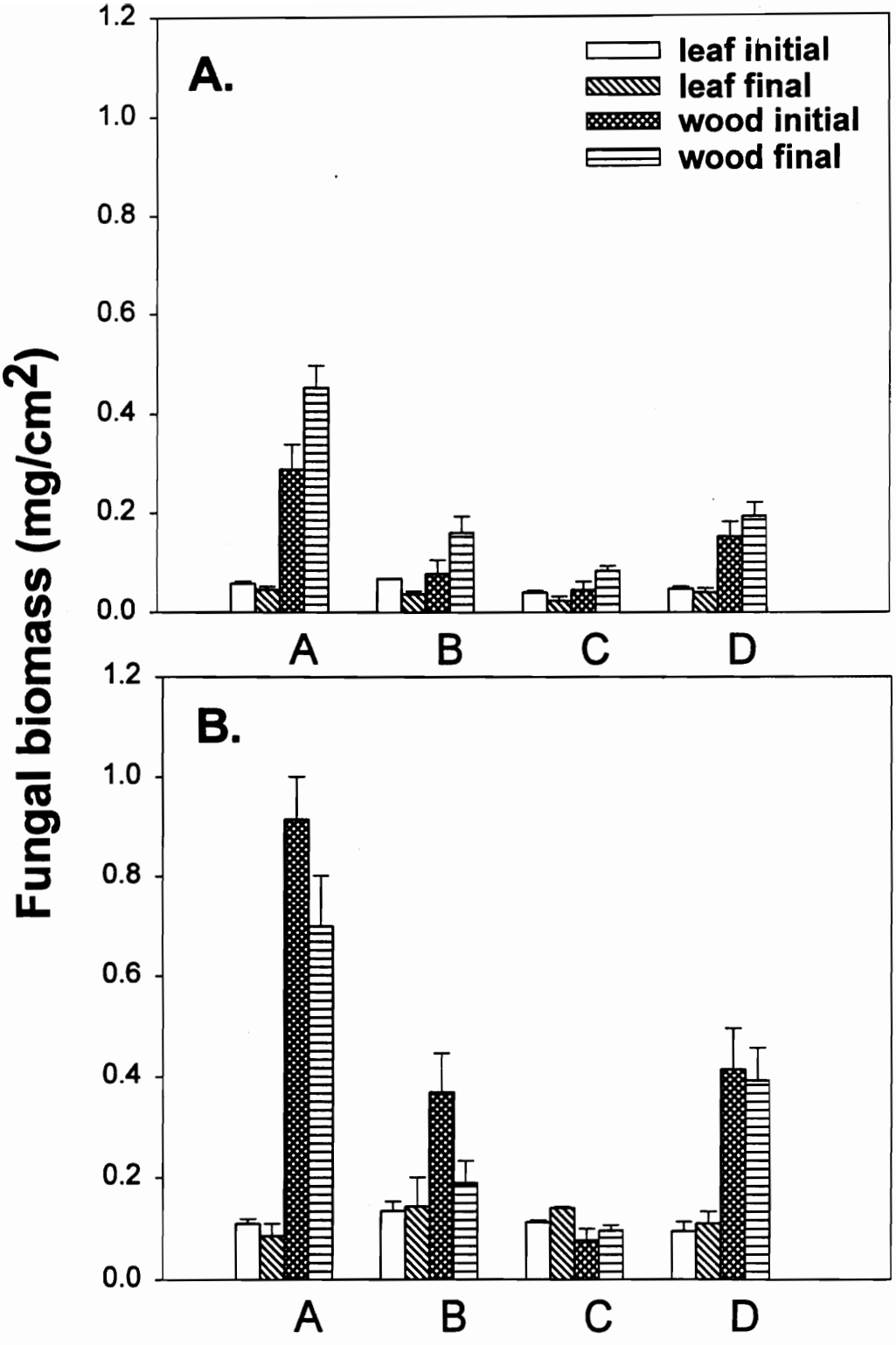

Fig. 3. Fungal biomass for A. 1-month substrates and B. 2-month substrates. A through D refer to substrates taken from 4 streams. (N=80)

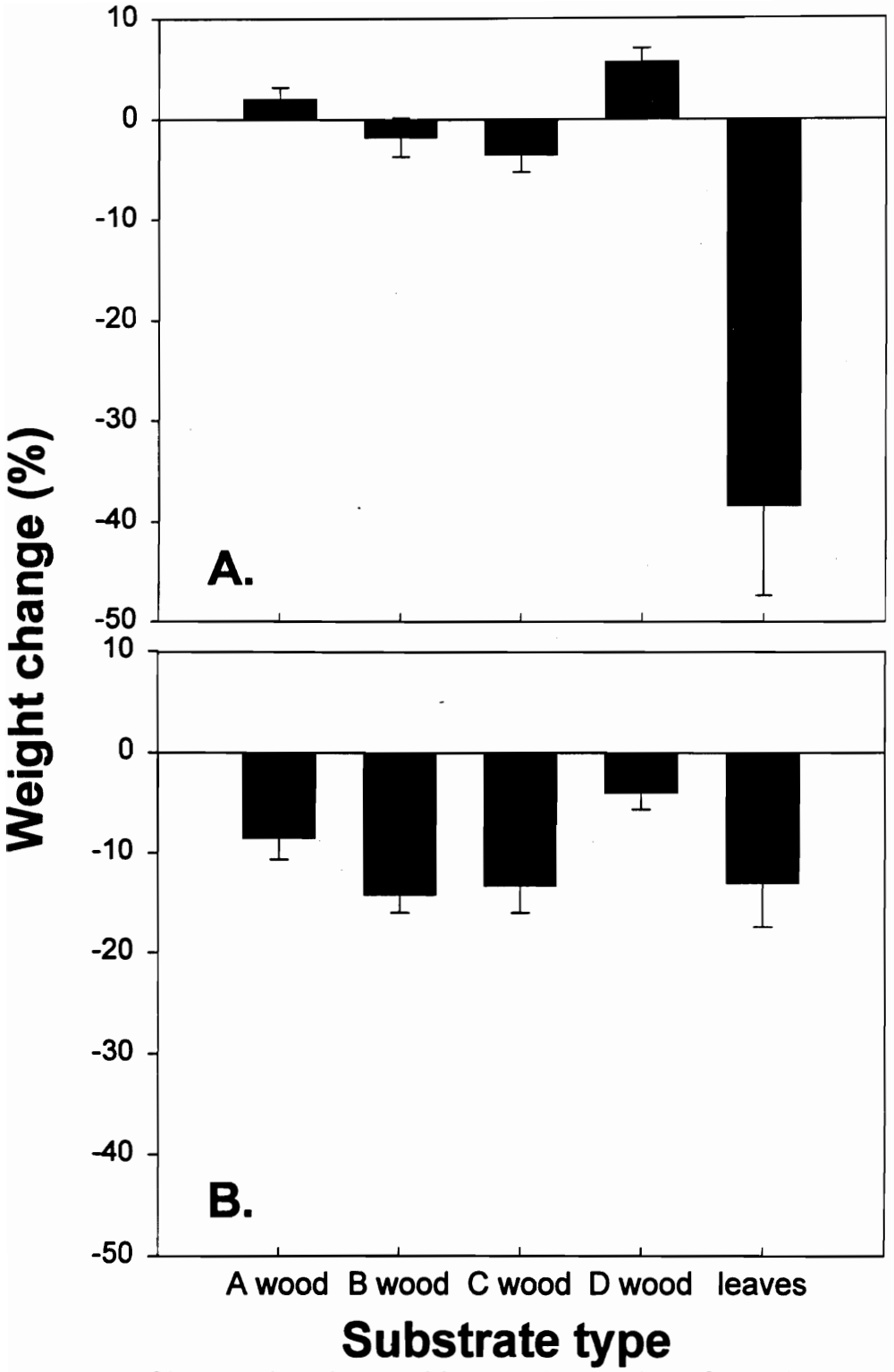

Fig. 4. Changes in substrate biomass (wet weight) for A. 1-month substrates and B. 2-month substrates. A through D refer to substrates taken from 4 streams. (N=48)

General Summary

Studies of wood biofilm structure and function were carried out in a South Island, New Zealand mountain stream to compare microbial development among wood strips, natural twigs from the forest floor, beech leaves, and stones (chapter 1). Similar microbial assemblages developed on wood strips, twigs, and beech leaves, but fungi did not grow on stones where diatoms were the predominant colonizer. Wood breakdown and colonization of buried and surface wood was examined and microbial colonists were mainly fungal hyphae, but actinomycetes and unicellular bacteria were also found. Microbial activity on wood estimated through incorporation of ^{14}C glucose indicated that biofilms exist on both surface wood and wood buried in the stream bed. Surface biofilms were more active than buried wood biofilms. Chironomid larvae, oligochaetes, and harpacticoid copepods were the most abundant animal colonizers. Wood surfaces appeared to be important sites of organic matter uptake and transfer in this forest stream. Wood may have a potentially significant role, because of its inherent stability, in supporting stream community metabolism, especially in New Zealand streams where retention of detrital material is poor.

I also compared the wood biofilms in 2 acid brown water (pH = 3.7 and 4.5) and 2 circumneutral streams (pH = 6.3 and 6.8) in New Zealand to determine the effect of acidity on wood biofilms (chapter 2). Fungal hyphae and actinomycete colonies were dominant colonizers observed in all 4 streams. There was no relationship between ^{14}C

glucose uptake and pH. Buried biofilms were not significantly different at 3-9 cm depth vs. 19-25 cm depth indicating a relatively uncompacted stream bed with adequate subsurface flow providing oxygen and nutrients to buried biofilms. Although chironomid larvae and harpacticoid copepods were the most abundant invertebrate colonizers at all sites, different chironomid species dominated at acid vs. circumneutral sites. Results from this study do not necessarily support the hypothesis that microbial activity on organic substrates is lower in acidic streams.

Wood biofilms were also studied in headwater streams at Coweeta Hydrologic Lab, Macon Co., North Carolina (chapters 3 and 4). These heavily shaded streams depend on organic matter inputs (leaves and wood) from the riparian zone to fuel stream ecosystem processes, and primary production is very low. Exclusion of leaf litter from one headwater mountain stream resulted in the enhancement of microbial activity on wood. Fungal biomass was 7 times as high and extracellular enzyme activity on wood was also higher in the stream where leaves were excluded. Measurement of relative activities of selected extracellular enzymes comparing carbon and nutrient acquisition suggested nutrient limitation in the reference stream. Results implied that nutrient limitation may have been responsible for low microbial respiration, fungal biomass, and extracellular enzyme activity in the reference stream. In an experiment using nutrient releasing substrates, the reference stream appears to be limited by both nitrogen and phosphorus, and estimates of nutrient uptake lengths concur that the exclusion stream had

longer uptake lengths for both phosphorus and nitrogen (as NH_4)(Webster et al. unpublished data).

Higher fungal biomass, microbial respiration, and extracellular enzyme activity may be a result of additional nutrient availability from groundwater inputs that are less rapidly immobilized due to the presence of fewer microbially colonized substrates (i.e. no leaves in the stream). In the reference stream with leaves, nutrients were rapidly immobilized due to increased organic substrate surface area. The presence of leaves in forested headwater streams mediates microbial activity on other organic substrates (e.g., wood) through the immobilization of a limited supply of nutrients from overlying streamwater. Results from my studies imply that heterotrophs colonizing organic substrates may be competing for necessary nutrients that are in short supply. Forested headwater streams at Coweeta have detritus based food webs, but results from this study imply that nutrients may be a governing detrital dynamics in this ecosystem.

Results from similar experiments using nutrient releasing substrates and wood in 5 New Zealand streams indicated that only 1 stream was nutrient limited in terms of wood biofilm activity. Yet the New Zealand streams were low-nutrient streams similar to Coweeta streams. In the New Zealand studies, ^{14}C-glucose uptake was used as the indicator of microbial activity, while microbial respiration was used in the Coweeta studies. It may be that glucose was not readily taken up by the particular fungi colonizing wood as is indicative of some "soft rot" fungi and therefore the effects of nutrient supplementation could not be detected. In contrast, Zygomycete fungi, that take up

glucose readily, may have been early colonizers, but then were displaced by other types of fungi by the time the 1-month study had concluded. The similarity of fungal communities in New Zealand compared to Coweeta streams is unknown but they undoubtedly different. Future studies on the culturing and identification of at least the major colonizers of wood could reveal much about the role of wood biofilms in nutrient turnover.

In Coweeta streams, the opportunity to study wood biofilms in the presence and absence of leaves has indicated that availability of nutrients for the organic matter in streams may dictate activity of biofilms colonizing the organic matter and rates of decomposition as a result of the microbes. I do not know if wood colonizers in the presence of leaves were the same microbes present when leaves were also in the stream. Further research into the seasonal (autumn vs. summer) assemblages of microbial decomposers is needed. Since wood is present year round for colonization, it is logical that wood could be used as the experimental substrate.

Two 3-week laboratory feeding studies were conducted with *Tallaperla* sp., a common stream shredder, to see if nymphs would grow when fed solely on wood and its associated microbial biofilm. Oak veneers and leaves were incubated for 1 and 2 months in 4 streams at Coweeta. *Tallaperla* grew equally well on wood either incubated for 1 month or 2 months. There were no significant differences between *Tallaperla* growth rates on wood and leaves. Fungal biomass on wood from feeding chambers at the end of each feeding study were not significantly different from beginning biomass indicating

that fungal production was keeping up with *Tallaperla* grazing, and that *Tallaperla* were not food limited in this study. Results indicate that *Tallaperla* can survive and grow while feeding solely on the microbial biofilm colonizing wood. In the absence of leaf litter, a common macroinvertebrate shredder can use wood biofilms as a food resource. Energy stored as carbon in wood can be transferred between trophic levels in a stream food web and the microbes colonizing wood serve as the vector for that transfer.

From October through May, both wood and leaves are present, in different proportions, in forest streams. Streams with low nutrient concentrations, like Coweeta, offer an opportunity to explore the possible competition for nutrients between wood and leaf biofilms occurring when microbial colonizers on wood and leaves are both in need of nitrogen and phosphorus in order to carry out decomposition. Results from my studies have indicated that nutrient availability may be limiting microbial activity. Further study of the link between heterotrophic biofilms and dissolved nutrients is needed in order to determine the factors controlling carbon cycling in forest stream ecosystems.

After leaves have decomposed by late spring, wood biofilms may become more important as a food resource for shredders. Wood may be viewed as an important bridge between pulses of high quality leaf litter. Wood biofilms in combination with omnivory by traditional leaf shredding invertebrates may be critical in stabilizing stream ecosystems by providing a temporally constant resource base. In summary, my studies have indicated that bottom up nutrient effects, via microbial biofilms, may play a regulatory role in detrital food chains, and ultimately carbon cycling, in streams.

Vita

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23 July 1996

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Personal Data: Date of Birth: 5 July 1966
Place of Birth: Ann Arbor, Michigan

Education:

1984-85	Northwestern Michigan College
1985-86	University of Michigan
1987-88	Michigan State University, B.S. in Zoology
1989-1992	Virginia Polytechnic Inst. and State University, M.S. Thesis topic: Microbial respiration on decaying leaves and sticks along an elevational gradient of a Southern Appalachian stream. Major Advisor: Dr. J.R. Webster.
1993	University of Canterbury, Christchurch, New Zealand. Rotary Foundation Graduate Fellowship for research study abroad. Topic: Biofilm development and invertebrate colonization of wood in forested mountain streams. Major Advisor: Dr. M.J. Winterbourn.
1992-present topic:	Virginia Polytechnic Inst. and State University, Ph.D. Dissertation The role of the biofilm colonizing small woody debris in forested stream ecosystems. Major Advisor: Dr. J.R. Webster.

Professional Experience:

1985-86	Research Technician, Behavioral Training in Primates, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI.
1988	Research Technician, Dept. of Physiology, Michigan State University, Lansing, MI.

1988	Internship, Surface Water Quality, Dept. of Natural Resources, State of Michigan, Lansing, MI.
1989	Research Technician, Stream Ecology, Dept. of Zoology, Michigan State University, Lansing, MI.
1989-1994	Teaching Assistant, General Biology, Principles of Biology, Freshwater Ecology, Field & Lab Ecology, VPI & SU, Blacksburg, VA.
1990-present	Research Assistant, VPI & SU, Blacksburg, VA.

Memberships in Professional Associations:

Societas Internationalis Limnologiae (SIL)
Sigma Xi
American Institute of Biological Sciences
Ecological Society of America
North American Benthological Society

Publications:

Tank, J.L. and M.J. Winterbourn. 1996. Heterotrophic activity and invertebrate colonization of wood in a New Zealand forest stream. New Zealand Journal of Marine and Freshwater Research 31:(in press).

Tank, J.L. and M.J. Winterbourn. 1995. Biofilm development and invertebrate colonization of wood in four New Zealand streams of contrasting pH. Freshwater Biology 34:303-315.

Webster, J.R., A.P. Covich, J.L. Tank, and T. V. Crockett. 1994. Retention of course particles in streams in the southern Appalachian Mountains. Journal of the North American Benthological Society 13(2):140-150.

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

Tank, J.L. and J.C. Musson. 1993. An inexpensive chamber apparatus for multiple measurements of dissolved oxygen uptake or release. Journal of the North American Benthological Society 12:406-409.

Book Reviews:

J.L. Tank and J.R. Webster. 1995. Book review of From the Forest to the Sea: the ecology of wood in streams, rivers, estuaries, and oceans. Chris Maser and James R. Sedell. Journal of the North American Benthological Society 14(1):213-215.

Webster, J.R. and J.L. Tank. 1991. Book review of The biology of particles in aquatic systems. R.S. Wotten (editor). Journal of the North American Benthological Society 10(3):339.

Abstracts and Presentations:

Tank, J.L., J.R. Webster, R.L. Sinsabaugh, and E.F. Benfield. Effect of leaf litter exclusion on microbial enzyme activity associated with wood biofilms in streams. Abstract accepted, to be presented at the Ecological Society of America Meeting, Providence, Rhode Island, August 1996.

Tank, J.L., J.R. Webster, and E.F. Benfield. Does the exclusion of leaf litter affect microbial biofilm development on wood in streams? Abstract accepted, presented at the North American Benthological Society meeting, Kalispell, Montana 1996. Bulletin of the North American Benthological Society 13:146.

Ramsey, A.E., J.L. Tank, and E.F. Benfield. Does a shredding stonefly (Tallaperla sp.) grow when feeding on wood colonized by a microbial biofilm? Abstract accepted, presented at the North American Benthological Society meeting, Kalispell, Montana 1996. Bulletin of the North American Benthological Society 13:262.

Tank, J.L. and M.J Winterbourn. Biofilm development and invertebrate colonization of wood in four New Zealand stream of contrasting pH. Presented at the North American Benthological Society meeting, Orlando, Florida, May 1994. Bulletin of the North American Benthological Society 11:213.

Tank, J.L. and M.J. Winterbourn. Heterotrophic activity and invertebrate colonization of wood in a New Zealand forest stream. Presented at the Ecological Society of America meeting in Knoxville, Tennessee, August 1994.

Webster, J.R, E.F. Benfield, T.P Ehrman, J.J. Hutchens, J.L. Meyer, M.A. Schaeffer, J.L. Tank, P.A. Turner, and J.B. Wallace. Organic matter processes along a 1st- to 4th-order stream gradient in the southern Appalachian mountains. Bulletin of the North American Benthological Society 10:106. Presented at the North American Benthological Society meeting, Calgary, Canada, May 1993.

Tank, J.L., J.R. Webster, and E.F. Benfield. Microbial respiration on decaying leaves and sticks along an elevational gradient of a southern Appalachian stream. Bulletin of the North American Benthological Society 9:267. Presented at the North American Benthological Society meeting, Louisville, Kentucky, May 1992. Received the Wildco Award for best student paper in basic research.

Tank, J.L. and J.C. Musson. A modified field apparatus for measuring benthic respiration in streams. Bulletin of the North American Benthological Society 8:136. Presented at the North American Benthological Society meeting, New Mexico, May 1991. Awarded an honorable mention for the Hydrolab Award for best poster presentation.

Webster, J.R., J.L. Tank, and T.V. Crockett. Retention of large particulate organic matter in streams at Coweeta Hydrologic Laboratory. Bulletin of the North American

Benthological Society 8:75. Presented at the North American Benthological Society meeting, New Mexico, May 1991.

Tank, J.L. The effect of elevational change on benthic microbial respiration rates in Ball Creek, Macon Co., NC. Presented at the All-Coweeta LTER meeting. June 1991.

Grant Proposals/Fellowships Funded:

Tank, J.L. and J.R. Webster. Microbial activity on wood in streams: exploring abiotic and biotic factors affecting the structure and function of wood biofilms. $10,948.00 (Funded December 1994 by NSF Doctoral Dissertation Research Program)

Tank, J.L. GSA Travel Fund for travel to NABS 1996, Kalispell, MT. Funded for $225.00 and matched by department for $100.00.

Tank, J.L. The role of the biofilm colonizing small woody debris in a forested stream ecosystem. Sigma Xi Grants-in-Aid of Research for $590.30. (Funded July 1994 and matched $500 by Biology Dept.)

Tank, J.L. Awarded a Cunningham Fellowship for August 1992- May 1996 for study at Virginia Polytechnic Institute and State University in the Ph.D. program in Ecology. Advisor: Dr. Jack Webster

Tank, J.L. Awarded Rotary Foundation Fellowship for February 1993- January 1994 to study at the University of Canterbury, Christchurch, New Zealand. Advisor: Dr. Mike Winterbourn

Tank, J.L. Effects of elevational change on benthic microbial respiration rates in streams. Funded by Sigma Xi for $400.00 May 1988. Matched by Department of Biology VPI & SU for $400.00.

Tank, J.L. GSA Travel Fund for travel to NABS 1991, Santa Fe, NM. Funded for $300.00 and matched by department for $100.00.

Professional Activities:

Selected as the University Graduate Student Representative to the Virginia Tech Commission on Graduate Studies and Policies 1994-1996.

Selected as the University Graduate Student Representative to the Graduate Appeals Committee 1994-1996.

Elected as an "at-large" member to the biology Graduate Student Advisory Committee 1995-1996.

Graduate student representative on the Cooperative Council for Women's Concerns SP 1991-Fall 1992.

Nominated by the Department of Biology for the Graduate Student Service Award, February 1992.

Selected to participate in the national teleconference "What is Graduate School?" in Washington DC , 3 Oct. 91, sponsored in part by NSF and GEM.
Elected as the GSA representative to the University Commission on Research, 1991-93
Elected Representative to the Biology Graduate Student Advisory Committee, 1990-91, 1991-92.
Graduate Student Assembly Delegate, 1989-90, 1990-91, 1991-92, Fall 1992.
Assisted Program Committee at NABS meeting, May 1990, hosted at VPI & SU.
Graduate Student Member of the Graduate Recruitment Committee, Fall 1990.

References:

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