

LOVE YOUR BIOFILM:IMPLICATIONS FOR RESTORATION OF STREAM ECOSYSTEMS

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ABSTRACT

A stream biofilm, or slime, consists of a complex mix of microorganisms including bacteria, algae, protozoa and fungi embedded within, or associated with, a polymer matrix. The role of biofilms in stream ecology has received little attention at the microbial level and the dynamics of the biofilm populations and interactions with larger organisms is only patchily described. Recently it has become clear that stream biofilms form a critical component in stream food webs. Components of the biofilm recycle organic matter; fix carbon, nitrogen and energy, and transform and cycle nutrients such as phosphates, and sulphur. A range of micro- and macrobenthic invertebrates feed on the biofilm material and derives significant nutrition from the microbial biomass. The nature of the biofilm and the relative composition in terms of microbial species may potentially affect the quality of a biofilm as a food source for grazing organisms and may in turn affect the diversity, abundance and distribution of macrobenthic invertebrates. Our work suggests that bacterial populations in biofilms vary between sites and between seasons and that nutritional quality of a biofilm varies considerably between sites within a stream.

KEYWORDS

Stream restoration, microbiology, biofilm, food web

1 INTRODUCTION

It is widely accepted that bacteria and other microorganisms play an important role in streams by recycling organic nutrients through degradation of plant and animal debris. Microorganisms more recently have also become increasingly recognized as an important food resource in streams, changing current perspectives on the way we view the energy flows within stream food webs. Where once it was believed that invertebrate species in streams consumed plant debris, we now know that many gain their nutrients from the highly nutritious microbial biofilms found on the aquatic surfaces. Such evolving knowledge has profound implications for management of stream ecosystems.

Much of the practical effort in stream restoration has focused on improving riparian vegetation, managing flows and assessing stream health by observation of macrobenthic invertebrate populations and diversity. Modifying such factors can have a dramatic effect on the stream ecosystem, for example increased shading affects the carbon flux of a stream. To date, however, neither the causes nor the specific implications of these often-dramatic effects on streams ecology and long-term sustainability are clear.

Microbial biofilms on stream surfaces are a critical trophic component of healthy stream ecosystems. Microbial biofilms are composed of microscopic heterotrophic, photosynthetic and chemotrophic bacteria, fungi, diatoms and algae in a complex polymer linked assemblage that coats most surfaces in water. Macroscopically biofilms appear as surface slimes on bed material, organic debris and aquatic plants. Protozoa, heterotrophic algae, macrobenthic invertebrates and some macro organisms intensively graze these biofilms. A very recent hypothesis (Sheldon et al 1997) is that the specific microbial composition of the biofilm will modify the nutritional quality of the material for grazing species. For example dominance of heterotrophic bacteria and nitrogen fixing cyanobacteria will provide higher levels of protein and carbohydrates, both as cell mass and in extracellular polymer, (low C:N ratio) than will a biofilm dominated by photosynthetic algae where polycaccharide (including cellulose) levels are high but protein relatively low (high C:N ratio). In this situation extracellular polymer is made up of polysaccharides, protein and nucleic acids, and is the material secreted by bacteria to enhance adherence to surfaces and provide a level of cellular shielding from the surrounding environment. The mass of extracellular polymer in a biofilm may be 10 times that of the bacterial cell mass (Hall and Meyer 1998). According to ecological principles stream biofilm composition will be modified by the physicochemical characteristics of the environment, predation by grazers and presence or absence of specific biofilm species.

Biofilm composition then, along with habitat factors, will influence the population level and composition of grazing organisms and so on through the food chain.

Key international research and our own studies supporting these concepts are described below

2 INTERNATIONAL STREAM BIOFILM FOOD WEB RESEARCH

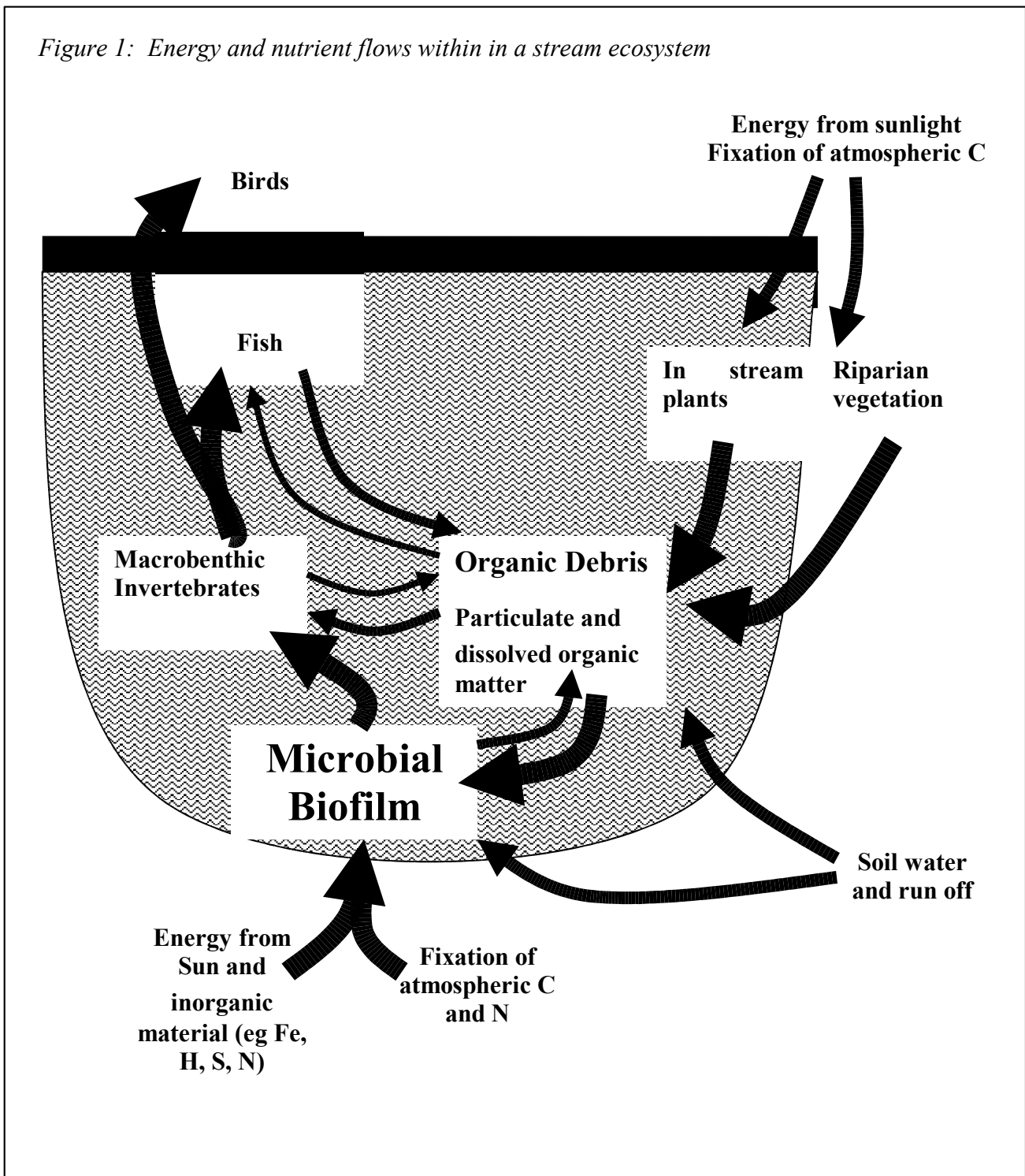
Stream biofilm research focusing on the role of bacterial components and the extracellular polymers they produce has begun to appear in recent years. Some key studies are identified and summarized here.

The assumption has long been made that many macrobenthic invertebrates largely feed on vegetation debris. A study in by Hall and Meyer (1998) in first order streams in North Carolina, USA, specifically investigated this assumption. Two comparable forest streams were selected and leaf litter excluded from a 170m length of one of the streams by netting and fences. ^{13}C labeled sodium acetate was added to the streams to trace the flow of carbon with and without detritus. Over the 18 months of the study, litter exclusion was found to be extremely effective, and labeled carbon was effectively incorporated into bacterial biomass in both streams. The carbon label was transferred to invertebrates with levels differing between broad feeding styles, scrapers, such as snails, and filterers showed high ^{13}C level while gatherers (e.g. choronimids) were moderately to highly labeled, and shredders had a relatively low ^{13}C content. The researchers suggested that much of the bacterial carbon used was probably exopolymers rather than bacterial cells. This work demonstrates that the microbial biofilm in a stream environment is an important food resource to a large portion of the invertebrate community. Quinn et al (2000) investigated the degradation of leaves of New Zealand native and introduced plants in streams, and concluded that leaves had a very high C:N ratio (>10:1) and required microbial "conditioning" (i.e. microbial colonization) to add nitrogen and as such improve nutritional quality. The study concluded that microbial activity, in both conditioning and decomposition, played an important role in leaf mass loss and carbon conversion in streams. Macrobenthic invertebrate communities in New Zealand streams have relatively low richness of shredders and higher browsing population (Thompson and Townsend 2000) suggesting that microbial activity and the biofilm food resources are of great importance to the ecology of our streams.

The nature of the biofilm, in terms of microbial composition, might logically be expected to affect the nutritional quality of the food resource. Evidence of the importance of this factor is shown by the work of Sheldon *et al* (1997) that investigated the extinction of native aquatic snails within the certain parts of the Murray River system in Australia. Sheldon developed the hypothesis that the river flow changes, resulting from regulation by dams and weirs, had led to changes in the nature of the food resources available to the snails. The authors argued that this food restriction resulted in population shift and extinction of some species. In this case the food resources in question were microbial biofilms present on the various surfaces in the river. The resulting investigation compared the composition and nutritional value (C:N ratio) of the biofilms, and determined the diet of the snails. The study suggested that stabilizing the water flow had two major possible effects on the stream biofilms. Firstly reduced flow variation would allow biofilms to reach maturity and remain in a mature algal dominated form rather than the early successional bacterially dominated form. The second major effect was an increased clarity of the water, which would enhance algal growth at the expense of bacteria again enhancing the algal component of the biofilm. C:N ratios and microbial composition of Murray River biofilm were high in areas where snails were extinct (C:N= 10:1, biofilm dominated by algae) but were comparatively lower where snails were still found (C:N = 4:1, biofilm dominated by bacteria) suggesting that the food quality, and as such biofilm composition, is a critical factor in macrobenthic invertebrate distribution.

The critical underpinning role of biofilms in stream ecology may be summarized as shown in Figure 1.

Figure 1: Energy and nutrient flows within in a stream ecosystem



3 STREAM BIOFILM RESEARCH: AUCKLAND

3.1 BIOFILM STUDY

We are currently involved in research to define the composition and key components of bacterial biofilms in relation to biological and physico-chemical components of healthy streams. The long term objective of this work is to investigate the restoration of ecological resilience in modified stream systems through biofilm manipulation. A component of the work investigating biofilms in unimpacted streams is described here.

3.1.1 METHODOLOGY

Four field-monitoring sites in stony bottom streams in native forest catchments within the Auckland region were established. Three sites in the Waitakere Ranges; Cascade Stream, Marawhara Stream, and Wekatahi Creek, and a further site; Mangatawhiri River, in the Hunua Ranges. These sites are part of the Auckland Regional Council long-term ecological monitoring network.

Biofilm samples were collected in one of two ways. In the first approach biofilms were collected from plastic slides mounted on concrete blocks ~5-10 cm under the water surface that had been in place for 4-5 weeks. Biofilms collected by this method provided an excellent representation of biofilm composition but yielded low biofilm mass and the material collected from these samples was predominantly used for molecular analysis of bacterial composition and microscopic observation of biofilm structure. Biofilms were harvested from the slides and DNA extracted from 0.1 g (wt) of biofilm using a Phosphate, SDS, Chloroform- Bead Beater method Miller *et al* 1999). Microbial community composition was assessed by DNA fingerprinting and sequencing of a 16srRNA gene clone library essentially as described by Amann *et al* (1995). Bacterial 16S rRNA genes were PCR amplified from each DNA sample using domain-specific primers. 16S rRNA gene libraries were generated and Restriction Fragment Length Polymorphism (RFLP) analysis used to reveal the diversity of 16S rRNA genes in each library. Each distinctive RFLP profile was designated as an operational taxonomic unit (OTU). For the second biofilm collection method rock surfaces were swabbed with sterile “specie sponges™” (NASCO). Relatively large amounts of biofilm can be collected by this method and samples were used for bacterial composition analysis and nutritional quality.

Nutritional quality of the biofilm was established through assessment of the C:N ratio and specifically based on carbohydrate (C) (meth ref) and protein analysis (N)(Mth ref).

3.1.2 FINDINGS

A comparison of biofilm sampling methodology was carried out to determine the effect of slide, and sponge techniques on sample representativeness. Biofilm collection by scrubbing surfaces with an electric toothbrush was included in the comparison. The 16SrRNA molecular fingerprint obtained for each sampling method was comparable showing that any of the methods could be effectively used for biofilm collection. Microscopic observation of the samples showed that diatoms, filaments and other large organism were severely damaged by the electric tooth brush method and the approach was discontinued. Molecular analysis of the microbial populations in the 4 streams were compared in samples collected in the same week. The population diversity in the biofilm was described as numbered OTU's . This form of description is used as organisms cannot be easily identified to species level from the genetic fingerprint without gene sequencing.

A total of 410 clones were obtained from samples from the 4 sites, within which 171 distinct OTUs were identified. Approximately one third of the clones were assigned to one of 9 “dominant”OTUs (i.e. the most frequently occurring OTU's). DNA sequencing of the 16S rRNA genes representing the 9 dominant OTUs revealed several α -Proteobacteria species, and species from the divisions cyanobacteria, diatom and candidate division TM7. The remaining two thirds of the clones yielded OTUs that were unique or represented a minor fraction of the clone library (Figure 2). Interestingly the microbial diversity at each site was quite different as defined by the occurrence of specific OTU's. The α -Proteobacteria are one of the major divisions of the Euacteria and contain many chemolithotrophic bacteria including those which fix nitrogen and utilize inorganic materials for energy as well as a range of heterotrophic bacteria. This group tends to be very metabolically diverse with members capable of utilizing otherwise difficult niches.

The Shannon-Weaver Diversity Index was used to compare the total microbial diversity in the biofilms at each site (Table 1). High microbial diversity in the biofilm sample was recorded at the Cascade and Mangatawhiri sites ($H = 3.85$ and 3.68 respectively) and moderate diversity ($H = 2.97$ and 2.99 respectively) at Wekatahi and Marawhara.

Figure 2: Population profile of microbial biofilm at each site based on operational taxonomic units (OTU's) derived by restriction digest of cloned 16SrRNA fragments. Coloured segments represent the 9 most frequently occurring OTU'S. White areas represent the proportion of the population in OTU's which occurred less than 3 times in the population clone library. The dominant groups include members of the α -proteobacteria (HL3H, HL6D, HL7F, HL8E, MU2F, HL3D), Cyanobacteria (HL3D), Chloroplasts (CU12A) and an unclutured group (CU8A).

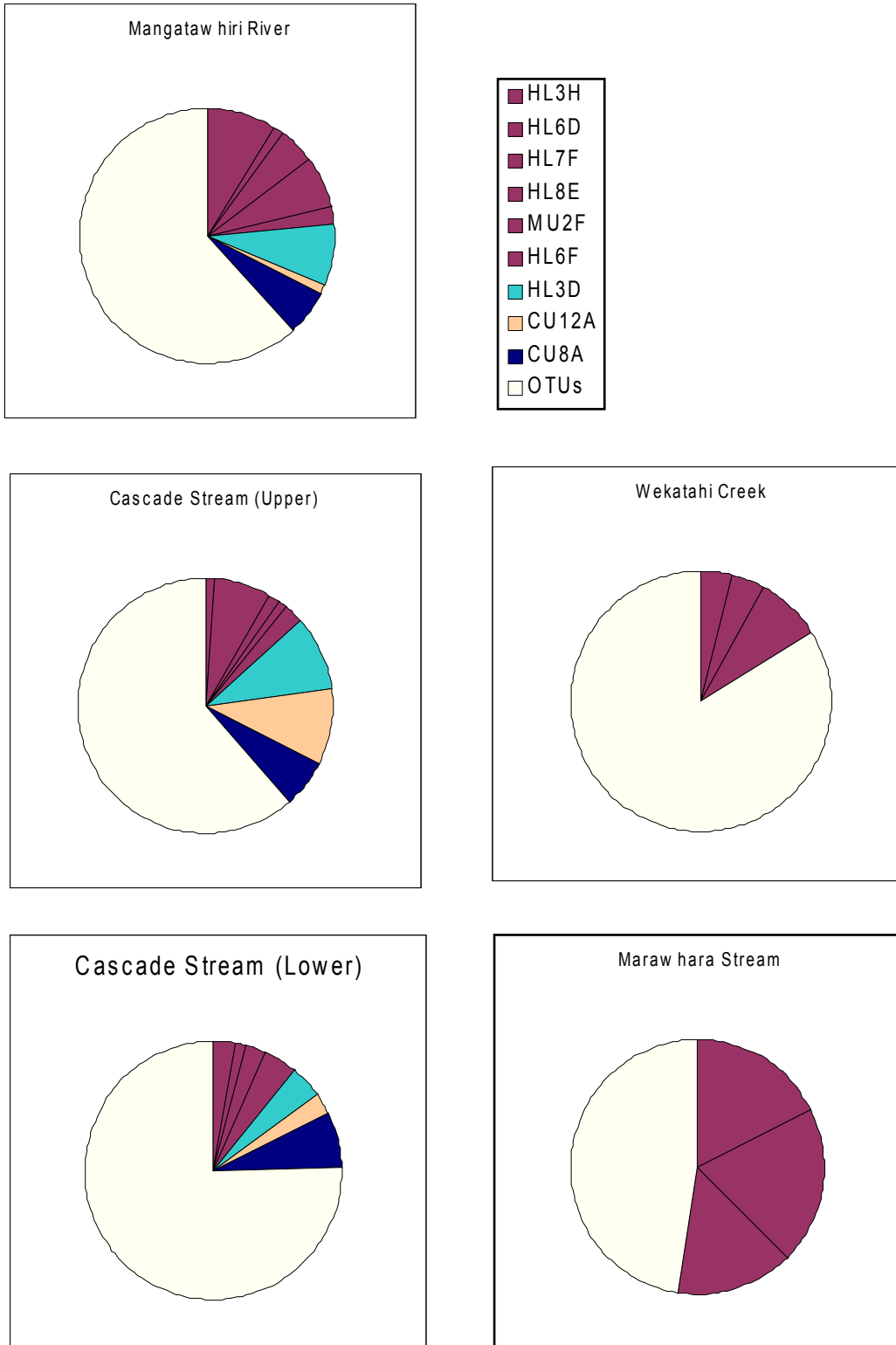


Table 1: Shannon-Weaver Diversity Index for operation taxonomic units derived by molecular evaluation of microbial biofilm samples from 5 sites.

Site	Diversity Index
Cascade Stream (Upper)	3.62
Cascade Stream (Lower)	3.85
Wekatahi Creek	2.97
Marawhara Stream	2.99
Mangatawhiri River	3.68

4 CONCLUSIONS

Microbial biofilms appear to be extremely diverse communities which are very important to the stream ecosystem as a functional nutrient and energy fixation and cycling component as well as a major food resource within the food web. As Cummings wrote in 1974 (cited by Hall *et al* 1998) “.. detritivorous macroinvertebrates derive much of their carbon from microbes in streams (the so called “peanut butter”) instead of from the detritus itself (the cracker)”.

The work described here shows that bottom up factors (ie food resources) are very significant in development of a stream ecosystem. Stream restoration approaches must account for both bottom up and top down effects if restoration is to be effective both in the short term and to be a sustainable long-term solution to stream ecosystem degradation.

Our ongoing research investigate the relationship between environment, biofilm composition and nutritional quality and the diversity and magnitude of the invertebrate populations with a view to enhancing the function of all stream components with a restoration management plan.

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