The role of forest stream corridor characteristics in influencing stream and riparian ecology

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Abstract

This PhD thesis seeks to consider conifer forestry stream corridor design in relation to both in-stream and riparian zone biodiversity and functioning. The contribution, availability and source of basal resources within varying corridor conditions are the focus of this project. This approach is combined with surveys of community diversity on a number of key trophic scales in order to determine how the corridor characteristics and their associated resource availability, affects community structure.

The effects of varying design and management of the riparian buffer zones within afforested stream systems on in-stream and overall habitat diversity and functioning remains largely unknown. Although guidelines have been implemented for several years (Forest and Water Guidelines, Forestry Commission), recommendations, although based on sound assumptions, are subjective assessments and tend not based on scientific research or data. As such, the premise of this project is to consider a variety of corridor physical parameters adjacent to low-order streams within two afforested catchments in South-West Scotland, between 2003 and 2005, in order to contribute to the understanding of system functioning within the limitations of forestry land-use and management.

A number of different approaches were employed in order to define the proportional contributions of allochthonous and autochthonous material within the benthos of the stream systems. This was done in order to define resource availability, biofilm characteristics, stream functioning and the role of corridor design in influencing resource availability. Yet, despite significant autochthonous productivity, allochthonous organic matter was the primary resource utilised by many taxa. However, conversely, light regime was found to be fundamental in shaping production and community structure within these ecosystems. Consequently, here I explore a number of different trophic scale responses to riparian conditions in order to define the biotic responses

to variation of resource availability, with the aim of contributing information which may aid in design and management of afforested riparian zones.

Declaration

I declare that the work done described in this thesis has been carried our by myself unless otherwise acknowledged. It is entirely of my own composition and has not, in whole or part been submitted for any other degree.

Stephanie Evers

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Contents

List of Figures

List of Tables

1 General Introduction

1.1 Introduction

Large-scale conifer afforestation of upland areas in the north and west of the UK has altered the ecological status of many rivers flowing within or draining extensive forest plantations (Clenaghan et al., 1998; Maitland et al.; 1990; Ormerod et al., 1986). Rapid expansion of UK forestry within the 1950s and 60s occurred during a period when environmental concerns were not given the priority they receive today (Broadmeadow and Nisbet, 2004). Commercial forestry plantation and forestry management expansion led to increasing concerns that large-scale alteration of land-use would be accompanied by a general degredation of water quality. Specifically, many concerns centered around the fact that trees were often planted in close proximity to water courses, reducing light availability to in-stream and riparian zones, reducing ground-flora vegetation cover and helping to destabilise river banks. These modifications to the riparian zone were associated with marked reduction in overall productivity as well as diversity of the in-stream and riparian zones (e.g. Peterken, 1996).

In 1986, a 'water workshop' organised by the Forestry Commission and the Water Research Centre at York addressed increasing concern over the forestry industry's impact on diffuse pollution, water acidification and reduced biodiversity. Following this meeting, the first set of best practice guidelines of forest and management and design were issued in 1988. The Forest and Water Guidelines have had four revisions (Forestry Commission, 1988, 1991, 1993 and the most current; 2003).

From the early 1990s, forest design planning became an integral part of forest planning and management. Initially, these design priorities were integrated into state owned forestry, but increasingly the private sector was also involved. This approach integrated conservation, recreation, landscape and water issues on a whole-forest scale. One key aspect to this process was the implementation of stream corridor and streamside re-design in order to form native woodland habitat and wildlife corridors (Farmer and Nisbet, 2004), with densely canopies being replaced by more open stands and broadleaf forestry (Forest and Water Guidelines, 2003).

The health and function of a stream ecosystem is not only dependent on the internal processes of the stream but also relies on the quality of habitat, functioning and energetics of the surrounding terrestrial habitat (Vannote et al, 1980). The riparian zone (the interface (transition zone) between streams, rivers and/or lakes and their surrounding terrestrial habitats) forms an integral part of stream functioning and should be of key concern when assessing stream health and functioning.

The Forest and Water Guidelines (Forestry Commission, 2003) try to balance the various interactions of riparian woodlands by recommending that about half the length of water channel is kept open to sunlight whereas the remainder is covered with dappled shade. However the effects of varying the design and management of the riparian buffer zone upon in-stream and overall habitat diversity, and ecosystem functioning remains poorly known (Farmer and Nisbet, 2004). As such, FWG recommendations although based on sound assumptions, are subjective assessments and tend not to be based on scientific research or data (Broadmeadow and Nisbet, 2004). In addition, the guidelines have been criticised for their restricted scope; being aimed towards conservation of fish, either specifically (e.g. Mills, 1980) or by implication (e.g. Forestry Commission, 1993). As the majority of these 'old style plantations' are now reaching the end of their rotational cycle, there is now the opportunity to redesign planting style in order to maximize biodiversity and system functioning.

The basic premise for this project was that rivers should be considered from the perspective of overall biological diversity, and as such, this project considered issues of biodiversity at different trophic scales to address energy cycling and system functioning within the habitats as a whole, in order to consider how variation of basic corridor/riparian design characteristics can be used to maximise system 'biointegrity'.

1.2 The role of riparian characteristics in influencing energy resources

Shading within streams of native broadleaf woodland may reduce primary production. However, allochthonous production is usually significant and is enhanced through high retention of organic material derived from woody debris (e.g. twigs, branches and fallen trees; Pozo *et al.,* 1998). Conversely, streams left "open"

(without an overhanging or nearby tree canopy cover) can have low availability of allochthonous material standing stock, and can often be poorly retentive of the organic matter entering the stream (Dobson and Cariss, 1999). However, in such open systems, there is a greater potential for autotrophic production with increased availability of Photosynthetic Active Radiation (PAR) within the stream system. Consequently, open systems are often characterized by high algal production, which provides food for primary consumers (Behmer and Hawkins, 1986).

Conifer forestry can cause significant alterations to the energetics driving stream functioning (Pretty and Dobson, 2004). Allochthonous leaf material can be a substantial energy resource for stream systems (Fisher and Likens, 1973). The processing rate of this allochthonous material is key to the rate at which it becomes available to the higher levels of the food chain (Peterson and Cummins, 1974). Within the stream, allochthonous detritus is colonised by aquatic fungi and bacteria and is subsequently processed by stream macro-invertebrates. However, the success of processing can be affected by a number of physical and chemical factors which include temperature, consumer populations, acidity and overall residence time in the system (e.g. Dobson and Cariss, 1999; Friberg and Jacobsen, 1994). Many of these variables are addressed within this study, as they are greatly influenced by the specific corridor design. In particular, the proportion of allochthonous material derived from coniferous sources has been found to be of significant influence. The chemical characteristic of allochthonous material itself has a direct influence on processing rates. For example, Gessner and Chauvet (1994) found that leaves from oak (*Quercus* spp.) had relatively high lignin and tannin concentrations and thus were processed slowly in comparison to alder leaves (*Alnus glutinosa*) which, being nitrogen-rich, are processed rapidly. Coniferous needles however, provide a poorer energy source for higher stream trophic levels than leaves from deciduous species (Sedell et al., 1975), having high levels of lignin (Berg *et al.,* 1982) and thick cuticles (Bärlocher et al., 1978). As a consequence, their process rates are much slower (e.g. Friberg and Jacobsen, 1994). The specific litter retention time can be a concern within commercial plantation forests as harvesting normally occurs before trees become old enough to significantly lose twigs and branches. Consequently, the retention time of allochthonous material is often low resulting in reduced availability and palatability of allochthonous resources. As such, these systems may receive little energetic benefit from coniferous-based detrital matter (Pretty and Dobson, 2004);

yet due to the proximity of the trees (and associated shading), can often also suffer from limited algal production (Friberg and Kjeldsen, 1994, Friberg, 1997).

1.3 Conifer forestry: A source of diffuse pollution

In the late 1940s and 1950s acceleration in conifer planting and the general expansion of the forestry industry was extensive and annual planting rates peaked in the early 1970s to >40000 ha yr^{-1} (Forestry Commission 2002a) in order to fulfil the role of a strategic reserve. As a consequence, approximately 20% of UK land is presently afforested (Neal et al., 2004).

Increasingly, plantation forestry has been recognised as a major source of diffuse pollution (Clenaghan et al., 1998, Maitland et al., 1990 and Ormerod et al., 1986). The main areas of concern focused not only on the plantations themselves but also the activities that surrounded them. British commercial forestry has been heavily criticised for degrading moorland habitats, degradation of soil and rivers, a lack of commitment and appreciation for conservation, and generally degrading the aesthetic appeal of the areas affected (e.g. Ramblers Association, 1980; Nature Conservancy Council 1986). In addition, concerns focused on aerial application of fertilisers, which contributes to nutrient enrichment and run-off (Swift, 1987). Cultivation, drainage-channel production and road building can all contribute to erosion and subsequent increased sedimentation in river and stream systems (Leeks and Roberts, 1987). In the 1980s increased environmental awareness led to concerns over the apparent increased ability of plantation trees to scavenge atmospheric acid pollutants and thus contribute to soil and surface water acidification (Stoner et al., 1984).

Catchment land use determines much of the variation in stream quality produced by varying levels of sediment and nutrient inputs, affecting physical habitat variables, and community composition (Omernik, 1976). The increase in proportion of agricultural and urban land use can cause increases in both sediment and nutrient inputs (Allan et al, 1997). The conversion of much of the UK's uplands to conifer forestry plantation land has similarly caused changes to deposition chemistry, soil, and runoff character as well as having a significant role in variation of community composition (Neal et al, 2004).

1.3.1 Acidification

Acid deposition is arguably the most controversial of all the impacts caused by widespread development of conifer forestry plantations (Nisbet, 2001). The ability of vegetation (particularly mature trees) to collect and concentrate elements has been documented some time ago (e.g. Miller and Miller, 1980). The extent to which atmospheric elements are absorbed depends on both the vegetation type and the transport mechanisms in place (Fowler, 1980). The concentration of most elements increases from canopy to forest floor for all tree species through mechanisms such as interception of acidic rain and crown leaching (Calder and Newson, 1979). However, drawdown and retention of pollutants is considerably greater in conifer plantations (Harriman and Morrison, 1982), exacerbated by the fast growth of the coniferous species.

During high discharge events, leaching of sulphate anions from soils into stream water occurs. If this excess sulphate is not balanced by calcium or magnesium ions then aluminium and hydrogen ions make up the deficit. Thus, the geological buffering capacity of the underlying rocks and soils can largely determine how much of an effect afforestation has on the level of acidification within the catchment and associated water-bodies (e.g. Sheppard et al, 2004; Stutter et al., 2004). The relative concentration of aluminium or calcium in forest soils will have a substantial role in determining the effect of acid runoff (e.g. Bache, 1984).

1.3.2 Trace Metal Contamination

Studies addressing the role and variation in trace metal deposition (e.g. Neal et al., 2004); demonstrate the importance of trace metals. For example, studies in Wales (Wilkinson et al., 1997) on Cr and Zn, and on Mn in Scotland (Heal, 2001), indicate that following harvesting operations, the deposition levels increase. Grieve and Marsden (2001), in a study of forest and moorland habitats, demonstrated that although there was much temporal and spatial variability, TOC, Al and Fe concentrations were significantly higher in streams draining forested catchments.

1.3.3 Sedimentation and soil disturbance

The classic study which first examined the ecological effects of catchment-scale conifer afforestation and the activities associated with it was undertaken in the Hubbard Brook Experimental Forest, an experimental site established in 1955 by the US Forest Service to study the hydrology of small catchments. The major emphasis in early studies was to determine the impact of forest land management on water yield and quality, and flood flow. Data arising from such studies have indicated that sediment release and riparian soil disturbance were, until recently, responsible for many water pollution incidents in UK forestry (Nisbet 2001).

Additionally, sedimentation and associated increases in water turbidity can severely reduce in-stream biodiversity (Vuori and Joensuu, 1996) and the effects may persist for many years after the disturbance event (Yount and Niemi, 1990). High levels of turbidity can reduce autochthonous photosynthetic rates by light attenuation within the water column (Cloern, 1987), and reduce breeding success of salmonid species through sedimentation of spawning gravels (Ziemer, 1991). Thus both bottom-up and top-down functionality of the ecosystem can be affected.

Disturbance causes the most severe effects during exposure of soils through weathering. Thus ploughing, channel production and felling events are associated with the mobilisation of soils and associated nutrients. The effects of soil disturbance are amplified when associated with channel drainage systems as the reduced residence times, increased flow rates and associated erosion rates increase the potential for soil and nutrient transport directly into streams and rivers. The risk of pollution from these sources is increased when operations are followed by a period of very wet weather (Nisbet, 2001).

1.3.4 Nutrient Enrichment

In both aquatic and terrestrial environments the quantity, availability and quality of organic mater and the rate of organic mater mineralisation in soils and sediments has been shown to exert strong control over the rates of nitrogen transformation (Jones *et al.,* 1995). In forested streams where allochthonous inputs can account for up to 98-99 % of total energy available to the stream ecosystem, organic carbon is the critical link between energy and nutrient dynamics (Kaplan et al., 1993). Dissolved organic carbon (DOC) can account for up to 30 – 75% of the total energy

inputs (Fisher and Likens, 1973) and thus, especially in nutrient limited systems the carbon and nitrogen cycles (and potential nutrient availability) are linked. The quantity of bacterial biomass is often controlled by the level of carbon availability as well as source quality (Bott et al., 1984). As a consequence, the carbon availability also dictates the bacterial nitrogen demand. Additionally, the rate of transformation of nitrogen is also mediated by micro-organisms reliant on oxidizable carbon supplies. Consequently, the rates of processes such as denitrification are reliant on the availability of organic carbon and are thus coupled with the carbon cycle.

Forestry can affect nutrient enrichment in a number of ways. Firstly, the direct application of phosphate and nitrogen fertilisers, required to achieve the satisfactory establishment of forests on certain soil types, can lead to significant nutrient leaching (Swift and Norton, 1993). Secondly, indirect release of nutrients (particularly phosphorus) into streams following large-scale felling operations (e.g. Staaf and Olsson, 1994; Dyck et al., 1987) can also present a problem in upland stream waters whose naturally nutrient-poor systems are often phosphorus limited. Phosphorus enrichment can, in extreme cases, produce excessive algal growths (e.g. Swift and Norton, 1993), resulting in wide fluctuations in dissolved oxygen, increased biological oxygen demand (BOD) and even fish death (Nisbet, 2001).

Buffer strip studies have demonstrated the importance of denitrification in removing nitrogen from anaerobic soils. Hubbard and Lowrance (1994) noted that a 7m buffer strip was effective at removing nitrate through plant uptake and denitrification. Pinay et al., (1993) found that 30m buffer strips were successful in reducing nitrate levels to below that of the shallow groundwater of riparian forests. Buffer strips along watercourses have also been shown to be effective at retaining phosphate leaching from forest soils following aerial applications.

1.3.5 Bio-monitoring of river water quality under the European Commission Water Framework Directive (WFD): existing approaches and the need for new methodologies

Bio-monitoring is recognised by WFD as an essential tool for use in both routine monitoring of freshwater ecosystem biointegrity ("ecological health"), and for assessment of impacts of pollution or other harmful pressures such as disturbance

upon these systems. Bio-monitoring procedures based on differing groups of organisms makes it possible to detect changes occurring in a freshwater system over differing timescales of impacts because the study organisms concerned integrate such effects over timescales ranging from days or less (e.g. microorganisms), to months or more (e.g. macrophytes). Within this study, using indicator species as well as assemblage structure (at different ecological trophic levels) allows utilisation of these benefits by establishing patterns of diversity associated with variable stream and riparian conditions. Such data can provide evidence and information on the effects of habitat disturbance and possible associated pollution events in streams draining forested areas.

Such methodologies are undoubtedly useful tools for river bio-monitoring purposes. However the WFD also recognises the need for continuous improvement in bio-monitoring technologies. For various reasons, dependent on factors such as size, geological factors, or pollution status (to mention only a few), assemblage-based methods may not provide an adequate picture of the state of health of a given stream especially when the pressures affecting biointegrity are intermittent and unpredictable. In these circumstances (and also as a complementary methodology to help assess the state of other types of stream system), any novel method which might aid determination of the bio-integrity state of a stream could be a valuable additional tool for water quality management. Thus, the following sections outline chapters which have utilised both traditional, and novel approaches to bio-monitoring in order to assess the influence of variation to conifer forest stream corridor design and forestry disturbance and to asses variability in ecosystem health through measurements of the specific community composition combined with assessment of both diversity and evenness

1.4 Study aims

This study aimed to explore the relationships involved in food-web dynamics within conifer forested streams and their associated corridors, in order to address the importance of corridor characteristics in determining and maintaining both biodiversity and production within food-webs. In addition to this, the project aimed to explore the relative importance of different energy sources driving ecosystem functioning in such systems. The findings can inform forest management strategies,

particularly in the context of riparian corridor design. This thesis presents the results of field studies undertaken in South-west Scotland between autumn 2003 and autumn 2005, aiming to cover the main subject areas as outlined below:

1.4.1 Chapter 2: Patterns in biodiversity and standing crop biomass of riparian vegetation, and the role of corridor physical characteristics in shaping this variation.

This chapter investigates variations in biomass and biodiversity of ground flora vegetation in stream corridors. I explore the role of riparian vegetation as a replacement for, or supplement to, basal energy resources, with the premise being that allochthonous litter availability and utilisation may be low from corridor coniferous sources. Defining the role of corridor characteristics in influencing standing stock biomass and diversity of ground flora vegetation serves to provide information on how these corridor habitat variables control allochthonous energy availability to other trophic groups and influence both vegetation and overall corridor diversity.

1.4.2 Chapter 3: Autochthonous primary production: using traditional approaches to characterising biofilm autotrophic components and the role of light in basal energy resource dynamics.

This chapter investigates temporal and spatial variation in availability and characteristics of in-stream biofilm material. Much of the focus of this study seeks to define the proportion of algal material within biofilm biomass. I use carbon and chlorophyll *a* analysis in two ways. Firstly, through the conversion of chlorophyll *a* concentrations, I calculate algal biomass within benthic biofilms of the study streams. Secondly, using C:Chl calculations, the proportional contribution of algal carbon to biofilm biomass is calculated. The physical and chemical parameters of the sites and temporal variation are explored in relation to biofilm content and biomass. In addition, algal species assemblages are considered, and dominant species discussed in respect to environmental variables and corridor characteristics (with particular reference to light availability) and changes to biofilm composition. Additionally,

advantages and limitations to using these relatively traditional approaches are considered here.

1.4.3 Chapter 4: Autochthonous primary production: development of a novel approach to characterising biofilm content and assessing the role of light in basal energy resource dynamics.

Here a novel approach is presented for differentiating allochthonous and autochthonous organic matter within benthic biofilms. The technique development is described here with information on the possible utilisation of isotopic signatures (δ^{15}N and δ^{13}C) and stoichiomatric measures (molar C:N), in order to delineate the source and contribution of organic matter to the biomass of benthic biofilms. This chapter assesses the comparative results from two study streams in order to describe changes to biofilm biomass and organic matter characteristics with corridor design over a temporal scale. This approach allows for the alternative measures explored to be examined in respect to production of a robust model. This approach is then combined with results from Chapter 3 in order to define the autotrophic proportion of the autochthonous compartment, in relation to the allochthonous organic matter. The results provide possible insight into biofilm functioning potential and the possible impacts to organic matter retention and processing by benthic biofilm biomass.

1.4.4 Chapter 5: Stream macro-invertebrate species assemblage structure and the role of physical, chemical and biotic factors.

Within this chapter, aquatic macro-invertebrates are used to assess variation in the habitat conditions available under the riparian and corridor characteristics associated with the different sites of this study. Invertebrate taxa were identified and characterised into Functional Feeding Groups (FFGs) according to morphological and behavioural traits, as described in the literature. Consideration of abundant taxa FFG provided information on dominant food resource preference, and thus, which basal resources were likely to be influencing the distribution and abundance of invertebrate communities and specific taxa. Multivariate ordinations are used to define gradients of habitat conditions which influence species composition and the physical conditions

important to each site assemblage. Additionally, diversity indices are used to assess the influence of environmental variables upon community diversity.

1.4.5 Chapter 6: Characterising the effect of felling on stream corridor habitat functioning

This study describes the immediate effect of a clear-felling event on stream and corridor habitats. As the felling was an unexpected event, the results are based on the ongoing ecological surveys of a single stream, the Black Burn. The data presented include benthic biofilm experimental data, combined with limited physical, chemical and macro-invertebrate assemblage results. Using the stoichiometric mixing model approach described in Chapter 4, stream biofilm composition and specifically the relative carbon sources and contributions that they make to stream biofilms, are used to provide information on the impacts of riparian disturbance to nutrient availability and retention within the stream system. The study focuses on temporal changes in biofilm composition post felling and discusses community assemblage modification in algae and chlorophyll production using a reference stream at an undisturbed location for comparative analysis. The impact on consumer species is investigated with analysis of macro-invertebrate populations before and after the event. I also consider the role of riparian vegetation on buffering the impact of the felling event: in particular the buffering role played by the plant community in terms of reducing nutrient and sediment runoff.

1.4.6 Chapter 7: The impact of riparian zone structure on native fish species growth and survival; a study of both natural populations and experimental stocking.

Salmonid fish species constitute the most economically important species within this study (Galloway Fisheries Trust, personal communication). Including an investigation into the impact of different riparian characteristics on higher predator species has benefits both to the local community but also to wider issues relating to declining fish stocks. In addition, the relative life span of these taxa means that they reflect the longer-term changes in environmental conditions. Experimental stocking of Atlantic salmon into selected sites characterising a variety of varying corridor

characteristics constitutes the primary data of this chapter. Survival success, as well as weight and fork-length data is included here for both years of fieldwork. This data is combined with the physical, chemical and ecological data from sites to determine relationships between site characteristics and the success of stocked salmon. In addition, natural populations of both Atlantic salmon and brown trout are also surveyed and the results produced here provide a wider scope for assessing population abundances with environmental variables, as well as information on habitats which can sustain and promote natural unstocked populations.

1.4.7 Chapter 8: Conclusions.

This chapter integrates the results from all chapters to discuses the overall role of stream corridor structure on ecosystem functioning. The key findings from organisms studied at each trophic level are integrated to provide information on the relationships between producer and consumer species with respect to conifer forestry stream habitat type. Additionally, I discuss possible optimal corridor characteristics which would contribute the greatest to overall system health and biodiversity. These results are then used to make recommendations for management protocols, and to define areas where improvements would have the greatest impact on the target habitats.

1.5 Field site details

Fieldwork for this project was undertaken between September 2003 and October 2005. The field area was located in Galloway, Southwest Scotland. This target area chosen lies within an area of extensive afforestation, covering approximately 27% of the region (Helliwell et al., 2001).

The two catchments studied were the Cree and neighbouring Bladnoch. Due to the nature of secured funding, the first year of study (Sep 2003 – Sep 2004) was limited to sites within the Minnoch catchment (a tributary of the Cree). Extension of funding at the end of year one, to provide a further two years of secured funding, allowed for expansion and some redesign of project parameters. As a consequence, further Cree catchment sites and also sites from within the neighbouring Bladnoch Catchment (Fig 1.1), were included.

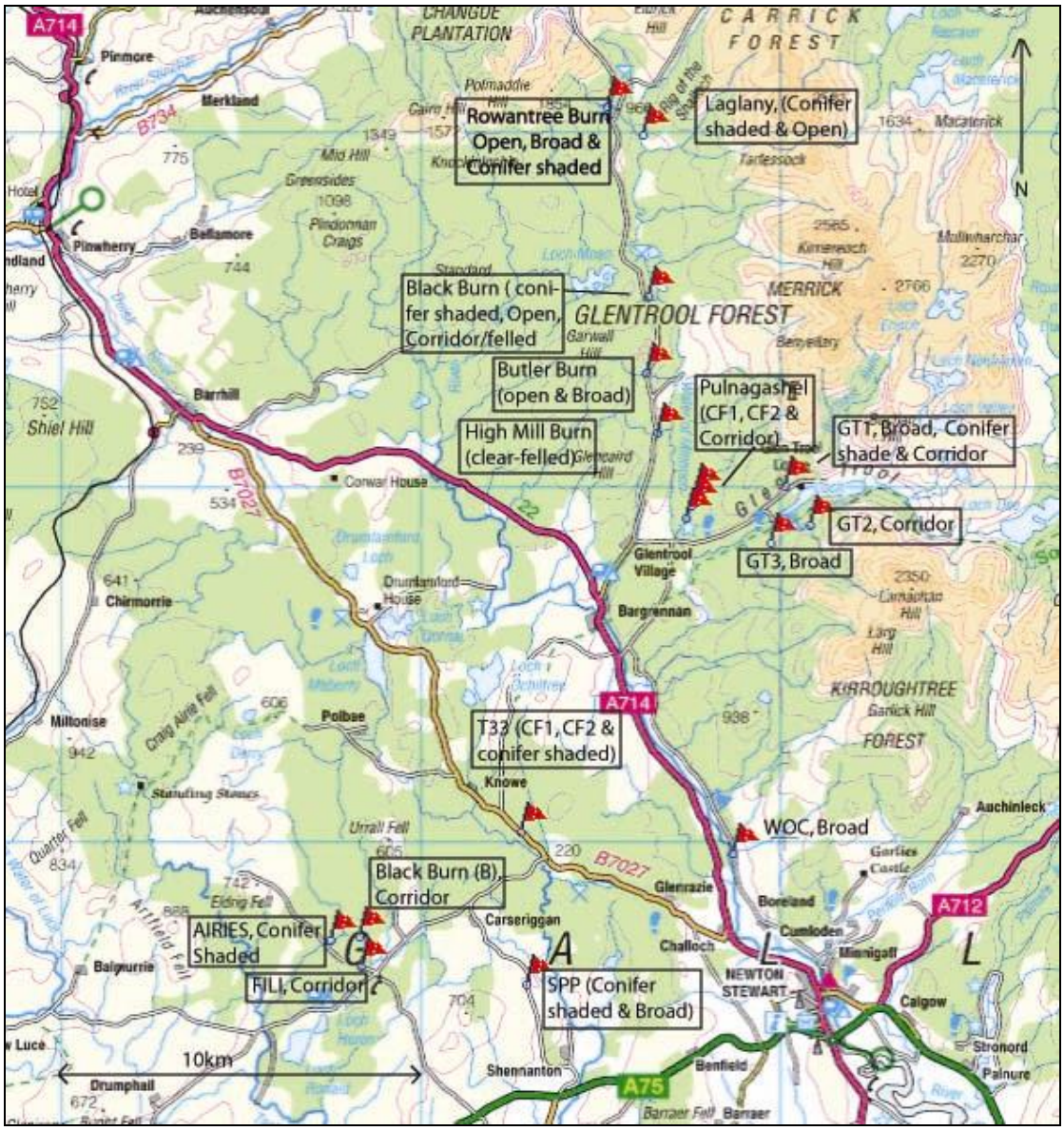

Fig 1.1. Map of all sites. Site/stream name illustrated with site types where multiple site locations found on a single stream (Map modified from Ordnanace Survey).

Streams in Galloway drain predominantly in a southerly direction from the Galloway uplands into the Solway Firth (Fig 1.2). There are seven river catchments of moderate size in the region, which include, from west to east, the Water of Luce (200 km^2), the River Bladnoch (340 km^2), the River Cree (370 km^2), the Palnure Burn (50 km^2), the Skyre Burn (25 km^2), the Water of Fleet (90 km^2) and the River Dee (1000 km^2).

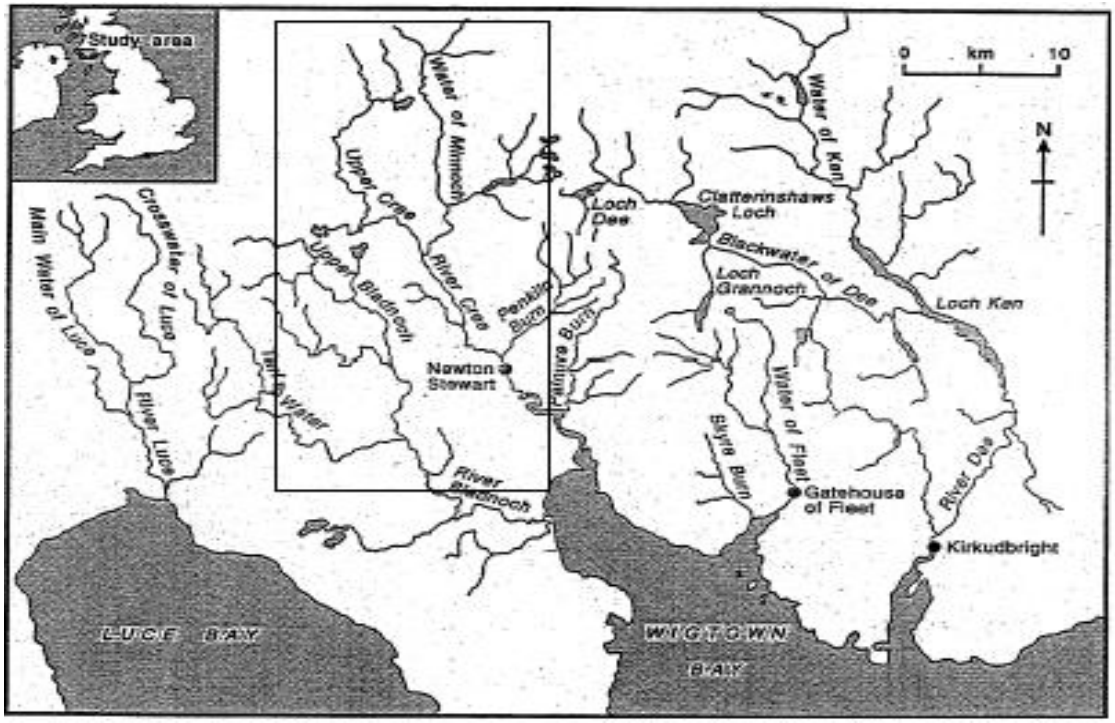

Fig 1.2. Catchment Systems within Galloway. Highlighted area includes the Cree Catchment (with Minnoch tributary) and Bladnoch Catchment. (Map modified from Puhr *et al.,* 1999).

Galloway has a relatively simple underlying solid geology, consisting of three main rock types: (1) granite intrusions of Tertiary age, (2) shales, mudstones and greywacke of Ordovician age and (3) shales, mudstones and greywacke of Silurian age. All these rocks have a low capacity to buffer external acid inputs (Edmunds and Kinniburgh, 1986). In addition to this, soils are generally thin, and consist primarily of podzols and peaty podzols; a large proportion of the more upland areas are covered by blanket peat (Bown et al., 1982). The annual precipitation is approximately 1400 mm.

1.5.1 The Cree Catchment

Widespread planting within the Cree catchment began in 1948. Initial development occurred upstream of Bargrennan (NX 350 768) between 1948 and 1954, resulting in forest cover totalling approximately 7% of the catchment. This was then expanded with a planting regime which accounted for a further 4 % additional cover, annually until 1965 (Tervet et al, 1995). After a pause in planting, further development occurred in 1977, and in the next 10 years, planting extended to its current range which covers approximately 65% of the catchment. The most prevalent of species used in the afforestation are non-native conifers, such as sitka spruce (*Picea*

sitchensis) and lodgepole pine (*Pinus contorta*). Deciduous tree stands are rare and distributed mainly within the more lowland areas of the catchment.

1.5.2 The Minnoch sub-catchment

The Minnoch is the largest tributary of the River Cree. Lying within the Galloway Forest Park, approximately 70% of the 141km^2 catchment is afforested. The river has a mean flow of 7.7 m^3s^{-1} but is subject to large spate events where flows reached approx 0.3 to ~ 60-90m^3s^{-1} from 2003 – 2005, inclusive. The geology of the catchment is Ordovician, with shales and greywackes, including rugged uplands (Fig 1.3).

Fig 1.3. Falls of Minnoch during spate flow event. (NX 37100 78600).

1.5.3 The Bladnoch Catchment

The second year of the project (March 2005 to October 2005) included fieldwork within streams of the Bladnoch catchment in S.W. Scotland. In recent years this catchment has been heavily afforested with coniferous species, mainly Sitka spruce *(Picea sitchensis*). However a much higher proportion of this development is under private land ownership and not undertaken by the Forestry Commission (Forestry Commission, personal communication). The soils include peaty podsols, peaty gleys,

peats and brown forest soils derived primarily from lower palaeozoic greywackes and shales. Flow here is also characterized by spate events. Flows ranged from approximately 0.4 to 100 - 115m^3s^{-1} from 2003 – 2005, inclusive. As the ranges for both rivers (Minnoch and Bladnoch) within the study are comparative, and the spate characteristic similar, variation in results from surveys are unlikely to be influenced by differential flow regimes between catchments.

The Bladnoch catchment was recently assigned SAC status (Special Area of Conservation), because the River Bladnoch supports a high-quality salmon population, which, unusually for rivers in this area, still supports a spring run of salmon (as apposed to late autumn migration immediately before spawning). The river drains a moderate-sized catchment (340 km^2) with both upland and lowland areas.

1.6 Site Selection

In total 28 sites were sampled within the study period. Due to the nature of funding acquisition, the sites were not all sampled over the same period. First year sites were confined to the Minnoch tributaries and a total of 15 sites were sampled from Sep 2003 to Sep 2004. Streams were all low order with an average width (± SD) of 2.20m (±1.03). All sites were selected on the basis of corridor characteristics broadly defined as the following:

- Broadleaf shaded
- Conifer shaded
- Conifer corridors
- Open
- Clear-felled

Three replicates of each site type were chosen. However, this type of selection process, based on broad categories, is liable to personal interpretation of site characteristics rather than on the basis of actual physical measurements. To compensate for this detailed measurements of corridor, stream and riparian characteristics allowed for analyses to be related either to these specific measured parameters or the more general site classifications based on more subjective observations.

In year two (Mar 2005 – Oct 2005), five of the original sites were abandoned due to one of two reasons. Firstly, two sites were removed as they had been unexpectedly felled (e.g. Laglany) and I felt this would confound results due to the extensive physical and chemical changes often associated with a large-scale disturbance event such as this. Secondly, expansion of the field area provided the opportunity for sites to be selected which were felt to be better representatives of the site types (as shown above).

Table 1-1 shows site locations (Ordnance Survey National Grid References) as well as year of sampling and indications of variation in general river and corridor widths. Where corridor widths exceed 100m, sites are open or clear-felled.

Table 1-1 Site details (Cree (top) and Bladnoch catchment (bottom), 2003 – 2005). Includes general site classifications and basic corridor characteristics as measured over the sampling period (± SE).

Sites	Grid Ref	Year(s) Sampled	Altitude (m)	River Width (m)	Corridor Width (m)
Laglany Open	358 902	Year 1	250	2.49 +/- 0.2	28.33 +/- 7.37
Laglany Conifer Shade	358 902	Year 1	252	2.91 +/- 0.27	14.00 +/- 5.05
Rowantree Broadleaf	352 906	Years 1and2	320	2.44 +/- 0.18	7.43 +/- 0.49
Rowantree Open	352 907	Years 1and2	330	1.95 +/- 0.09	33.12 +/- 2.9
Rowantree Conifer Shade	351 907	Years 1and2	340	2.65 +/- 0.12	8.16 +/- 0.58
GT1 Broadleaf	401 799	Year 1	100	2.90 +/- 0.33	6.00 +/- 0.31
GT1 Conifer Shade	402 798	Years 1and2	100	2.37 +/- 0.11	6.22 +/- 0.29
GT1 Corridor	402 800	Years 1and2	100	1.46 +/- 0.06	12.24 +/- 0.44
Butler Broadleaf	362 832	Year 1	210	2.55 +/- 0.17	9.33 +/- 0.42
Butler Open	363 832	Year 1	200	1.14 +/- 0.01	100 +
High Mill Burn Clear-felled	365 814	Year 1	190	3.35 +/- 0.08	100 +
Pulnagashel CF1 Clear-felled	374 793	Years 1and2	130	2.40 +/- 0.13	100 +
Pulnagashel CF2 Clear-felled	375 797	Years 1and2	120	2.73 +/- 0.11	100 +
Pulnagashel Corridor	374 792	Years 1 and2	110	3.34 +/- 0.18	9.93 +/- 0.56
GT2 Broadleaf	407 789	Year 1	130	6.81 +/- 0.18	25.00 +/- 0.00
Black Burn (m) Conifer Shaded	361 852	Years 1and2	210	2.23 +/- 0.12	9.01 +/- 0.20
Black Burn (m) Open	360 852	Years 1and2	210	1.77 +/- 0.03	31.32 +/- 1.06
Black Burn (m) Corridor/Cleared	360 851	Years 1 And 2	220	1.40 +/- 0.12	100 +/- 0.00
Wood of Cree Broadleaf	385 701	Year 2	50	1.88 +/- 0.19	7.68 +/- 1.25
GT3 Broadleaf	396 784	Year 2	100	1.31 +/- 0.25	7.14 +/- 0.75
T33 CF1 Clear-felled	328 703	Year 2	100	0.93 +/- 0.09	91.11 +/- 2.81
T33 CF2 Clear-felled	327 702	Year 2	100	1.11 +/- 0.17	64.44 +/- 5.62
T33 SH Conifer Shade	327 703	Year 2	90	1.10 +/- 0.15	36.44 +/- 5.20
SPP Broadleaf	329 659	Year 2	60	1.78 +/- 0.15	3.22 +/- 0.07
SPP Conifer Shaded	329 658	Year 2	60	1.32 +/- 0.12	2.72 +/- 0.03
Black Burn (B) Corridor	283 673	Year 2	110	3.27 +/- 0.14	23.55 +/- 3.09
AIRIES Conifer Shaded	275 672	Year 2	120	1.35 +/- 0.03	7.22 +/- 0.98
FILI Corridor	284 664	Year 2	100	1.24 +/- 0.01	16.55 +/- 1.61

2 Patterns in biodiversity and standing crop biomass of riparian vegetation, and the role of corridor physical characteristics in shaping this variation

2.1 Abstract

The riparian zone constitutes the aquatic/terrestrial interface zone adjacent to a water body. The area adjacent to a stream channel forms an essential component in the functioning of many stream ecosystems. Overhanging vegetation creates a valuable food source within the stream ecosystem in the form of organic detritus from terrestrial origins. The transfer of energy between the aquatic and terrestrial zones constitutes one of the key mechanisms driving the biodiversity of the aquatic environment and controlling the balance between allochthonous and autochthonous energy sources to the stream.

Sampling of riparian vegetation using replicate quadrat surveys and 400 cm^2 biomass sampling indicated variable biomass and diversity correlations. Biomass was positively correlated with increasing light and altitude. Consequently, using broad site-type classifications, riparian vegetation biomass was significantly higher within 'open' sites. Spatial variation of vegetation within sites was measured using quadrats positioned adjacent to the water edge, and three meters from the water edge. Proximity to either the streamside or the corridor edge respectively, did not translate to variability of either diversity or biomass of the riparian ground vegetation. The corridor characteristics measured did not predict changes in vegetation biomass. However tree height and riparian tree diversity did correlate with a positive increase in ground vegetation diversity. Conifer-shaded sites provided the least favourable conditions for riparian vegetation diversity.

A significant ($P < 0.001$, $n = 62$, $r^2 = 0.1961$) positive linear relationship was observed between invertebrate diversity and vegetation biomass. Neither vegetation biomass nor diversity could be used to predict changes in invertebrate abundance. Therefore, as the difference in riparian vegetation diversity was negligible between all sites, except 'conifer shaded', considering results for both invertebrate and riparian vegetation diversity preferences, suggested that by promoting riparian

vegetation biomass through increased light availability increases invertebrate diversity, whilst also maintaining relatively high diversity of riparian ground-cover vegetation.

2.2 Introduction

The riparian zone is the interface (transition zone) between aquatic and terrestrial habitats associated with a river. Natural riparian zones are amongst the most diverse, dynamic and complex terrestrial habitats in the world, but they are very sensitive to environmental changes (Petts, 1990; Naiman and Décamps, 1997). This area adjacent to the stream channel forms an essential component in the functioning of many stream ecosystems (Murphy *et al.,* 1994). Significant changes in riparian zones can, in turn, significantly affect the diversity of biological communities in both the adjacent terrestrial and aquatic habitats (Risser, 1990).

As with many transitional habitats ("ecotones"), the aquatic terrestrial transition zone (ATTZ) supports an assemblage of species, the distribution of which is controlled by the gradients of resources and other environmental factors occurring along the ATTZ ecotone. Individual species occupy locations along the ecotone where physicochemical conditions are most appropriate for their survival. The riparian zone provides a unique habitat in which the combination of terrestrial and aquatic conditions supports a community structure with both allochthonous and autochthonous components (Moss, 1980).

As well as creating an important habitat for stream corridor species, one of the most ecologically important roles of this ecotone is to provide biological, chemical and physical connectivity between water and land. For example, in the provision of allochthonous food material for in-stream species (Bilby and Bisson, 1992), overhanging vegetation contributes organic detritus: a valuable food source within the stream ecosystem. The ecologically diverse habitat produced promotes increase of 'drift' food sources (e.g. flying insects) from terrestrial origins (Sagar and Glova, 1995). The transfer of energy between the aquatic and terrestrial zones is one of the key mechanisms driving the often-high biodiversity found within riparian habitats (Bilby and Bisson, 1992; Vought *et al.,* 1994).

Open spaces within woodland, including river corridors are important areas supporting the diversity of woodland flora and fauna (Peterken and Welch, 1975). Rides, glades and riparian habitats all contribute substantially to the woodland diversity of vascular plants by creating 'edge' habitats. These habitats support a wide

variety of species, often more than either the open or the shaded habitats alone (Peterken and Francis 1999).

Under the current Forest and Water Guidelines (Fourth Edition: Forestry Commission, 2003) the management protocol for forested areas where timber harvesting occurs dictates that "buffer" strips should be left adjacent to rivers, streams, and lakes to protect aquatic habitats. Specifically, the guidelines state that in order to provide adequate protection for the aquatic habitat, buffer zones should be a minimum of 20m either side of the channel for watercourses of 2m width or greater. A buffer zone of 10m should be provided for those streams with a width of 1-2m and 5 meters for those streams below 1m width (unless of importance for fish spawning in which case the 10m buffer strip applies). In addition, aims are established for the planting of a variety of native tree species (for defined locations and soil types), thus providing variation in the level of shading from tree species. The guidelines also specify that light should be maintained at such a level as to allow for continuous cover of ground and bank-side vegetation and that, overall, an average of 50% of the watercourse should be left open.

Streamside vegetation provides an important additional function within stream systems. Specifically it (1) buffers and filters nutrients and pollutants which would otherwise potentially impact on stream water chemistry (Pinay *et al.,* 1990); (2) stabilises stream banks (Naiman and Decamps, 1997); (3) influences channel form, size and flow regime (Giller and Malmqvist, 1998); and (4) influences water temperature and light regimes through shading effects (Gordon *et al.,* 1992). All of these may have direct effects on in-stream primary productivity, and the balance between autotrophy and heterotrophy in the system, thus influencing the balance between allochthonous and autochthonous energy sources of stream corridors. The size of the water body also has an influence on these functions, with a greater influence from riparian vegetation likely in small watercourses than in larger ones (Giller and Malmqvist, 1998).

The spatial extent of the riparian zone is generally associated with the fluctuation of the water level and flood regime. Along streams, export of terrestrial organic matter into streams and the influence of riparian physical characteristics may be from a wider spatial area then just the immediate bank-side (Coroi et al 2004). The wider

riparian zone area has also great potential to influence in stream functioning. Away from the immediate bankside, the wider riparian zone also contributes to buffering of organic matter and sediment to streams, stabalisation of banks, influences in-stream autotrophic productivity (through shading by riparian trees) and contributes to the production and delivery of allochthonous litter to the stream (e.g. autotrophic litter and drift invertebrates)(Petts, 1990, Pinay et al., 1990, Naiman and Décamps, 1997 and Giller and Malmqvist, 1998). It is in both spatial scales that I consider the riparian zones in this study. Consequently, streamside vegetation survey data collected here included both the immediate aquatic – terrestrial transitional zone species, closest to the water edge, and also an area more distal from the water (3 meters), more often typified by a greater proportion of terrestrial derived vegetation types, yet proximate enough to the water to contribute to stream function. Therefore, both sub-stations (bank-side and 3 meter) are sampled in order to determine firstly the characteristics of vegetation likely to contribute to stream allochthonous carbon supply (as leaf litter etc), and secondly, any spatial variation of vegetation assemblage structure and biomass contributing to the overall biodiversity and production within the afforested catchment riparian zones.

Detritus produced from conifers is generally of low palatability and food quality for aquatic detritivore species (Maltby, 1992). In addition, the decomposition and processing rate for conifer needles is relatively slow (Friberg and Jacobsen, 1994; Sedell *et al.,* 1975). Short residence times have been recorded for needles in upland streams (Dobson and Hildrew, 1992) and may result in coniferous material rarely having sufficient time to decompose to a point where it is palatable for insect and other detritivores (Dobson and Cariss, 1997). Consequently, despite abundant over-storey litter resources, the potential quantity of allochthonous energy provided to streams by conifer forests may in practice be very low (Cariss and Dobson, 1997). Depending on its structure and species composition, the ground flora community within the riparian zone may hence provide a large proportion of the energy delivered from the terrestrial zone, in such forest streams. The abundance of this allochthonous material and the diversity of ground-flora may thus significantly influence the functional capacity of the corridor and stream food-web.

Natural tree-dominated riparian zones constitute the least disturbed riparian zones (Brinson and Verhoeven, 1999). Native riparian forests are able to support high

biodiversity, as they serve as refuges for both in-stream and terrestrial species. The diversity and relative abundance of the riparian ground-flora has to be examined for each of the different habitat types present in the conifer-afforested system, in order to provide information of the effect of conifer forest proximity, overall conifer forestry land use and changing variation in light intensities on this potentially important allochthonous resource.

Finally, aquatic and riparian flora and vegetation have long been used as components in river classifications (Naiman *et al.,* 1992, King and Caffrey, 1998; Holmes *et al.,* 1999). As aquatic vegetation is usually very limited in oligotrophic and meso-oligotrophic rivers and in low order upland streams, bank vegetation could be useful in the classification of the watercourses and the potential resources available to them.

This chapter has the following aims:

1. To determine the potential riparian vegetation biomass production within varying types of forest riparian zone in order to determine relationships between potential allochthonous resources available to in-stream consumers and corridor design.
2. To assess riparian vegetation diversity in order to determine variation with corridor design and to contribute to knowledge of total corridor biodiversity.
3. To compare conifer and broadleaf riparian zones in order to determine the role of forest land use on riparian vegetation biomass, diversity and species evenness.

2.3 Materials and Methods

2.3.1 Study Sites

The study was undertaken in Galloway, Southwest Scotland, within a single catchment (the Cree) in year one (autumn 2003 – summer 2004) and an additional second catchment (Bladnoch) in year two (2005). Initially, low order streams within the Minnoch tributary of the Cree Catchment were investigated. Riparian zone sites were confined to areas adjoining low order streams. Sites were chosen to represent the different habitats available within the afforested catchment. Broadly, these

categories were: conifer shaded, conifer corridor, open, clear-felled and broadleaf shaded. However, these characterisations are subjective and therefore the measured physical variables of each site are used to determine site differences, rather than relying solely on visual characterisations.

2.3.2 Fieldwork Methodology

Riparian vegetation measurements were always taken on the same side of the stream (true right side of the channel: facing downstream). Samples were taken on the bank edge and three meters from the edge. Three replicates were taken of each riparian location within a 10m sample-site length of stream bank to encompass the variation in environmental conditions found within the riparian zone. The study area included both the narrow strip of ground which is directly influenced by changes in the soil moisture level and also the more distal areas which are influenced both by the nutrients and moisture delivered by the stream, and also the additional physical variations in light intensity (due to the potentially close proximity to corridor/ plantation tree species).

Light (PAR) was measured at each site as a percentage of incoming light at an adjacent open site. Simultaneous readings were taken at each location (simultaneity was ensured by using mobile phones, or timed readings with synchronised watches in areas too far apart for verbal communication, or where no phone signal was present). The open site readings provided a measure of 100% light at time of sampling. Changes in light intensity with weather and season etc. are thus taken into account. Light measurements were taken during each site visit to estimate mean % light intensity throughout the year, as well as individual light measurements for each sampling date. All measurements were taken at a one-meter height from the ground within the centre of the bank-side sampling area. In addition, physical characteristics of the corridors were recorded for tree height (three visual estimates per visit), mean corridor width (three measures of trunk to trunk distance across corridor per site/visit), identification of tree species within the site, altitude (available from GPS readings), and stream/corridor orientation (facing downstream).

Replicate $1m^2$ quadrats were used to record ground-cover vegetation parameters (Fig 2.1). Vascular plants and bryophyte species were identified and their cover-abundance recorded using the Domin Scale (Table 2-1) (Dahl and Haduč, 1941).

Quadrats were placed randomly within each site, with positions relative to the streamside roughly following the example illustrated in Fig 2.1. A total of three replicates per location for both bank-side and 3m sites were sampled, thus providing a pooled total of six per station.

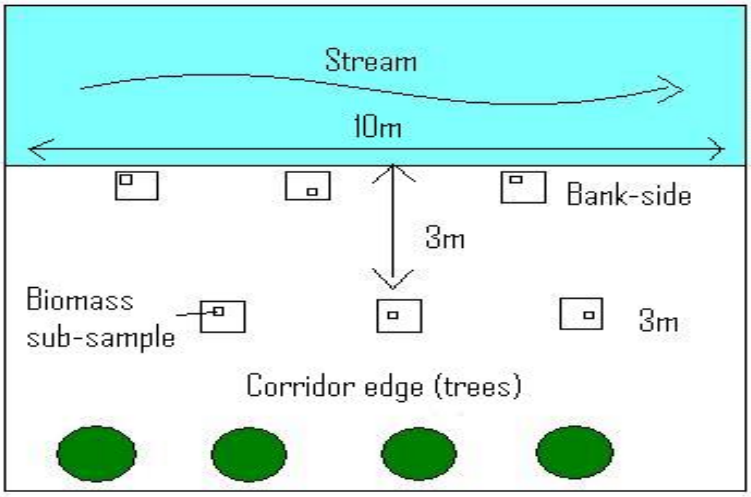

Fig 2.1. Positioning of riparian vegetation quadrats relative to stream and corridor edge.

During the second sample season (2005), at, a randomly selected sub-sample square was placed within each quadrat to provide a 20x20cm biomass sub-sample. Vegetation biomass was removed to ground level and stored in a cool box during fieldwork. Biomass samples were then oven dried at 70^{O}C for four days in order to calculate the mean vegetation dry-weight per m^2 for both bank-side and three-meter locations.

Table 2-1 Domin Scale of Cover (Dahl and Haduc, 1941).

Domin Scale	Percentage vegetation Cover
10	91 - 100
9	75 - 90
8	51 - 74
7	34 - 50
6	26 - 33
5	11 - 25
4	4 - 10
3	< 4 and many individuals
2	< 4 and several individuals
1	< 4 and few individuals

Plant species richness represents number of plant species found within all three quadrats from each site location (bank-side or three-meters). Abundance measures represent the mean score given to each species within all three quadrats.

2.3.3 Data Analysis

Diversity (H) was calculated using the Shannon-Wiener equation (Equation 1), which integrates species richness and abundance scoring to produce a measure of diversity and evenness of distribution ("equitability").

Equation 1

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Where:

- ni The number of individuals in each species; the abundance of each species.
- S The number of species (species richness).
- N The total number of all individuals: $\sum_{i=1}^{S} n_i$
- Pi The relative abundance of each species, calculated as the proportion of the individual of a given species to the total number of individuals in the community: $\frac{n_i}{N}$

2.4 Results

Measurements of the physical features of corridor design are shown in Table 2-2. Additionally, information on site names and locations is included.

Table 2-2 Physical parameters of corridor characteristics (mean ± S.E). Sample size (n) equals three replicates per sample visit (as indicated in the far right column) for each measurement variable (n = No. Visits x 3).

Site	% PAR	Riv Width	No. Tree	Cor width	Tree height	Altitude	Grid Ref	No. Visits
AIR	13.68 +/- 2.32	1.36 +/- 0.06	1	7.22 +/- 1.56	29.44 +/- 2.00	120.00 +/- 0.00	275 672	3
BBB	92.38 +/- 4.86	3.28 +/- 0.22	1	23.56 +/- 4.89	16.33 +/- 3.51	111.67 +/- 1.67	283 673	3
BBMCF	81.14 +/- 10.12	1.33 +/- 0.16	1	100.00 +/- 0.00	2.83 +/- 0.50	217.50 +/- 2.50	360 851	6
BBMOP	82.61 +/- 2.06	1.71 +/- 0.08	1	30.48 +/- 1.45	21.67 +/- 2.04	208.75 +/- 1.25	360 852	6
BBMSH	8.83 +/- 2.80	2.20 +/- 0.15	1	8.85 +/- 0.28	19.17 +/- 1.44	207.50 +/- 2.50	361 852	6
BUTBR	49.11 +/- 12.69	2.55 +/- 0.28	2	8.00 +/- 0.00	10.00 +/- 0.00	210.00 +/- 0.00	362 832	3
BUTOP	95.16 +/- 4.84	10.49 +/- 9.35	1	100.00 +/- 0.00	6.00 +/- 0.00	200.00 +/- 0.00	363 832	3
FILI	67.26 +/- 7.09	1.24 +/- 0.03	4	16.56 +/- 2.56	11.22 +/- 1.37	100.00 +/- 0.00	284 664	3
GT1BR	46.00 +/- 15.28	2.90 +/- 0.52	5	7.00 +/- 0.00	12.00 +/- 0.00	100.00 +/- 0.00	401 799	3
GT1CO	54.30 +/- 12.31	1.47 +/- 0.09	2	11.71 +/- 0.50	18.50 +/- 1.00	96.67 +/- 3.33	402 800	6
GT1SH	8.82 +/- 2.47	2.37 +/- 0.18	1	6.83 +/- 0.57	20.28 +/- 2.12	96.67 +/- 3.33	402 798	6
GT2	51.80 +/- 25.10	5.95 +/- 0.39	5	20.00 +/- 0.00	15.00 +/- 0.00	200.00 +/- 0.00	407 789	3
GT3	26.95 +/- 12.54	1.31 +/- 0.40	3	7.14 +/- 1.19	10.33 +/- 1.68	96.67 +/- 3.33	396 784	3
HMB	86.84 +/- 9.30	3.23 +/- 0.07	0	100.00 +/- 0.00	0.00 +/- 0.00	190.00 +/- 0.00	365 814	3
LAGOP	89.73 +/- 5.15	2.86 +/- 0.48	1	5.00 +/- 0.00	20.00 +/- 0.00	250.00 +/- 0.00	358 902	3
LAGSH	18.64 +/- 15.68	2.49 +/- 0.45	1	30.00 +/- 0.00	20.00 +/- 0.00	252.00 +/- 0.00	358 902	3
PCF1	88.34 +/- 2.03	2.18 +/- 0.17	1	100.00 +/- 0.00	1.00 +/- 0.45	128.33 +/- 1.67	374 793	6
PCF2	86.93 +/- 3.34	2.37 +/- 0.14	1	66.00 +/- 20.82	8.00 +/- 4.90	114.00 +/- 2.45	375 797	6
PCOR	38.35 +/- 8.64	2.73 +/- 0.34	2	7.53 +/- 2.00	24.00 +/- 6.68	104.00 +/- 2.45	374 792	6
RBR	29.06 +/- 8.12	2.44 +/- 0.27	3	7.44 +/- 0.73	17.89 +/- 3.02	316.67 +/- 3.33	352 906	6
ROP	72.34 +/- 8.82	1.95 +/- 0.15	3	40.46 +/- 5.34	9.89 +/- 1.35	326.67 +/- 3.33	352 907	6
RSH	14.30 +/- 4.26	2.59 +/- 0.22	2	9.00 +/- 1.00	9.07 +/- 2.73	338.00 +/- 2.00	351 907	6
SPPBR	33.58 +/- 25.18	1.79 +/- 0.24	5	3.22 +/- 0.11	12.33 +/- 1.90	60.00 +/- 0.00	329 659	3
SPPSH	31.87 +/- 30.43	1.32 +/- 0.19	4	2.72 +/- 0.06	19.44 +/- 0.56	60.00 +/- 0.00	329 658	3
T33CF1	38.18 +/- 9.49	0.94 +/- 0.14	1	88.89 +/- 5.88	1.56 +/- 0.78	67.67 +/- 32.33	328 703	3
T33CF2	61.78 +/- 2.22	1.12 +/- 0.28	1	66.67 +/- 10.18	22.44 +/- 3.20	67.67 +/- 32.33	327 702	3
T33SH	41.95 +/- 10.12	1.10 +/- 0.24	1	46.56 +/- 15.91	30.00 +/- 0.96	60.67 +/- 29.33	327 703	3
WOC	24.45 +/- 17.11	1.89 +/- 0.31	5	14.47 +/- 7.95	19.44 +/- 1.11	34.00 +/- 16.00	385 701	3

2.4.1 Biomass

Mean dry weight biomass for the 2005 sampling season (Fig 2.2) showed significant (ANOVA, P = 0.001, n = 62) increase in July (797g/m^2) compared with March (310g/m^2) and September (494g/m^2). However, with all stream site data pooled, there was no significant difference between bank-side and 3m quadrate biomass (ANOVA, P = 0.980).

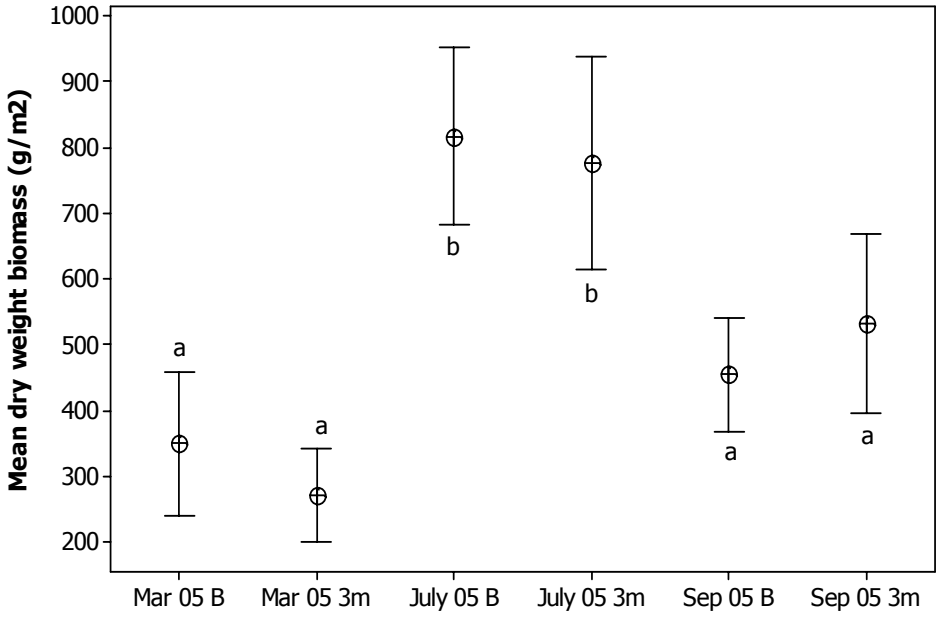

Fig 2.2. Temporal variation in riparian vegetation production in 2005. Mean dry weight biomass (g/m^2) ± 95% confidence interval. Significance of differences between groups determined using ANOVA ($P < 0.001$, $n = 62$). Tukey test (95% confidence) specified differences as signified here with differing lettering (a and b).

Regression analysis was applied to the mean biomass samples of each river location (pooling B and 3m sub-sites) to compare standing crop of riparian vegetation with the physical parameters of the corridor measured (% light, corridor width, tree height, tree diversity and site altitude). Linear and polynomial relationships were both explored and, throughout the chapter, the approach which yielded the greatest significance is presented. Regression analysis was used to reveal relationships between the two variables in question. Regression is a statistical tool, more powerful then Pearsons correlation and able to determine accurately the strength of any relationships observed. Additionally, this approach is useful in providing a both a P value and r^2 value for determining both the significance and variance of a relationship.

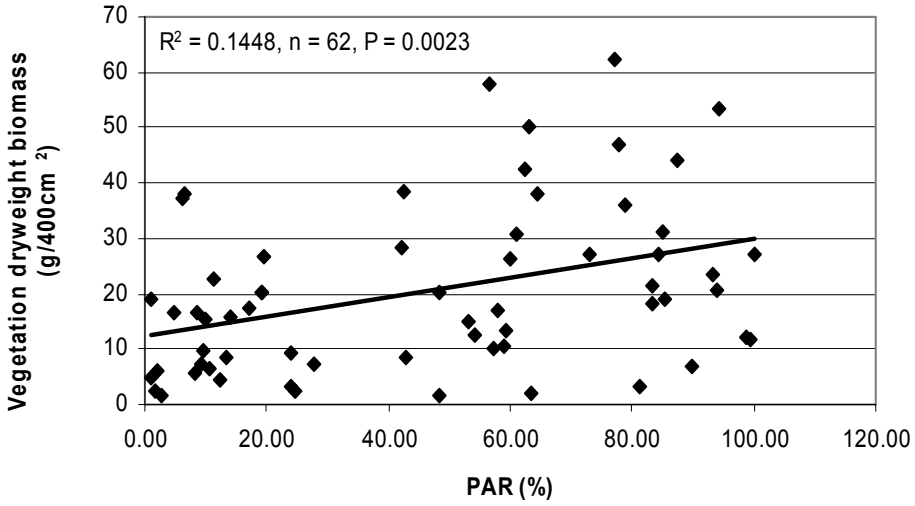

Fig 2.3. Significant positive linear relationship between light (% PAR) of the riparian zone sampling area and the dry-weight biomass of riparian flora (g/400cm^2) in 2005.

A significant positive linear relationship was found between PAR measurements and ground-flora biomass (Fig 2.3) indicating the importance of light intensity to riparian vegetation production. Although there is a substantial degree of variation in this relationship (as indicated by the low r^2 value), there is evidence to suggest that whatever other variables are controlling production within the riparian zone at high light levels, at low light intensities production of biomass is limited by light.

Most other corridor characteristics yielded correlation coefficient values too low to permit prediction of biomass responses (corridor width; P = 0.067, tree height; P = 0.13; tree diversity; P = 0.25), with the exception of altitude (Fig 2.4), where P = 0.001.

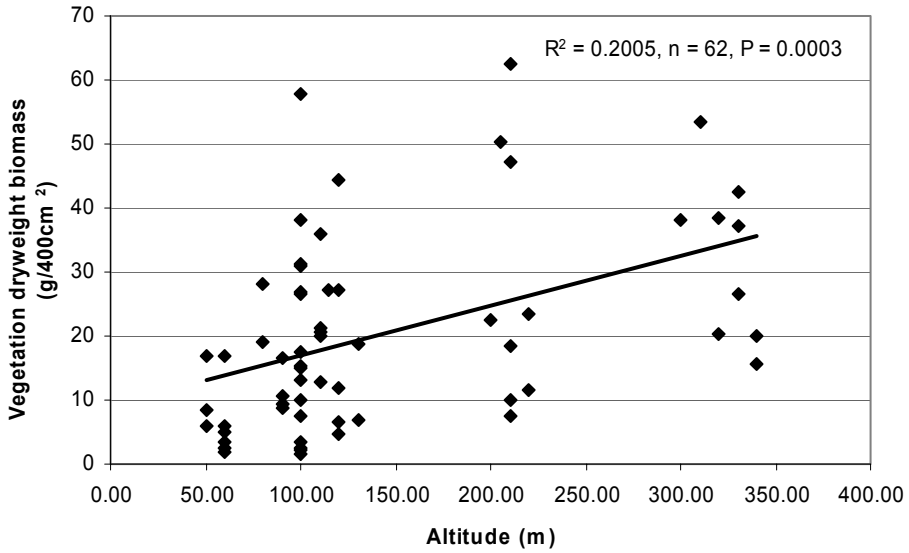

Fig 2.4. Significant positive linear relationship between altitude (m) of site and the dry-weight biomass of riparian flora (g/400cm^2) from samples taken in 2005.

Direct delivery of terrestrial plant matter to the stream environment is likely to be a good indicator of the relationship between riparian vegetation and allochthonous material supply to the stream. Here the riparian mean biomass is compared with visual estimates of the proportion of stream overhung by riparian vegetation. Fig 2.5 indicates a significant polynomial relationship between riparian biomass and proportion of stream overhung. Analysis using linear regression also yields a significant result (r^2 = 0.2812, n = 62, P < 0.001). However the r^2 value of the polynomial relationship is greater and may reflect reduced proportional overhang with increasing stream width (independent of biomass variation).

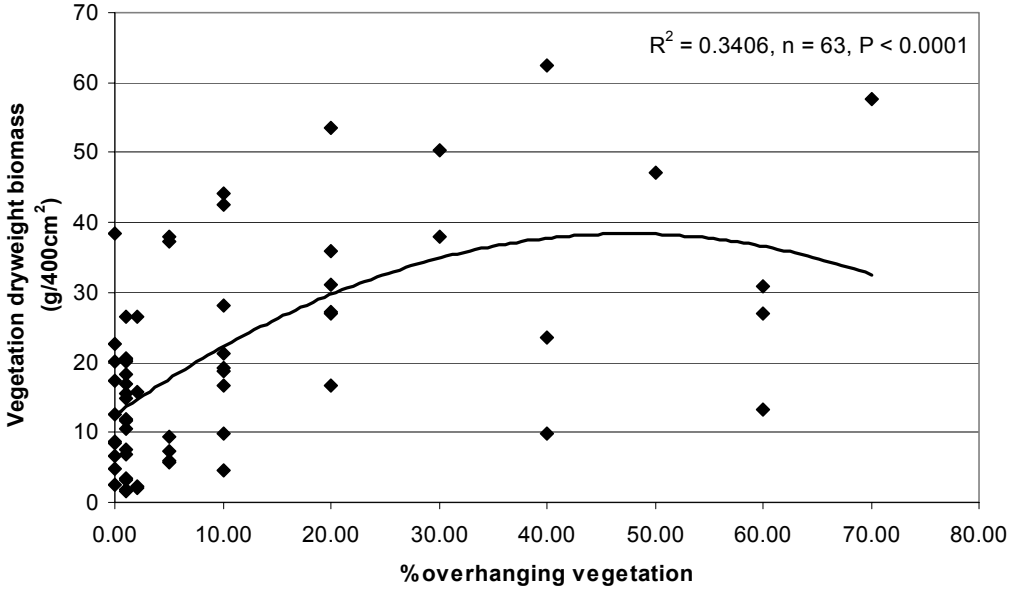

Fig 2.5 Significant polynomial relationship between bank vegetation biomass (g/400cm^2) and the estimated proportion of stream overhung by riparian flora (%) in 2005.

Sites were classified into categories according to their riparian characteristics: broadleaf, clear-felled, conifer corridor, open and conifer shaded. Fig 2.6 uses these categories to determine broad scale differences in the standing crop production of each site type and indicates a significantly greater biomass production at open sites.

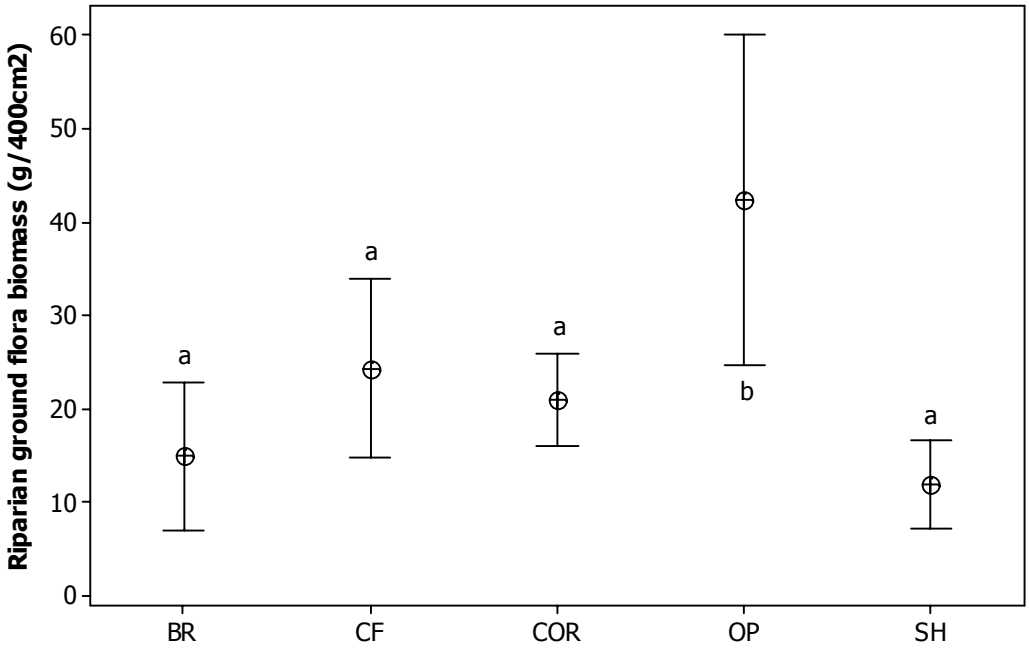

Fig 2.6. Variation in mean riparian ground flora biomass (± 95% confidence interval) within site classifications for 2005 (BR = Broadleaf, CF = clear felled, COR = corridor, OP = open and SH = Shade). Significant variation determined through ANOVA (P < 0.001) Tukey test used to indicate 'OP' has significantly greater riparian biomass (g/400cm^2). Significance indicated through differing lettering (a and b).

2.4.2 Species richness

Species number and diversity index (H) data for the study sites are given in Table 2-3.

Table 2-3. Species richness and Shannon-Weiner diversity index (H) scores for each site (± S.E). Bank-side and 3 meter sub-sites have been pooled. Sites have been ordered according to site-type classification.

Site	Species richness	Diversity (H)	Descriptor
Rowantree Br Mean	10.91 +/- 0.9	2.13 +/- 0.10	Broadleaf Shade
GT1 Broad Mean	9.66 +/- 1.22	2.07 +/- 0.11	Broadleaf Shade
Butler Broad mean	7.33 +/- 0.71	1.73 +/- 0.08	Broadleaf Shade
Wood of Cree Mean	11.33 +/- 1.28	2.12 +/- 0.39	Broadleaf Shade
GT3 Mean	11.00 +/- 2.42	2.08 +/- 0.22	Broadleaf Shade
SPP Broad Mean	7,86 +/- 1.44	1.79 +/- 0.17	Broadleaf Shade
High Mill Burn Mean	10.00 +/- 0.36	2.11 +/- 0.05	Conifer Clearfelled
Pulnagashel CF1 mean	7.25 +/- 0.66	1.74 +/- 0.11	Conifer Clearfelled
Pulnagashel CF2 mean	6.80 +/- 0.55	1.60 +/- 0.09	Conifer Clearfelled
T33 CF1 Mean	8.66 +/- 1.58	1.85 +/- 0.23	Conifer Clearfelled
T33 CF2 mean	11.66 +/- 1.76	2.22 +/- 0.14	Conifer Clearfelled
Black Burn (m) Cor/CF mean	7.00 +/- 0.51	1.73 +/- 0.06	Conifer Corridor/Clearfelled
GT1 Cor Mean	8.66 +/- 0.83	1.85 +/- 0.09	Conifer Corridor
Pulnagashel Cor Mean	6.70 +/- 0.53	1.63 +/- 0.10	Conifer Corridor
GT2 Mean	12.25 +/- 1.84	2.26 +/- 0.15	Conifer Corridor
Black Burn (B) mean	8.50 +/- 2.01	1.85 +/- 0.21	Conifer Corridor
FILI mean	10.50 +/- 1.87	2.07 +/- 0.20	Conifer Corridor
Laglany Shade Mean	3.5 +/- 1.2	1.052 +/- 0.34	Conifer Shade
Rowantree Sh Mean	8 +/- 0.6	1.84 +/- 0.07	Conifer Shade
GT1 Shade Mean	6.08 +/- 0.75	1.49 +/- 0.12	Conifer Shade
Black Burn (m) SH mean	6.16 +/- 1.27	1.56 +/- 0.16	Conifer Shade
T33 SH Mean	6.33 +/- 1.25	1.60 +/- 0.19	Conifer Shade
SPP Shade Mean	4.66 +/- 0.67	1.32 +/- 0.13	Conifer Shade
AIRIES Mean	4.66 +/- 1.08	1.13 +/- 0.24	Conifer Shade
Laglany Open Mean	7.5 +/- 1.3	1.73 +/- 0.20	Open
Rowantree Op Mean	8.83 +/- 0.66	1.94 +/- 0.07	Open
Butler Open Mean	9.16 +/- 0.79	1.91 +/- 0.13	Open
Black Burn (m) OP mean	6.00 +/- 0.73	1.56 +/- 0.12	Open

Temporal and spatial variation of the riparian species diversity (H) is shown in Fig 2.7, indicating that mean diversity of riparian vegetation was lowest during the March 2005 sampling season. There is also no indication of significant variation in the bank-side or 3 metre sampling station diversity indices.

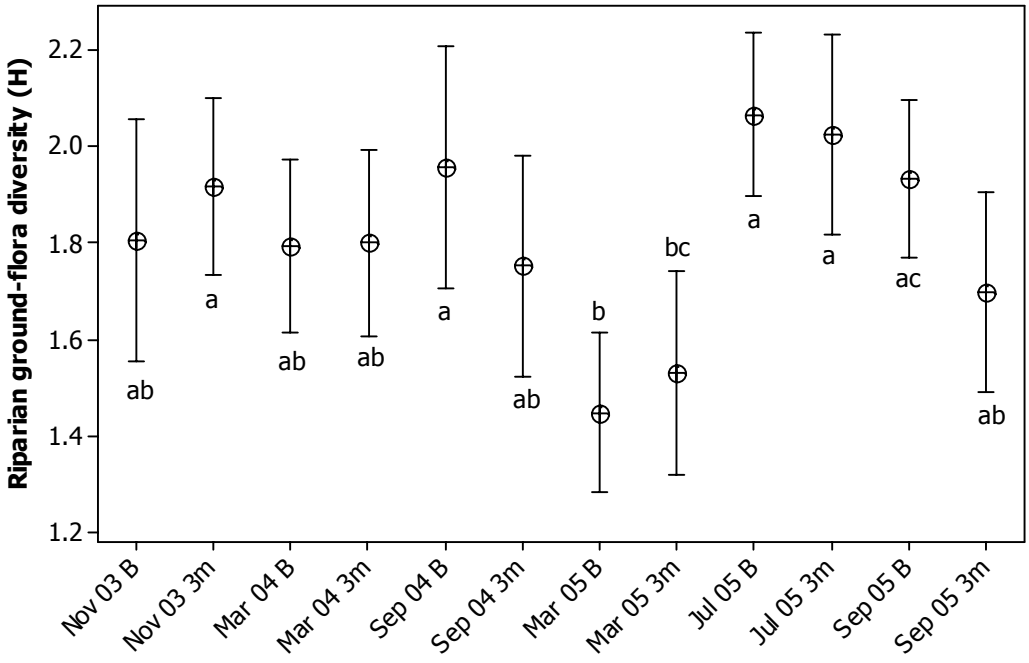

Fig 2.7. Temporal and spatial (Bank-side = B and 3m sites = 3m) variation in riparian vegetation diversity (H). Diversity as calculated by Shannon–Weiner index ± 95% confidence interval. Significance differenced between groups determined using ANOVA (P < 0.001). Tukey test (95% confidence) specified differences as signified here with differing lettering (a, b and c).

Consideration of the same corridor parameters analysed in conjunction with dry-weight biomass data were compared with the diversity of the ground-flora species assemblage data. Regression analysis of corridor variables found temporal variation in the riparian vegetation response to PAR. Samples from 2004 (Cree only) showed a significant positive linear relationship with increasing PAR (Fig 2.8). However, those samples pooled for 2005 (both Cree and Bladnoch) showed no significant relationship (correlation coefficient r, P = 0.96). This led to questions regarding the appropriateness of pooling the species data from both catchments within analyses.

ANOVA testing of the two catchments individually for both 2004 and 2005 combined indicated that there was no significant difference in the diversity of either catchment (P = 0.185). This was used as justification to pool the riparian vegetation diversity (H) data. The results of this can be seen in the following figures (Fig 2.8, Fig 2.9 and Fig 2.10). Pooling the data increases n, and also increases the applicability of the relationship to a wider spatial scale. However, there are also limitations to this

approach, specifically the increase of variance in the relationships, which will be discussed further, later in the chapter.

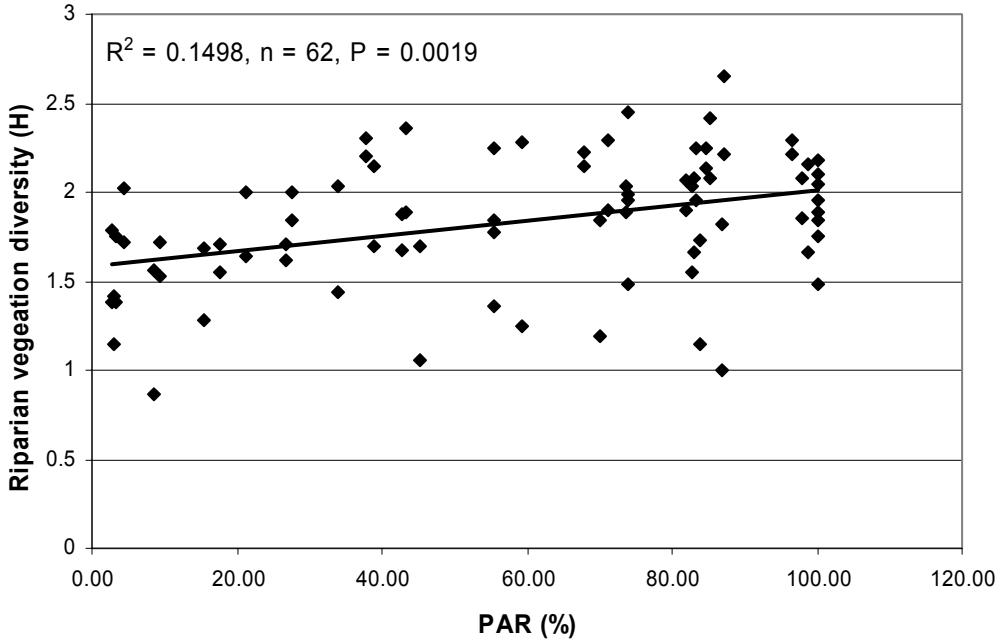

Fig 2.8. Significant positive linear relationship between light (% PAR) and riparian vegetation diversity in 2004 Cree samples (H).

In analysis of the other corridor characteristics, correlation coefficients of r provided mixed results. Estimated tree height was correlated with riparian ground flora diversity (H) using a polynomial negative curve, and as such, tree height appears to have a negative impact on the under-storey vegetation assemblage (Fig 2.9). This result is consistent with the negative linear relation between light and vegetation diversity found in year one of the study (Fig 2.4). This trend is not reflected however in corridor width data, as analysis of corridor width did not predict diversity (corridor width; $r^2 = 0.0066$, n = 213, P = 0.24).

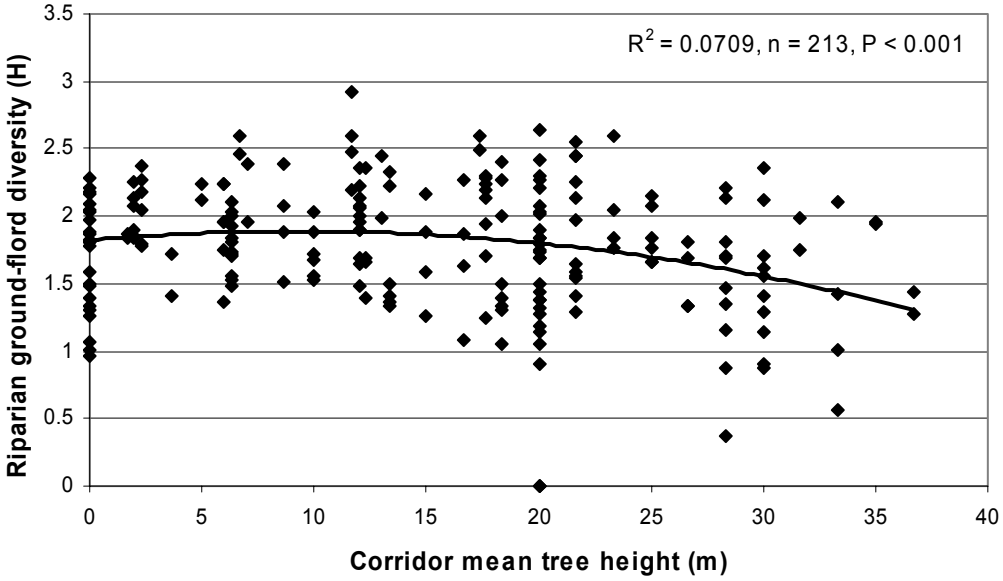

Fig 2.9. Significant negative polynomial relationship between mean estimated tree height (m) and riparian groundflora vegetation diversity (H). Pooled data from both catchmets and both 2004/2005.

Analysis of over-story tree species richness and ground flora diversity (Fig 2.10), and the overall differences in diversity within assigned site type groups (Fig 2.11), both suggest that broadleaf or species rich corridors support a greater diversity of riparian ground flora species. In year one, riparian sampling occurred either in spring or autumn (October, March and September), whereas 2005 sites include a greater number of broadleaf locations and a mid summer sample date (July). Therefore, trees would have been in full leaf and yet mean under-storey vegetation diversity of broadleaf sites is the highest of all site types (Fig 2.11), consequently reducing the direct negative influence of light on 2005 analyses.

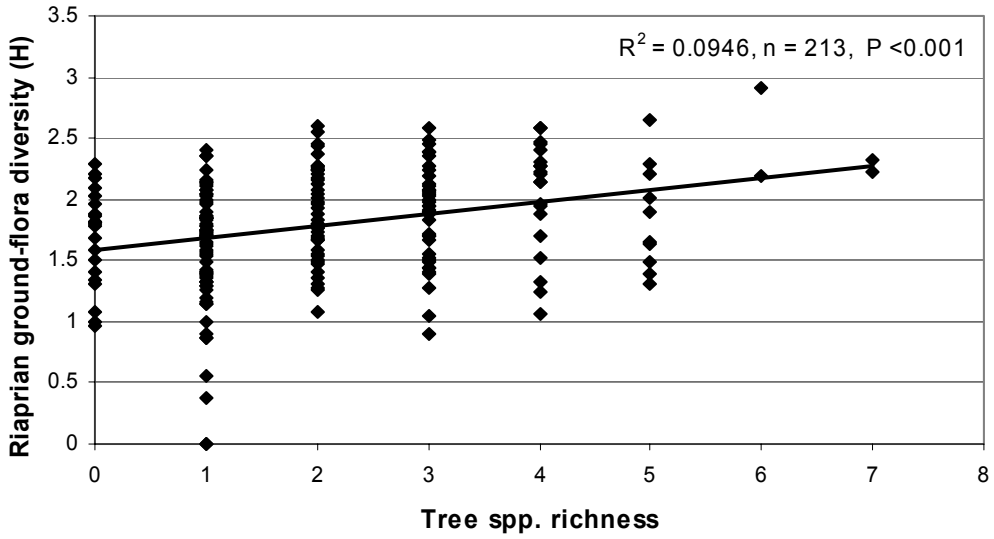

Fig 2.10. Significant positive linear relationship between tree species richness of site and riparian groundflora vegetation diversity (H). Pooled data from both catchmets and both 2004/2005.

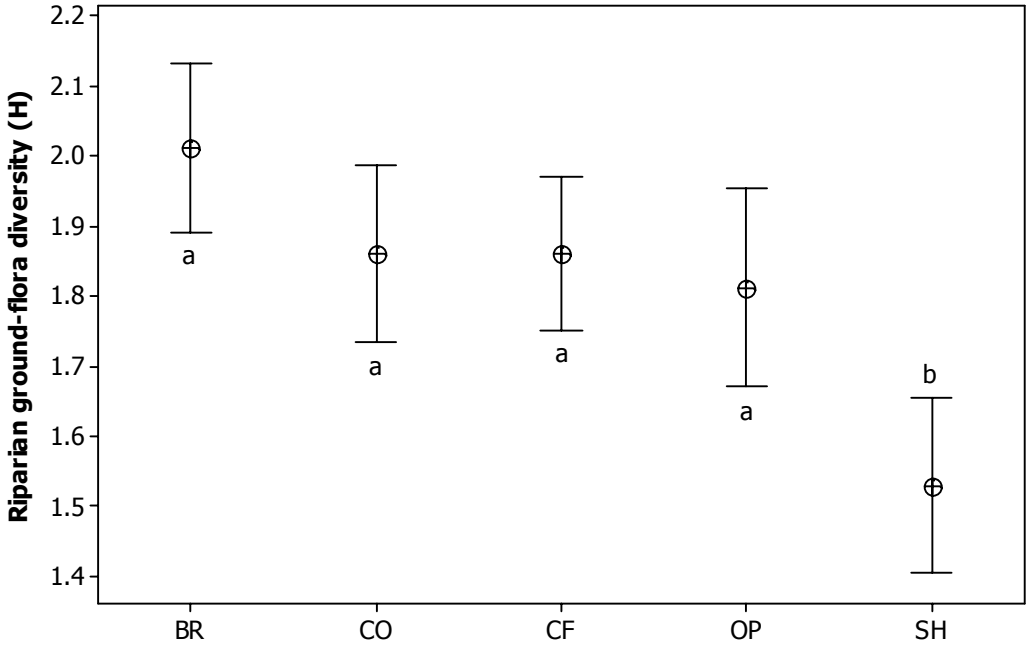

Fig 2.11. Variation of mean riparian ground flora diversity (H) (± 95% confidence interval) within site classifications (BR = Broadleaf, CF = clear felled, COR = corridor, OP = open and SH = Shade). Significant variation determined through ANOVA (P < 0.001). Tukey test used to indicate 'SH' has significantly lower riparian diversity (H). Pooled data from both catchmets and both 2004/2005.

2.4.3 Species ordination using Canonical Correspondence Analysis (CCA)

To explore the response of vegetation species assemblage structure to the riparian environmental variables, a canonical correspondence analysis (CCA) was carried out (Ter Braak, 1986) using the package CANOCO (Ter Braak and Smilauer, 1999). Classification using TWINSPAN analysis (Hill 1979) was also applied to the data, and groups assigned by TWINSPAN (Table 2-5) were identified within the CCA ordination to help assess environmental influence on the vegetation.

Within CCA 1 (Fig 2.12) inter-set correlations (Table 2-4) indicate that river width, altitude, corridor orientation, "East", % light, corridor width, tree height and tree diversity all contribute significantly to the assemblage structure of riparian ground-flora species. The majority of species are clustered on the opposing side of the ordination to increasing altitude, suggesting that this variable has a negative influence on species numbers.

TWINSPAN analysis separated the species list into four separate groups. However by overlaying this result on the CCA analysis, Fig 2.12 indicates that two of the groups identified (1 and 4), are clearly correlated with opposing environmental gradients. TWINSPAN groups 2 and 3 are primarily central to the ordination and as such show relatively little specific preferences. However, group 1 follows a gradient of over-storey tree diversity (often found in the broadleaf sites), tree height and low altitude (negative association with axis one and two -Table 2-4), whereas group 4 is closely associated with increasing light / corridor width (positive association with axis 2-Table 2-4). Both TWINSPAN groups 1 and 4 are of comparable size; indicating that the greatest diversity is seen at one or other extreme of the physical conditions associated with the two opposing gradients. TWINSPAN groups 2 and 3 are both much smaller in comparison, suggesting that intermediate conditions yield the lowest overall vegetation community assemblage diversity.

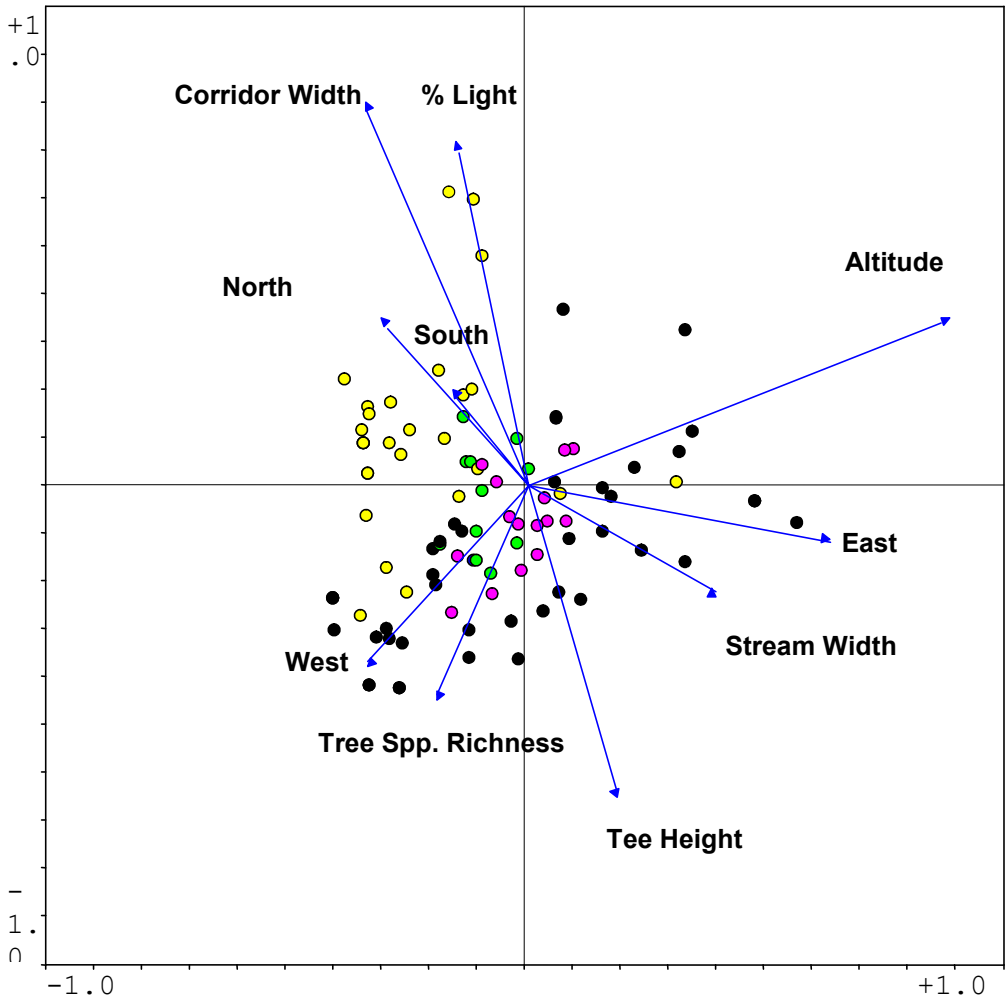

Fig 2.12. Canonical Correspondence Analysis (CCA) 1 (species vs. environmental variables): Eigenvalues for ordination axis one; 0.3484, axis two; 0.263, axis three; 0.263 and axis four; 0.1403. Sigificance of ordination defined through Monte-Carlo analysis (P = 0.005). TWINSPAN groups (Table 2-5), Group 1 – black, Group 2 = pink, group 3 = green and group 4 =yellow.

Table 2-4. CCA Inter set correlations of environmental variables with axes.

Variable	AX1	AX2	AX3	AX4
% Light	-0.125	0.556	-0.2482	0.3322
Stream Width	0.3369	-0.1785	-0.2742	0.4014
Corridor Width	-0.2919	0.6301	-0.1807	-0.0934
Tree Height	0.159	-0.5125	-0.054	-0.0007
Altitude	0.7415	0.2724	0.2078	-0.1065
Tree spp. Richness	-0.1639	-0.3509	0.6114	0.1791
North	-0.2525	0.2634	-0.0447	0.2166
East	0.5448	-0.0957	0.1231	0.0166
South	-0.1319	0.1508	-0.0129	0.1296
West	-0.289	-0.2934	-0.0964	-0.3647

Table 2-5. TWINSPAN species groups. Group one and two differentiations produced an eigenvalue of 0.388, groups three and four yielded an eigenvalue of 0.337. Within CCA1 (Fig 2.12), Group 1 – black, Group 2 = pink, group 3 = green and group 4 =yellow.

TWINSPAN Group 1	TWINSPAN Group 2	TWINSPAN Group 3	TWINSPAN Group 4
Anemone nemorosa	*Athyrium filix-femina*	*Agrostis stolonifera*	*Acer pseudoplatanus*
Blechnum spicant	*Brachythecium rivulare*	*Deschampsia caespitosa*	*Agrostis canina*
Calluna vulgaris	*Brachythecium rutabulum*	*Digitalis purpurea*	*Ajuga reptans*
Cardamine flexuosa	*Chrysosplenium oppositifolium*	*Holcus lanatus*	*Anthoxanthium odoratum*
Carex flacca	*Conopodium majus*	*Minium hornum*	*Anthriscus sylvestris*
Cotoneaster simonsii	*Dryopteris filix-mas*	*Primula vulgaris*	*Caltha palustris*
Dactylis glomerata	*Knautia arvensis*	*Ranunculus flammula*	*Carex diandra*
Dicranum majus	*Lonicera periclymenum*	*Rubus fruticosus*	*Carex rostrata*
Equisetum sylvaticum	*Luzula sylvatica*	*Viola palustris*	*Carex vesicaria*
Erica tetralix	*Lysimachia nummularia*		*Chamaenerion angustifolium*
Eriophorum angustifolium	*Oxalis acetosella*		*Cirsium palustre*
Festuca ovina	*Potentilla erecta*		*Cirsium vulgare*
Festuca pratensis	*Pseudoscleropodium purum*		*Epilobium palustre*
Festuca vivipara	*Pteridium aquilinum*		*Filipendula ulmaria*
Galium saxatile	*Sorbus aucuparis*		*Galium pulustre*
Geranium robertianum			*Juncus acutiflorus*
Hedera helix			*Juncus effusus*
Hieracium			*Myrica gale*
Hyacinthoides non-scripta			*Pellia* spp.
Ilex acquifolium			*Potamogeton polygonifolius*
Lamium purpureum			*Ranunculus ficaria*
Lathyrus spp			*Rumex acetosella*
Luzula multiflora			*Rumex obtusiflous*
Luzula spicata			*Salix lapponum*
Molinia caerulea			*Senecio aquaticus*
Philonotis fontana			*Senecio jacobaea*
Picea sitchensis			*Silene dioica*
Picea abies			*Stellaria alsine*
Polytrichum commune			*Stellaria media*
Quercus petraea			*Taraxacum officinale*
Rhytidiadelphus squarrosus			*Tussilago farfara*
Sphagnum spp.			*Valeriana officinalis*
Stellaria holostea			*Veronica montana*
Stellaria palustris			
Succisa pratensis			
Teucrium scorodonia			
Urtica urens			
Vaccinium myrtillus			
Veronica chamaedrys			

2.4.4 Comparison of CCA-derived assemblages with National Vegetation Classification communities

The species assemblage groups defined by TWINSPAN in CCA1 were analysed using TABLEFIT, to compare the assemblages identified by TWINSPAN with standard species assemblages of the UK National Vegetation Classification (NVC: Rodwell 1991, 1992). Each group is given a 'goodness of fit' score to describe how well it compares to standard NVC groups. The results are shown in Table 2-6.

Four distinct vegetation classification types were assigned. However, from the 'Goodness of Fit' score, it is clear that these vegetation types are not strongly comparable to nationally recognised vegetation assemblages, suggesting a mixture of nationally-recognised communities present within the sample areas. Group 3 has the best goodness of fit score. Such low fits to NVC categories are not unusual when sampling protocols are utilised which have physical habitat parameters as their criteria for site selection. In such circumstances it is common to have representatives of more than one NVC community type present within the sampling unit, inevitably leading to a reduction of the goodness of fit to NVC category scores (which are defined using phyto-sociological procedures, on purely botanical site selection criteria: Rodwell 1991, 1992).

Table 2-6 TABLEFIT classifications (Hill, 1989). Goodness to fit scores (GTF) are classified as: 80-100 = Very good; 70-79 = Good; 60-69 = Fair; 50-59 = Poor; 0-49 = Very poor. Table indicates both NVC and CORINE classification systems

Classification	NVC Community
2.4.4.1.1.1.1 Group 1	M15 - *Scirpus cespitosus - Erica tetralix*
NVC Sub-community	*Vaccinium myrtillus*
CORINE	C31.11 "Northern Wet Heath"
GTF Score	23
Group 2	W10e - *Quercus robur - Pteridium aquillinum - Rubus fruticosus*
NVC Sub-community	*Acer pseudoplatanus - Oxalis acetosella*
CORINE	C4.21 "Atlantic oakwood and bluebell"
GTF Score	33
Group 3	*Holcus lanatus - Deschampsia cespitosa*
NVC Sub-community	
CORINE	C37.213 "Deschampsia meadow"
GTF Score	58
Group 4	*Iris pseudacorus - Filipendula ulmaria*
NVC Sub-community	*Juncus effusus*
CORINE	C37.1 - "Meadowsweet grassland"
GTF Score	35

2.4.5 Site TWINSPAN classification and CCA ordination

From the TWINSPAN classification, eight groups of sites have been defined (Table 2-7). Indicator species have been assigned to each group (as described in the Table legend). The presence (positive association) or absence (negative association) of the indicator species qualifies the site (a specific assemblage of species) into each group classification.

Additionally, a second CCA (CCA2) ordination considers samples (site/date combinations) in relation to environmental variables (Fig 2.13). This ordination also shows overlaid sample-groups as produced by the TWINSPAN ordination of Table 2-7.

Table 2-7. TWINSPAN classifications of sites (in terms of vegetation assemblage) within both the Cree and Bladnoch catchments from November 2003 – October 2005. At the third level of division (iteration three), eight groups are produced. Separation of groups were all significant with eigenvalues of > 0.30 Group 1 is characterised by the positive association with *Viola palustrus*. Group 2, is positively associated with the presence of *Juncus effusus*. Group 3 is separated using the positive association with *Molinia caerulea*. Group 4 is associated with *Juncus acutiflora*, *Cirsium palustre, Viola palustris* and *Juncus effusus* at high abundance. Group 5 is characterised by the presence of *Pteridium aquilinum*, whereas Group 6 is characterised by the presence of *Oxalis acetosella*. Group 7 is indicated by *Deschampsia caespitosa* and abundant *Sphagnum* spp. Finally, group 8 includes the following indicator species: *Calluna vulgaris*, *Potentilla erecta* and abundant *Vaccinium myrtillus*.

Group 1	group 2	Group 3	Group 4	Group 5	Group 6		Group 7	Group 8
GT3BJ5	PCF1BM4	LOPBN3	BUTOPBN3	GT2BM4	LSHBN3	BBMSHM5	RSH3N3	RBRBN3
GT33J5	PCF13M4	PCF1BM5	BBMCF3J5	BBMOPBJ5	PCF1BN3	BBMSHM5	PCF23M4	RBR3M4
SPPBRBJ5	PCF2BM4	LOP3N3	FILI3J5	GT23M4	PCO3M4	SPPSHBM5	HM3S4	ROPBS4
SPPBR3J5	LOP3S4	PHELLBN3	FILIBS5	GT2BS4	GT1SHBS4	SPPSH3M5	GT1CBM5	RBR3J5
GT1CBS5	BUBRBS4	PHELL3N3	BUTOP3N3	GT23S4	GT1C3M5	AIRBM5	GT1SHBJ5	RBR3N3
GT3BS5	BUBR3S4	LOPBM4	BUOPBM4	BBMOPBM5	SPPBRBM5	AIR3M5	PCF2BS5	ROPBN3
GT33S5	PCF1BS4	LOP3M4	BUOP3S4	BBMOPM5	BBMSH3J5	BBMSHBJ5	GTBSHBN3	ROP3N3
	PCF13S4	LOPBS4	T33CF13M	BBB3M5	T33SHBJ5	PCO3M5	GT1SH3N3	RSHBN3
	PCF2BS4	GT3BM5	FILIBM5	BBMOP3J5	WOCBS5	WOCBM5	HMBBN3	GT1BR3N3
	PCF23S4	BBBBM5	BBMCFBJ5	BBB3J5	SPPSH3S5	WOC3M5	HMB3N3	RBRBM4
	PCF2BM5	FILI3M5	T33CF1BJ	AIRBJ5	LSH3N3	T33SHBM5	RSHBM4	ROPBM4
	GT33M5	PCF23J5	T33CF13J	AIR3J5	GT1BRBN3	T33SH3M5	HMCFBM4	ROP3M4
	PCF1BJ5	BBMCFBS5	T33CF2BJ	BBMC3S5	GT1CBN3	SPPSHBJ5	GT1SHBM4	BUOP3M4
	PCF13J5	AIRBS5	T33CF23J		GT1C3N3	SPPSH3J5	GT1SH3M4	HMCF3M4
	PCF2BJ5		BBBBJ5		BUTBRBN3	BBMSH3S5	GT1CBM4	RBRBS4
	BBMSHBS5		FILIBJ5		BUTBR3N3	GT1CO3J5	RSHBS4	RBR3S4
	PCF1BS5		BBMCBS5		PCF13N3	T33SHBS5	RSH3S4	ROP3S4
	PCF13S5		T33CF1BS		LSHBM4	GT1C3S5	HMBS4	GT1BR3S4
	BUBR3M4		T33CF13S		LSH3M4	PCOBJ5	RSHBM5	RBR3M5
	BUOPBS4		T33CF2BS		RSH3M4	PCO3J5	RSH3M5	ROPBM5
	T33CF1BM		T33CF23S		BUBRBM4	WOCBJ5	BBMCFBM5	ROP3M5
	BBB3S5		BBBBS5		PCOBM4	WOC3J5	BBMCF3M5	RBRBJ5
			FILI3S5		GT1BRBM4	T33SH3S5	GT1SHBM5	ROPBJ5
					GT1BR3M4	PCO3S5	GT1SH3M5	ROP3J5
					GT1C3M4	SPPBRBS5	PCF13M5	RBRBS5
					PCOBS4	SPPBR3S5	PCF23M5	RBR3S5
					PCO3S4	SPPSHBS5	T33CF2BM	ROPBS5
					GT1BRBS4		T33CF23M	ROP3S5
					GT1SH3S4		RSHBJ5	
					SPPBR3M5		RSH3J5	
					PCOBM5		GT1SH3J5	
					T33SH3J5		PCF23S5	
					GT1COBJ5		RSHBS5	
					WOC3S5		RSH3S5	
					AIR3S5		BBMCF3S5	
					GT1COBS4		GT1SHBS5	
					GT1CO3S4		GT1SH3S5	
					RBRBM5		PCOBS5	

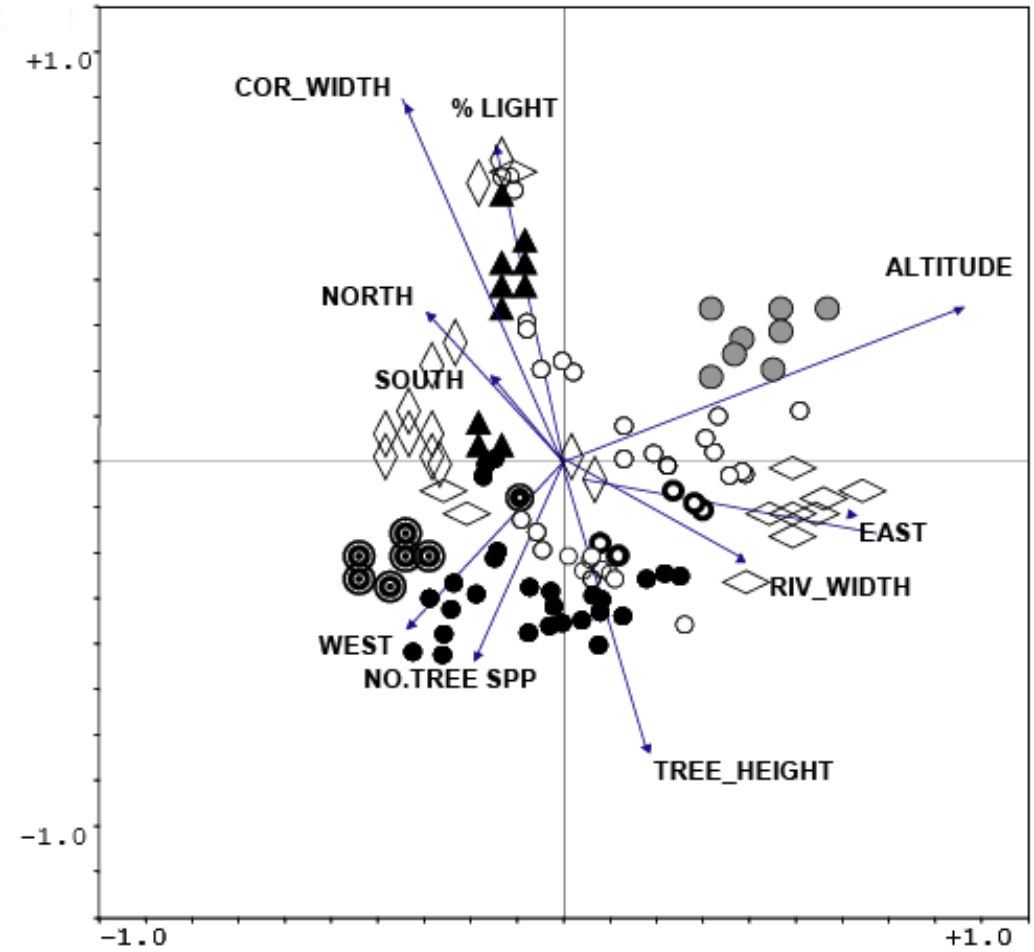

Fig 2.13: CCA 2 Sites in relation to environmental variables. TWINSPAN group membership as shown in Table 2-7. TWINSPAN Group symbols: Large layered circles = group 1, Black triangles = group 2, Horizontal diamonds = group 3, Vertical diamonds = group 4, dark edged circles = group 5, Black circles = group 6, White circles = group 7 and grey large circles = group 8.

Sample points are identified with differential symbols to illustrate TWINSPAN sample group membership (Table 2-7). Broadly, the samples can be split into those associated with broadleaf and low light conditions, and those of more open and light intensive habitats. For example, TWINSPAN groups 6 and 1 are indicated by the presence of shade adapted *Oxalis acetosella* and *Viola palustris,* respectively. In addition, these sites, located towards the bottom left of the ordination plot, are closely correlated with increased tree biodiversity, increased tree height, low altitude and low light intensity (percent light). Conversely, TWINSPAN groups 2 and 4 are indicated by the presence of *Juncus effusus* (for group 2) and *Juncus articulatus*, *Cirsium palustre, Viola palustris* and abundant *Juncus effusus* (for group 4); species which better represent an open and wet habitat.

2.5 Discussion

Biomass of riparian vegetation could be related to the potential delivery of riparian material to the in-stream habitats and thus, the contribution of allochthonous material to in-stream consumers. Comparisons of riparian vegetation biomass and the proportion of overhanging vegetation of the stream yielded a positive relationship (Fig 2.5). It was suspected that the significance and associated r^2 values of this relationship were affected by the relative width of the watercourses and, as such, future measurements should be modified to account for stream width. However, the significant relationship shown in Fig 2.5 is evidence that the proportion of allochthonous material overhanging the stream banks was related to the riparian ground-flora standing crop and thus the light level (PAR) of the riparian zone (Fig 2.3).

Overhanging material provides a direct source of allochthonous carbon as well as increasing the delivery of drift invertebrates, which can be essential components of consumer diets. For example, stream consumers, such as fish, depend for up to half of their diets on terrestrial invertebrates that fall into streams (Elliott 1967, 1973; Cloe and Garman, 1996*;* Johansen *et al.,* 2000; Kawaguchi and Nakano, 2001). These areas of overhanging vegetation also act as refuges for invertebrates and fish (Eklöv and Greenberg 1998) and are often utilised in the emergence of the adult forms of many aquatic invertebrate (see Sabo and Power 2002; Kato *et al.,* 2003*)*. In this context, it seems clear from the results presented above that the design of riparian zones in forest corridors should take better account of the desirability of enhancing light availability to increase the abundance of riparian ground-flora, and especially and overhanging vegetation. This could be achieved directly through promotion of wider corridors and reduction of riparian tree cover at bankside locations.

It was hypothesised that there would be a direct link between riparian biomass and the quantity and type of allochthonous material present within the stream water column. Experimental collection of detrital matter available within the water column was explored through use of detritus traps, set during two months of the 2005 sampling season (May and June). However this experiment failed to be consistently effective, mainly due to large variation in stream water level, resulting in many traps

being lost, broken, filled to capacity before collection, blocked or positioned above the current water level at the time of recovery. Thus, making comparisons of riparian biomass or riparian environmental conditions with allochthonous organic matter availability impossible. Details of the data are in Fig 10.1 of the appendix, and although the total dry-weight collected cannot be successfully used in analysis, the results do illustrate that within sites, allochthonous biomass from a monthly flow can yield up to 1037g (within a $100cm^2$ cross-section area per month) dry-weight organic matter. This maximum value was from Wood of Cree, a broadleaf, low altitude site and so it seems likely that the biomass collected was of riparian tree origin rather than ground-flora.

Both production and diversity have different significances to the functioning of the stream and corridors under study. Specifically, ecosystem ecological integrity is often measured using diversity within a specific indicator trophic group, yet between trophic groups (across a food chain), ecosystem integrity and overall system biodiversity may be more heavily influenced by the productivity and biomass of a specific producer/prey trophic group. The overall aim of this project was to examine the conditions which best support the biodiversity, bio-integrity and overall functioning of the in stream and riparian habitat of forest streams. Thus, here diversity and standing crop were examined to determine which set of environmental variables best supported the greatest diversity within a single trophic group, yet also across the whole system. However, to attain maximum system biodiversity, diversity of a single trophic group (e.g. riparian vegetation), may have to be essentially 'sacrificed' in favour of conditions favouring greater biomass production.

Diversity of riparian vegetation data was pooled for all sample points (both catchments and both sample seasons). This allowed for greater applicability of the results to a wider scale, and reduced the danger of too great a focus on systems in isolation. However, although the greater sample size gained by this approach decreased P values, and increased significances, the high level of variance in the relationships was apparent with the r^2 values obtained. Such a result may have been amplified by limitations to the physical parameters measured. For example, correlations between light levels and diversity will inevitably be effected by the lack of differentiation between broadleaf and coniferous shading. Improvements to the study should perhaps consider this problem in greater detail. However, separation of

sample sites into generic site-type classifications aided in deciphering potential variation between the role of light at these sites. However, future analysis of data using multi-regression analysis would provide some information on the significance of the physical corridor parameters and diversity within the boundaries of either season or catchment, and thus, provide a greater detail of information on the relationships occurring, and reduce the inevitable increased variance associated with pooled data sets.

Although vegetation standing-crop was measured only in 2005, greatest biomass was found at higher altitude locations (Fig 2.4) within areas of greatest light availability (PAR) (Fig 2.3). Many studies have noted the reduced diversity of ground-flora species within upland, open, moor land habitats and the dominance of grass species such as *Deschampsia flexuosa* (Bokdam and Gleichman, 2000). However, within this study high diversity could be found within two very different habitat types; low altitude broadleaf areas and higher altitude light intensive areas. Yet intermediate conditions were less favourable to assemblage diversity (Fig 2.12 and Table 2-5). However, using broad classification of sites according to the riparian characterises indicated that open areas yielded the greatest standing crop biomass (Fig 2.6), and although only riparian vegetation diversity at conifer shaded sites was deemed significantly lower then the other sites (Fig 2.11), mean vegetation biodiversity was greatest at broadleaf sites. As such, there is a suggestion that a trade off between biomass and diversity exists. Specifically, that as suggested within this study, and similar studies (e.g Bokdam and Gleichman, 2000), management must prioritise between promotion of maximum overall diversity within the ecosystem and maximum diversity within the riparian vegetation trophic group. Overall, it would appear that increase of light level here did not significantly reduce the diversity of the riparian vegetation trophic group. Yet under specific circumstances, it may be of greater benefit to reduce light level in order to promote the growth of specific species assemblages (or rare species) and/or to promote the maximum vegetation diversity (as found within broadleaf sites), at the expense of overall standing crop biomass.

Benthic macro-invertebrates arguably represent the key consumers of allochthonous material which is likely to be entering the food-web through riparian vegetation delivery routes. The populations, assemblage structure and overall abundance and diversity are explored in detail in Chapter five. At this point however Fig 2.14

indicates a significant positive linear relationship observed between riparian biomass and benthic macro-invertebrate diversity.

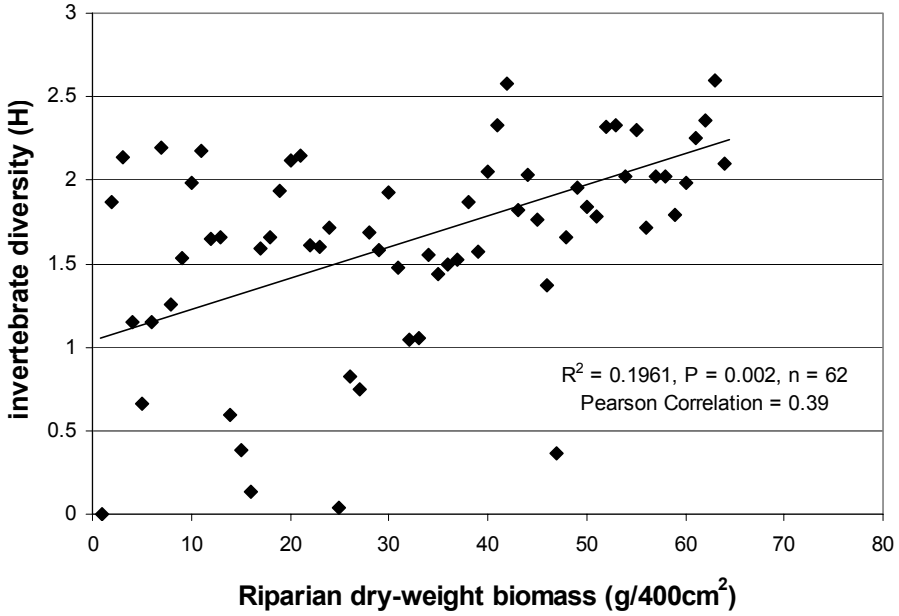

Fig 2.14. Significant positive linear relationship between riparian ground-flora biomass and in-stream benthic macro-invertebrate diversity (H). r^2 suggests that approximately 20 % of variation in data is described by this relationship. While Pearson's Correlation shows a week positive correlation of 0.39. Although the r^2 value suggests high variance in this relationship (suggesting additional influences to invertebrate diversity), there is still high significance, indicating that riparian vegetation is an important parameter to be considered.

However, when the diversity of riparian vegetation is explored in the same manner, there is no relationship (r^2 = 0.003, n = 62, P = 0.89) between riparian vegetation diversity and invertebrate diversity. Similarly, neither vegetation biomass nor diversity is related to overall invertebrate abundance (P = 0.8, n = 62, r^2 0.005 and P = 0.5, n = 62, r^2 = 0.0064, respectively). Thus, these results suggest that in order to maximise the diversity of invertebrate species as a function of riparian vegetation, riparian vegetation biomass should increase irrespective of riparian vegetation diversity. Thus, although a scenario of relatively low light conditions, characterised by diverse and broadleaf riparian over-storey trees and greater tree height provide conditions promotes the greatest riparian ground-flora diversity, these conditions do not promote high biomass production. As such, over storey tree-derived allochthonous detritus material associated with broadleaf tree cover does not appear to promote greatest diversity of in-stream macro-invertebrate species within this system.

Although increased biomass and associated overhang of ground flora vegetation from within the riparian zone promotes a significant increase of aquatic invertebrate diversity, it is not possible to presume that this result can be directly related to an increase in the availability of allochthonous biomass. Availability of allochthonous organic matter within the water column was at a maximum at broadleaf sites (see rejected detritus traps experiment results – Fig 10.1 of the appendix). Thus, there is the suggestion that allochthonous organic matter derived from broadleaf over-story trees is not as beneficial to invertebrate community composition as the allochthonous material derived from riparian ground flora. This result may indicate that broadleaf tree-derived allochthonous litter may be of lower palatability. Alternatively, the non-food benefits of riparian overhanging vegetation described earlier (e.g. habitat complexity, delivery of drift invertebrates and modification of flow patterns) may be of greater benefit to the in-stream assemblage then the delivery of allochthonous organic material. Consequently, the design of corridor characteristics should consider the importance of both the potential variability in palatability of allochthonous resources to consumer groups, and the other benefits gained by riparian ground flora vegetation overhang. In specific circumstances, where key species are of concern, management may wish to orientate habitat design priorities and requirements to either the in-stream or riparian zones in order to maximise the benefits to specific species of concern. However, within this study, as diversity of riparian vegetation was statistically comparable at open sites to broadleaf sites, it is suggested that to accomplish maximum optimal overall diversity across both trophic levels. Management should consider increasing riparian zone light intensity and "openness".

3 Assessment of the autotrophic contribution of algae to benthic biofilms within two low order streams in South-west Scotland.

3.1 Abstract

The chapter discusses stream biofilm characteristics obtained through measurements of benthic organic matter standing crop and diversity within two catchments in South West Scotland subject to extensive afforestation. Understanding resource availability supporting the specific community structure found within differing habitat conditions, and particularly light regimes, is key to delineating optimal forest stream corridor design.

Stream biofilms can also influence primary production, community respiration, microbial breakdown of detritus and the retention of nutrients, thereby playing a major role in overall ecosystem functioning.

The aims of this chapter are to describe and quantify the characteristics of biofilm resources within study streams, through specific measurements of:

- Temporal and spatial variation in the composition, general characteristics and overall production of the baseline resource,
- Variation in autotrophic production and diversity with changing corridor characteristics,
- Potential physical factors which can affect biofilm elemental composition,
- Variability in the functioning capacity of the biofilm with changes in compositional characteristics.

A two-year sampling programme of biofilm analysis was undertaken in the Black Burn (BB: Cree catchment) and a one-year replicate in stream T33 (Bladnoch catchment), using artificial, semi-flexible substrates for biofilm growth and settlement experiments. Although the uppermost site of BB was felled prior to sampling within 2005, this approach provides information on in-stream resources and specifically autotrophic biomass, and also modifications to biofilm characteristics through measurements of:

- Chlorophyll data,
- Total carbon
- Diversity of the algal component of the biofilm; determined through microscope analyses.

Biofilm biomass significantly increased in summer 2004 and 2005 in BB biofilms. There was spatial variability in biofilm production with significantly greater mean overall biomass at BB sites. Additionally, intra-site analysis indicated higher biomass settlement in BBCF, 2005. Analysis of chlorophyll *a* concentration indicated that T33 and BB 2004 biofilms had the greatest mean chlorophyll *a*. Analysis of algal species composition indicated that sites in BB 2005 were significantly different from those of T33, in their algal community composition and had comparably reduced algal diversity.

Analysis of C:Chl indicated that very few of the BB biofilms had high algal contributions. Highest C:Chl was found at BB 2005, indicating minimal algal proportion. Applying a conversion factor to chlorophyll data allowed for the prevalence of algae (mg/m^2) to be calculated. This approach indicated that biofilms from T33 had the highest algal biomass of all locations/sample years. Although estimations of T33 and BB 2004 were comparable, most BB 2005 samples were significantly chlorophyll *a* (Chl *a*) depleted. As overall autotrophic proportion of biofilm material within BB 2005 was low, biofilms were dominated by detritus and heterotrophic material. Consideration of this previous work suggests that in relation to the present study, the autotrophic production limitation found post-felling, may be contributing to reduced nutrient retention, processing and overall buffering of excess nutrients by in-stream biofilms

3.2 Introduction

Stream biofilms can play a major role in overall ecosystem functioning as they affect primary production, community respiration, microbial breakdown of detritus and the retention of nutrients (Sabater et al., 2002). Biofilms are generally made up of a matrix of algal cells, particulate organic matter at different stages of decomposition, and microbial organisms (bacteria, protozoans and fungi). Within this matrix, autotrophic algae convert atmospheric CO_2 to carbohydrates and other photosynthetic products, while bacteria and other fungi are essential to the breakdown of detritus. Bacterial production is directly influenced by this autotrophic production. Yet, bacterial populations are controlled by food availability and protozoan predation. Predation by these hetrotrophic consumers often forces bacterial colony formation and thus, aids in the overall biofilm formation (Arndt et al., 2003). Primary production and detrital decomposition are influenced by nutrient availability. In streams, dissolved nutrients are continuously delivered to colonised surfaces via unidirectional water flow. Nevertheless, nutrients (primarily nitrogen, N and phosphorus P) are often limiting to algae, bacteria and fungi in these systems (e.g. Pringle et al., 1986, Tank and Webster, 1998). Additionally, algal biomass production is heavily influenced by a suite of other factors which include light (Hill, 1996), temperature, invertebrate grazing (Steinman, 1996), and scouring caused by increased flow (Biggs and Close, 1989, Peterson, 1996). This can produce variation in the weight of biomass, and also the relative contribution made by each component (i.e. algae, bacteria, fungi and allochthonous detritus) of the biofilm to ecosystem functioning.

Many studies of benthic systems assume that periphyton is primarily composed of algal cells (e.g. Frost and Elser, 2002; Bowman et al., 2005), However, recent studies have demonstrated that algal cells can be a minor component of 'periphyton' (e.g. Frost et al, 2001; Hamilton et al., 2001). Consequently, this chapter aims to quantify the proportional contribution of dominant energy sources available in streams through consideration of benthic biofilms. In order to achieve this objective, a number of potential approaches to characterising the baseline resources utilised by benthic biofilms will be explored.

Biofilms are essential to purification of river waters (Pusch et al., 1998) as they take up and retain organic and inorganic nutrients from the waters passing over them (Burkholder et al., 1990, Flemming, 1995). However, several factors can affect the functioning of biofilms as pollutant removers. Physical effects can include water velocity, water temperature and light penetration; chemical effects include pH and nutrient availability; and the biological effects include grazing pressure, biofilm biomass and the relative proportion of heterotrophs to autotrophs (algae) within the biofilm (Stevenson, 1996). Romani and Sabater, 2000 showed that benthic heterotrophs within the biofilm use by-products excreted by the autotrophs. Benthic algae and bacteria actively exude substantial quantities of organic carbon, primarily as exopolymeric substances (e.g. Hoagland et al., 1993). These exudates can constitute a large proportion of the carbon acquired by algae and bacteria (e.g. Goto et al., 1999). This nutrient production from within the biofilm has been found to increase the capacity of biofilms to function as water purifiers (Romani and Sabater, 2000). A ratio of around 3:1 of autotrophs (e.g. algae) to heterotrophs (e.g. bacteria) was found to facilitate the largest increase in the ability of the biofilm to break down allochthonous detritus (Romani and Sabater 2001).

The ability of the biofilm to produce energy (through the breakdown of allochthonous detritus and through primary production by the algae) and purify water is greatly affected by the biomass and thickness of the biofilm, where increasing biomass increases primary production. An increase in grazing by aquatic invertebrates decreases the thickness of the biofilm and so reduces its functional capacity (Mullholand et al, 1994). However, invertebrate grazing is not uniform (e.g. Tuchman and Stevenson, 1991) and so changes in the algal composition due to selective grazing can also greatly affect biofilm functioning. For example, Marti et al. (2004) found that Chlorophyceae (green algae) had a greater photosynthetic and nutrient uptake capacity than diatoms. Thus, hypothetically, selective grazing of Chlorophyceae by invertebrates, could result in a less productive biofilm algal component.

Amongst the microbial organisms present in biofilms are Hyphomycete fungi. These diverse micro-fungi are important decomposers and play a crucial role in the conditioning of leaf matter for invertebrate consumption (Barlocher and Kendrick, 1976). Microfungi can account for up to 17% of the detrital leaf mass and,

depending on the area of the stream, may account for as much of the stream production as bacteria or invertebrates (Gessner, 1997). In coniferous forest streams, conifer needles can support a substantial number of hyphomycete fungal species (as much as broadleaf species), though the rate of this colonisation is slow and so requires long periods (1-2 years) of submersion within the system to produce significant effects (Barlocher, 1992). Needle detritus retention is generally low in conifer forest plantations as the reduced natural dropping of woody material and debris (which would be the most efficient mechanism for retention: Trotter, 1990) is often limited when trees are felled and removed at the young age associated with modern plantation cycles (Cariss and Dobson, 1999).

The degree to which different streams are dependent on autochthonous or allochthonous carbon inputs has long been of interest to stream ecologists. Sampling bias towards woodland streams with large visible terrestrial inputs led to the concept that allochthonous sources dominated in low order streams. More recently, it has been suggested that the importance of autochthonous carbon in supporting consumer production in streams has been underestimated (Minshall, 1978), particularly in forested headwater streams (e.g. Hawkins et al., 1982; Mayer and Likens, 1987).

Since both autochthonous and allochthonous inputs are regulated primarily by the density of riparian vegetation, measuring algal production under different canopy conditions can give insight into the relative importance of autochthonous carbon inputs. Within conifer-forested catchments the degree of allochthonous and autochthonous production is extremely variable and both can often be limited (Dobson and Cariss, 1999). However, if primary production is very low, then autochthonous carbon cannot be a significant energy source in the system. If primary production is substantial, then autochthonous carbon has at least the potential to contribute to invertebrate and fish production.

Large-scale anthropogenic activities such as logging can produce fundamental alterations to the supply of limited resources to the aquatic habitat, such as increased light (e.g. Hill et al., 2001) and nutrients (Rounick and Winterbourn, 1982). It has been suggested that the increase in solar flux, which is associated with forest clearance, results in an increase in primary production, which then extends to an

increase in stream invertebrates and vertebrates (Murphy, 1998). Whether increased solar flux is brought about as a result of active forest clearance, or as a consequence of wide or open riparian zones, the expected positive influence on algal biomass and overall levels or autochthonous primary production has been widely reported in past studies (e.g. Gee and Smith, 1997). As light availability is one of the primary physical characteristics which can be directly influenced by design and management of stream riparian zones, an ability to quantify the response by in-stream primary producers to variation in light regimes, will provide information on the potential resource production increase which can be achieved through specific changes in riparian corridor design. Chlorophyll *a* measurements, as well as cell counts of algae and bacteria, have successfully been used to illustrate greater productivity in light-grown biofilms and to underpin suggestions that light availability has a significant impact on the production and functioning of a heterotrophic biofilm (Romani and Sabater, 1999). Here, however, I use Chl *a* concentrations as a proxy for algal biomass, as has been done is many previous studies (e.g. Romani and Sabater, 2000; Frost et al., 2005; Rosenfeld and Roff, 1990), and seek to determine changes in standing crop with light regime. It is not possible to demonstrate changes in productivity without measures of primary production and respiration, and consideration of control mechanisms such as grazing and scouring.

The importance of being able to quantify the algal contribution to the biofilm is emphasised when considering interaction between the autotrophic and heterotrophic components of the biofilm. Several studies suggest that this relationship has significant potential to influence functioning capability. Algae are important in increasing surface area for bacterial colonisation (Geesey et al., 1978) as well as in the production of metabolites used as an energy resource by bacteria (Haack and McFeters, 1982). As a consequence, the enzymatic activity of the bacterial community appears to be directly correlated to the chlorophyll concentration and biomass of the autotrophic component of the biofilms (Romani and Sabater, 1999). For example, in dark-incubated biofilms, the bacterial response to any chlorophyll accumulation/addition is rapid (Romani and Sabater, 1999). Biomass of algae is also important as low-light grown biofilms require a much greater overall chlorophyll density to support the same bacterial population and enzyme activity than autotroph-rich biofilms grown under light-intensive conditions (Romani and Sabater, 1999). Thus increased light intensity promotes growth of biofilms with abundant algal

populations, whose production of polysaccharides accumulating within the matrix may act as an organic matter reservoir (Freeman and Lock, 1995) for internal bacterial activity. Such biofilms often show increased potential to adapt to variable, and specifically, reduced aquatic nutrient supplies. Consequently, where primary productivity in biofilms is high enough to supply the heterorophic component with sufficient exudates, an autotrophic biofilm can become relatively 'self sufficient' in its energy production.

Thus, it could be hypothesised, that with highly autotrophic biofilms, the response of the heterotrophic component of light-grown biofilms to increases in water nutrient levels would be reduced. The consequence of the increased availability of internal metabolites for biofilms showing high autotrophic production would be a reduced requirement to draw down organic material from nutrient enriched waters. As such, dark-grown and low autotrophic biofilms may retain a greater proportion of organic material despite reduced overall functioning.

Such studies illustrate the point that it is not possible to assume that changes in environmental conditions (e.g. increased light availability following tree felling) necessarily produce a predictable biological response from each ecosystem component within a given time period. Algae and macro-invertebrates, for example, may respond to a change in light availability on quite different time scales (e.g. Gee and Smith 1997). In order to determine the true impact of altered light regime on the bio integrity ("ecological health") of the stream, biological monitoring using diversity, abundance and species evenness, of both groups of organisms at different trophic levels in the food chain would be desirable.

A study of the impact of stream corridor characteristics on biodiversity would be incomplete without generating an understanding of biofilm response to changing and changed environmental parameters. However, in order to be consistent with past methodologies (and to permit comparison with results in the wider literature), more traditional approaches to characterising autochthonous benthic primary production were applied in this study. In addition, measures of the carbon content of the biofilm provide information on the quantity of basic energy resources available to higher trophic consumer groups. In summary, the approaches used in this chapter aimed

to characterise biofilms using chlorophyll *a* measurements, dry-weight biomass, biofilm carbon content, and microscopic analysis of algal species composition.

3.3 Aims

1. To estimate algal biofilm component from chlorophyll measurements in order to gain information on algal productivity. This provides information from which the biofilms can be classified as either heterotrophic or autotrophic, thus allowing inference of the functioning capacity of the biofilm.

2. To quantify the resource quality of the biofilm, as described by carbon and nitrogen content, providing information on the quality of biofilm material available for use by organisms at higher trophic levels within the stream system.

3. To assess temporal and spatial variation in algal community composition in relation to environmental factors, in order to determine the relative importance of physical, chemical, and biotic factors in predicting assemblage and diversity of stream periphyton

3.4 Methods

3.4.1 Study Site

The study was undertaken in two low order streams in Galloway (Fig 3.1): the Black Burn within the Cree catchment, (2004 and 2005); and T33 within the Bladnoch catchment (sampled in 2005 only).

Fig 3.1. Regional location of the study in Galloway modified from Ordnance Survey.

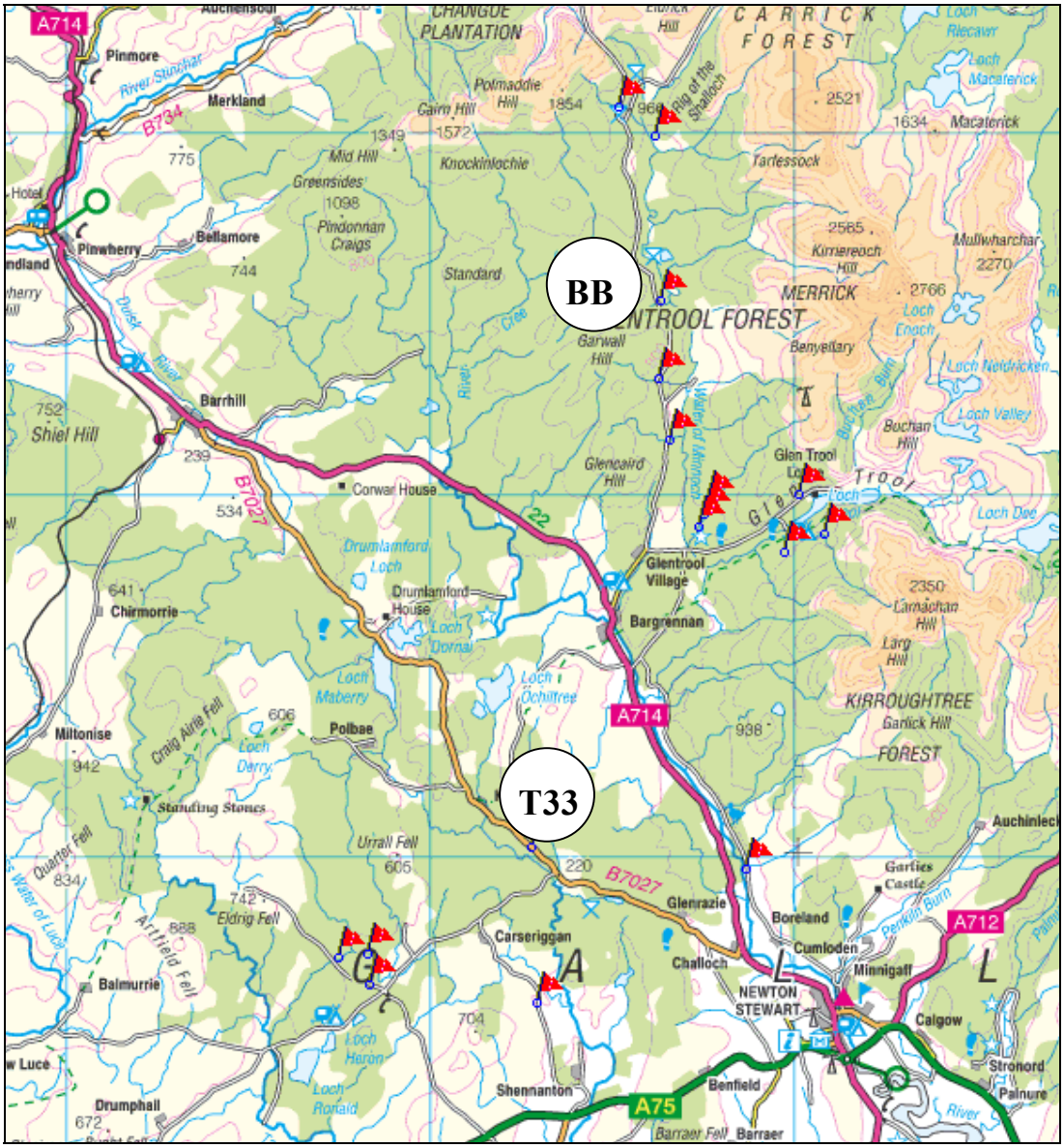

Fig 3.2 Black Burn (BB) and T33 site locations (other sites studied within the project as a whole are flagged), within Galloway Forest Park

3.4.2 Black Burn

A two-year sampling programme of biofilm analysis was undertaken in the Black Burn (Fig 3.3, Table 3-1). This small, upland stream catchment was characterised by intensive coniferous forestry. This stream encompassed a range of corridor widths within a study stretch length of approximately 300m. This was advantageous for a study where the primary objective was to study variation in baseline resources available under different corridor conditions, as water chemistry changes along its length were minimal.

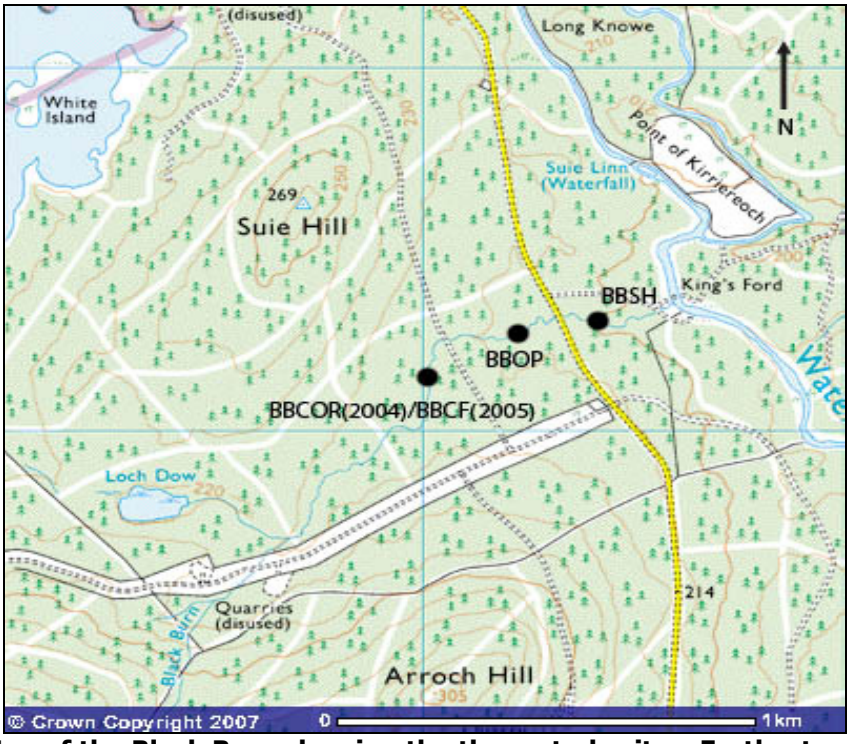

Fig 3.3. Map of the Black Burn showing the three study sites. Furthest upstream is BBCOR/BBCF (corridor/clearfelled) – referred to as BBCF post felling event in winter 2004/2005. Middle site is BBOP (open) and furthest downstream is BBSH (conifer shaded).

Table 3-1. BB site location descriptions and positions (UK National Grid references)

NX 36602 85358	BBSH	Downstream of bridge, forest overhangs
NX 36566 85333	BBOP	Upstream of bridge, open corridor
NX 36050 85239	BBCOR/CF	Upstream of track crossing burn, open corridor/ clearfelled in 2005

Within the Black Burn, three different sites were studied (Table 3-1). Sites were located in close proximity to each other to minimise variation in water chemistry but varied in corridor characteristics; from heavily shaded to open corridor and clear-felled. Thus, variation existed between the sites in the amount of cover by riparian trees (Norway spruce, *Picea abies*) and therefore in light availability and other corridor features (Table 3-2).

Table 3-2. Mean values for physical parameters measured for the three Black Burn sites over sampling periods in 2004 and 2005 (± standard error).

Physical Parameters	BBCOR (2004)	BBCF (2005)	BB OP	BB SH
Light (%)	56.50 +/- 3.54	90.18 +/- 6.41	82.61 +/- 2.06	8.83 +/- 2.80
Stream wet width (m)	1.05 +/- 0.07	1.40 +/- 0.19	1.71 +/- 0.08	2.20 +/- 0.15
Stream depth (cm)	24.50 +/- 6.36	22.00 +/- 6.91	17.42 +/- 1.19	16.42 +/- 1.02
Bedrock (%)	20.00 +/-7.01	21.33 +/- 5.67	25.87 +/- 3.17	10.50 +/- 2.31
Boulders/cobbles (%)	45.00 +/- 7.01	36.67 +/- 12.02	42.50 +/- 4.79	35.00 +/- 2.89
Pebbles (%)	30.00 +/- 14.14	16.67 +/- 8.82	24.25 +/- 9.44	32.50 +/- 4.79
Sand (%)	5.00 +/- 0.00	3.33 +/- 3.33	3.75 +/- 2.39	12.50 +/- 6.29
Silt/Clay (%)	0.00 +/- 0.00	23.33 +/- 14.53	7.50 +/- 7.50	10.00 +/- 7.07
Riparian tree diversity	1.00 +/- 0.00	1.50 +/- 0.29	1.50 +/- 0.29	1.50 +/- 0.29
Overhanging vegetation (%)	20.00 +/- 0.00	20.00 +/- 0.00	27.75 +/- 9.22	2.75 +/- 2.43
Corridor width (m)	32.5 +/- 3.54	90.33 +/- 14.95	30.48 +/- 1.45	8.85 +/- 0.28
Corridor tree height (m)	22.50 +/- 3.54	2.56 +/- 0.59	21.67 +/- 2.04	19.17 +/- 1.44
Site altitude (m)	220.00 +/- 0.00	220.00 +/- 0.00	200.00 +/- 0.00	210.00 +/- 0.00

3.4.3 T33

The three T33 sites are located within the Bladnoch Catchment. T33 is a similar low order stream to the Black Burn (Fig 3.4 and Table 3-3). Both streams (BB and T33) have very similar geologies: Ordovician, with shales and greywackes. The highly siliceous granite bedrock covered by thin, patchy, organic rich and generally acidic soils, offers only limited ability to neutralise acid inputs from the atmosphere (Wright et al., 1994) and, as a consequence, the soils from both sites are naturally acid (Edmunds and Kinniburgh, 1986).

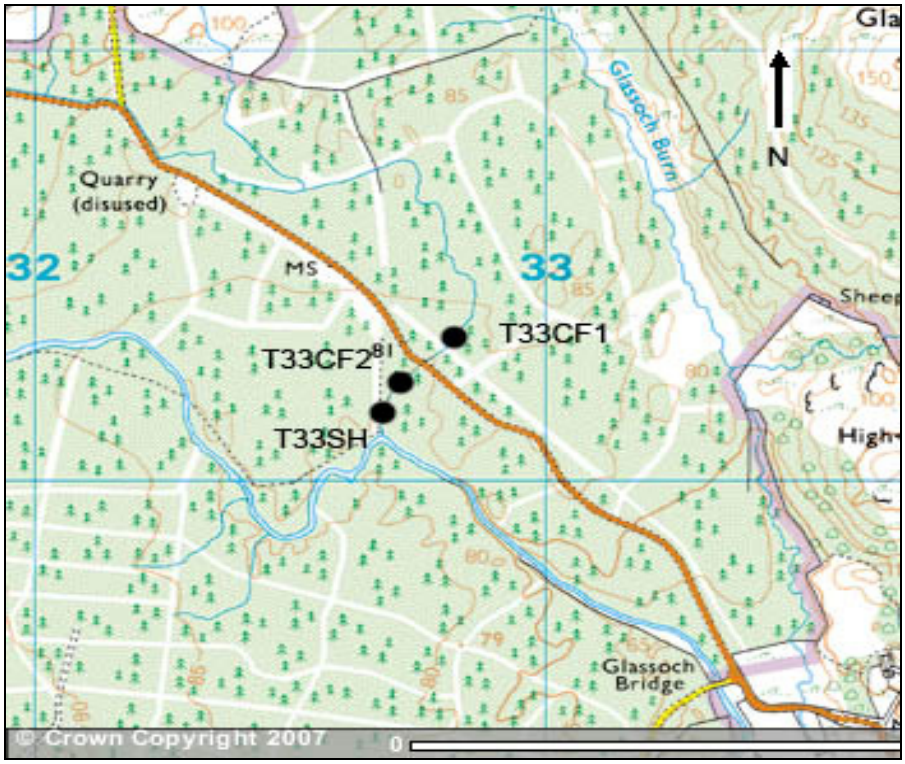

Fig 3.4. Map of T33 showing the three study sites. Furthest upstream is T33CF1 (clearfelled), middle site is T33CF2 (clearfelled) and furthest downstream is T33SH (conifer shaded).

T33 was added to this study in 2005 to offer a stable comparative site as Black Burn was experiencing influences of the clear-felling event adjacent to the uppermost site (BBCOR/CF).

Table 3-3. T33 site location descriptions and positions (UK National Grid references)

NX 328 703	T33CF1	Upstream of road, open, previously clearfelled
NX 327 702	T33CF2	Downstream of road, open/ forest clearance debris
NX 327 703	T33SH	Furthest Downstream site, conifer shaded/ clearance debris

T33 runs through a large open area characterised by widespread bankside growth of tall wet-meadow vegetation (National Vegetation Classification M23 *Juncus effusus* meadow) which produces a relatively high level of shading of the stream bed despite the lack of tree canopy cover (Table 3-4). The area was previously conifer plantation. Forestry clearance occurred between April 2002 and March 2003 at the two upstream sites (T33CF1 and T33CF2).

Table 3-4. Mean measurements for physical parameters measured for the three T33 sites over sampling periods in 2005 (± standard error).

Physical Parameters	T33CF1	T33CF2	T33SH
Light (%)	38.18 +/- 9.49	61.78 +/- 2.22	41.95 +/- 10.12
Stream wet width (m)	0.94 +/- 0.14	1.12 +/- 0.28	1.10 +/- 0.24
Stream depth (cm)	3.33 +/- 0.38	5.78 +/- 0.40	10.89 +/- 2.26
Bedrock (%)	0.00 +/- 0.00	0.00 +/- 0.00	10.00 +/- 3.33
Boulders/cobbles (%)	5.00 +/- 2.89	13.33 +/- 8.33	30.00 +/- 11.28
Pebbles (%)	83.33 +/- 3.33	46.67 +/- 11.67	43.33 +/- 12.02
Sand (%)	5.00 +/- 2.89	10.00 +/- 5.77	10.00 +/- 5.77
Silt/Clay (%)	6.67 +/- 3.33	31.67 +/- 9.28	6.67 +/- 3.33
Riparian tree diversity	0.67 +/- 0.67	1.33 +/- 0.33	1.00 +/- 0.00
Overhanging vegetation (%)	44.00 +/- 21.20	24.00 +/- 11.37	2.00 +/- 1.53
Corridor width (m)	88.89 +/- 5.88	66.67 +/- 10.18	46.56 +/- 15.91
Corridor tree height (m)	1.56 +/- 0.78	22.44 +/- 3.20	30.00 +/- 0.96
Site altitude (m)	70.00 +/- 0.00	69.00 +/- 0.00	65.00 +/- 0.00

3.4.4 Sampling Protocol

Field based studies which have quantified biofilm/periphyton growth and settlement within lotic systems mainly use one of two methods: sampling of the natural stream substratum (e.g. Cazaubon and Loudiki, 1986; Singer et al, 2005) or using some form of artificial substrate sampler. Using the former, Cazaubon and Loudiki (1986) found that micro-distribution around the substrate itself varies strongly with current and light penetration, and with the shape and size of the riverbed substrate. The variability in results from both the intra-site and inter-site samples of the Cazaubon and Loudiki (1986) study made reliability and replication of results difficult. In addition, with increased flow intensity, natural substrates are often found to be unstable and subject to movement, resulting in the frequent loss of material (e.g. Cazaubon, 1988).

The desire for reproducible, quantitative data has prompted the design of sampling techniques using artificial substrates (e.g. Wetzel, 1964; Tank and Winterbourn, 1995; Fellows et al., 2006). These offer the advantages of uniform size and textural properties. However varying results between artificial and natural substrates has generated much debate as to how representative artificial substrates are (e.g. Castenholz, l961; Stockner and Armstrong, 1971; Foerster and Schlichting, 1965)

Within this study, it was decided to use artificial, semi-flexible substrates. The artificial substrate comprised a 20 x 20 cm linoleum square, superglued onto 2.5 kg

weights and subsequently placed in the stream (Fig 3.5) in a position orientated to minimise turbulent flow and decrease the risk of tile loss during high flow. These substrates were smooth enough to allow for easy scraping of the surface for algal/biofilm removal, yet had a slightly pitted texture to promote a more normal settlement/growth pattern, than would be expected on a smooth glass slides - which are the more common substrate used in artificial substrate experiments (e.g. Wetzel, 1964; Tank and Winterbourn, 1995; Fellows et al, 2006). This approach allowed for consistency in sampling methods and substrate growing conditions between sites, and in addition, were of a large enough surface area to promote sufficient biomass settlement for multiple analyses.

Fig 3.5. Biofilm artificial substrate tiles in the Black Burn at the end of a settlement period.

At each site, four tiles were placed randomly throughout a five-meter stream stretch. The tiles remained in situ, within the stream for approximately one month per sampling period, commencing 23 December 2003. Subsequent replacement and removal is outlined in (Fig 3.5) for the two streams.

Table 3-5. Treatments used for biofilms collected at both Black Burn (O = yes, / = no) and T33, throughout 2004 and 2005. Abbreviations for treatments are: T.CHL = Total Chlorophyll, D.W = Dry-Weight, I.D. = microscopic examination of sample/algal I.D. and E.C = Elemental Composition; isotopic and stoichiometric measurements (explored further in chapter four).

			Black Burn Treatments				T33 Treatments			
Deployment	**Removal**	**No. Days**	**T.CHL**	**D.W**	**I.D**	**E.C**	**T.CHL**	**D.W**	**I.D**	**E.C**
22/11/03	23/12/03	31	O	O	/	O	/	/	/	/
23/12/03	24/1/04	31	/	O	/	O	/	/	/	/
24/1/04	20/2/04	27	/	/	/	O	/	/	/	/
20/3/04	24/4/04	34	O	O	/	O	/	/	/	/
24/4/04	29/5/04	35	/	O	/	O	/	/	/	/
29/5/04	26/6/04	27	O	O	/	O	/	/	/	/
26/6/04	2/8/04	36	O	O	/	O	/	/	/	/
7/3/05	31/3/05	24	O	O	O	O	O	O	O	O
31/3/05	5/5/05	35	/	/	/	/	O	O	O	O
5/5/05	1/6/05	26	O	O	O	O	O	O	O	O
1/6/05	15/7/05	44	O	O	O	O	O	O	O	O
15/7/05	7/8/05	22	O	O	O	O	O	O	O	O
7/8/05	16/9/05	39	O	O	O	O	O	O	O	O
16/9/05	18/10/05	32	O	O	O	O	O	O	O	O

At each sampling trip, tiles and attached weights were removed from the stream (handled by the weight only, to minimise disturbance of the biofilms). Tiles were stripped from the weight and placed into a labelled re-sealable plastic bag. Samples were then stored in a darkened cool box to minimise sample degradation during transportation back to the lab. The biofilm tiles were kept in the dark and refrigerated (at 5 ºC) until processed (usually the following day).

3.5 Laboratory Analysis methods

3.5.1 Biomass

The biofilm material was scraped from the artificial substrates**,** using a glass microscope slide and distilled water until no visible remains of biofilm material were present on the tile upper surface. The diluted liquid organic slurry was placed in a centrifuge at 3635G for 12 minutes. The post centrifuge supernatant was decanted from the samples. From the concentrate remaining, a 2ml volume was removed from each sample for composition/species analysis. The remaining biomass was placed in pre-weighed beakers, and freeze-dried (usually 48 hours maximum). Weighing of the beakers post freeze-drying allowed for total biomass to be quantified. Approximately, 2mg of dried biomass was retained for stable isotope and elemental analysis. Some samples were too small to achieve this weight and so a lesser amount was removed

(this could sometimes be the entire sample). The remaining sample (up to 200mg) was used for chlorophyll analysis.

3.5.2 Carbon Content

Weight % carbon and nitrogen were derived from biofilm samples using a sub-sample of approximately 2 mg of biofilm material (weighed out to 0.01mg precision) of the dried material was loaded into an 8 x 5 mm tin capsule and crimped closed. Using continuous-flow isotope-ratio mass-spectrometry (CF-IRMS), the crimped capsules were processed for measurements by combusting in a Carlo Erba C/N/S analyser interfaced with a Finnigan Tracer Matt CF-IRMS. These analyses were carried out at the Scottish Universities Environmental Research Centre (SUERC) in East Kilbride.

3.5.3 Chlorophyll Analysis

Chlorophyll *a* was extracted following methodology primarily following Wetzel and Likens (1991). Approximately 200 mg of freeze-dried biofilm material was added to 10 ml of acetone, to extract chlorophyll *a* pigment. This solution was mixed vigorously and refrigerated overnight at 4^{o}C. To separate the acetone (for chlorophyll assay) from the biomass, the sample was centrifuged in a sealed tube at 1000rpm for 5 minutes, allowing removal of the supernatant post centrifugation with a pipette. 2.7ml (90%) acetone/chlorophyll solution and 0.3ml (10%) distilled water was added to a glass spectrophotometer cuvette, covered, and inverted to mix. Absorbance of the solution was analysed using a spectrophotometer (Schimadzu).

Calculating chlorophyll a concentration in the acetone extract:

1. Measurements of absorbance were taken at 750 and 665 nm. Measurements at 665nm were used to calculate total chlorophyll *a* concentrations and measurements at 750nm (with and without acid) are subtracted to account for turbidity in the sample.

2. Each sample was blank corrected by measurements first with a blank composed of 10% distilled water and 90% acetone.

3. The chlorophyll content was corrected for phaeo-pigments by acidification; HCl (0.1 ml of 6mol per ml of extract) was added directly to the cuvette following 665 nm and 750 nm measurements, then covered and inverted to mix. The tube was left to stand for 5 min.

Chlorophyll *a* calculations followed methodology outlined in Wetzel and Likens (2002) and equations following Lorenzen (1967):

$$\text{Chl a } (\mu g/l) = \frac{k * F * ((A_{665_0} - A_{750_0}) - (A_{665_A} - A_{750_A}))}{z}$$

where:

k = absorption coefficient of chlorophyll a = 11

F = factor to equate reduction in absorbency to initial concentration, 1.7:1.0, or 2.43.

Z = path length of the cuvette or cell in cm, so usually 1cm, thus 1.

However, this value produced is expressed in µg/L. Consequently, the chlorophyll concentration (in mg/L) was converted into an absolute amount expressed as mg Chl *a* / mg of biofilm biomass (following equation), allowing for further calculation to mg /m^2 using biofilm biomass data measurements.

$$\text{Chl a (mg/mg biofilm)} = \frac{[\text{Chl mg/l}] * V * DF}{\text{mg biomass used in extraction}}$$

V = extract volume, in litres

DF = Dilution Factor - The Increase in calculated concentration that would have arisen from using a larger cell length (calculated by dividing absorbance readings by cell length).

3.5.4 Tile composition analysis (species / detrital measurements)

In order to determine possible links between characteristics of the biofilm and the algal species composition, samples were analysed for taxa assemblage structure and estimates of taxa abundance (only in 2005 samples). The 2ml wet sub-sample

retained from the biofilm tile was analysed as quickly as possible after collection. Samples were stored in an Eppendorf tube, retaining an area of a headspace to allow for continued respiration (to help preserve samples until analysis). The samples were mixed with 5ml water and a drop of the solution was placed on a glass slide for microscopic identification of algal species and % detritus (using x 10 magnification). Algal unit counts (one cell = one unit for unicellular organisms, one filament = one unit for filamentous taxa) and identifications covered three fields of view, following Prescott (1978), Belcher and Swale (1978) and Belcher and Swale (1979).

3.6 Results

3.6.1 Biomass

Black Burn

Mean biofilm biomass in the Black Burn was significantly (ANOVA P <0.001) greater in summer samples compared with spring/winter. Mean biomass on 2004 BB tiles increased from a mean of 163.2 ± 28.1mg/m^2 (± S.E) in Dec – April to 1419.8 ± 6.2mg/m^2 (± S.E) in May-Aug. Using these seasonal cut-offs defined through Tukey test (95 % confidence) of the ANOVA groups described above, pooled samples from spring/winter and summer indicated no significant inter-site differences in biomass from pooled seasonal data (Dec 03 to April 04, P = 0.658; summer samples, P = 0.912). However, from visual assessment, Black Burn Shade and Corridor indicate some senescence of algae into the autumn (Fig 3.6), but this was not reflected in the middle site (Open), which continued to be productive into August.

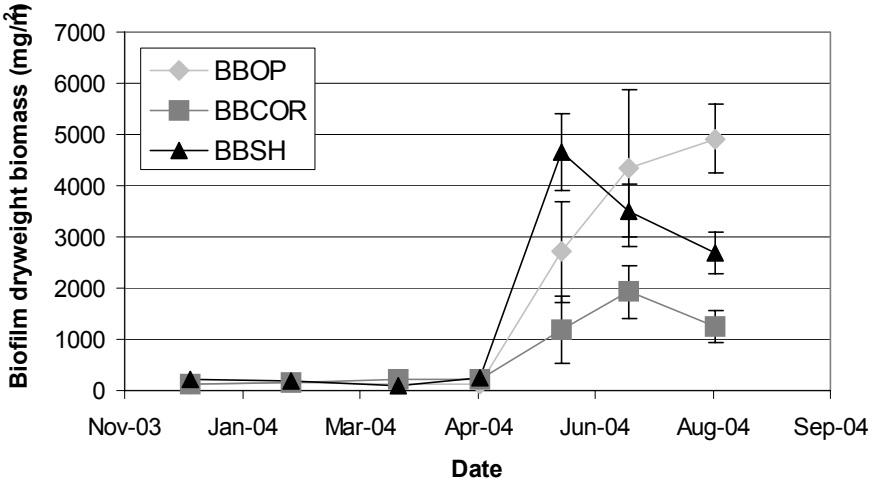

Fig 3.6. Temporal changes of Black Burn biofilm dry-weight biomass per m^2 during 2003 - 2004 (mean ± standard error).

Analysis of both 2004 and 2005 data (Fig 3.7) revealed that the overall mean biomass of Black Burn biofilm was approximately 7700 ± 2009.3mg/m^2 (± S.E). However, contributing to this result is the significant increase in biofilm dry-weight in the second year of the study (ANOVA, $P < 0.001$), prior to returning to levels comparable with mid summer of 2004 (ANOVA, $P = 0.192$). The Black Burn site most affected by biofilm biomass increase was the furthest upstream, which was also the site closest to an adjacent forest clearance event (in the winter of 2004/2005). It is suggested that this large increase in biofilm biomass was a consequence of increases in allochthonous nutrient runoff related to the disturbance event.

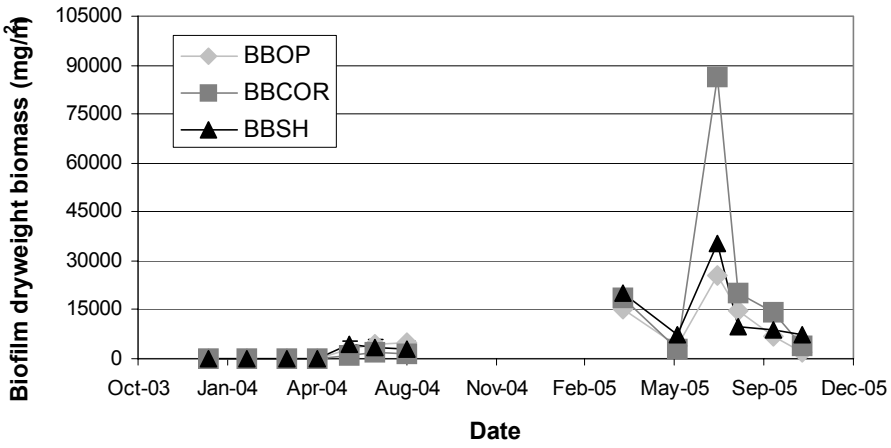

Fig 3.7. Black Burn temporal variation during 2003 - 2005 of dry-weight biomass per m^2 of tiles (mean ± standard error) Note changed y-axis scale reflecting the considerable increase in biomass production in year two.

3.6.2 T33

All three of the T33 sites show similar patterns of biofilm standing crop biomass (Fig 3.8), with no significant difference in the biofilm biomass retrieved from each of the three sites (ANOVA, P = 0.127). The same y-axis scale has been used in Fig 3.7 and Fig 3.8, in order to show comparative biomass levels between the two sites and years. There is a much greater standing crop at T33 in comparison to pre-felling BB. However biofilm biomass increased significantly in 2005 BB biofilms compared with T33 and BB2004.

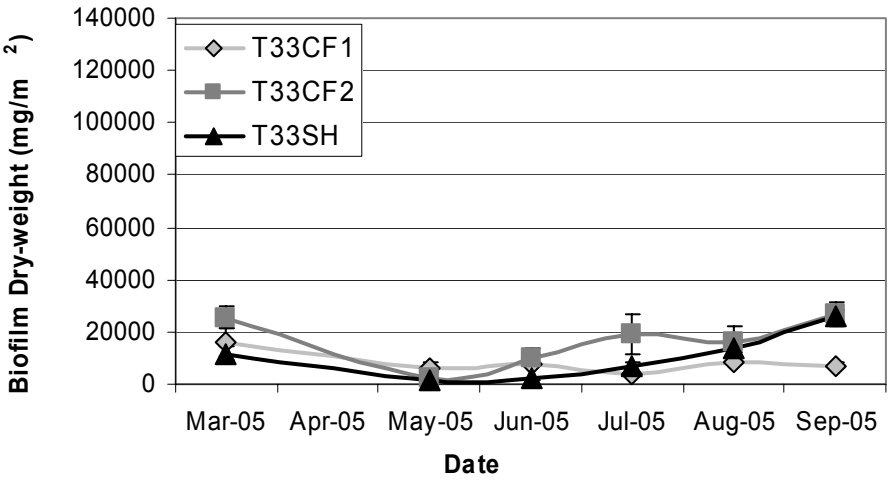

Fig 3.8. T33 biofilm dry-weight biomass (mg/m^2) sampled in 2005 (mean ± standard error).

When data from all samples within 2005 is pooled, significantly greater biofilm biomass production was found at BBCF compared to the most upstream of the T33 sites (T33CF1) (Fig 3.9). Thus, despite the temporal variation in the sites (Fig 3.7 and Fig 3.8), BBCF is still clearly distinguishable in terms of basal resource production from T33CF1.

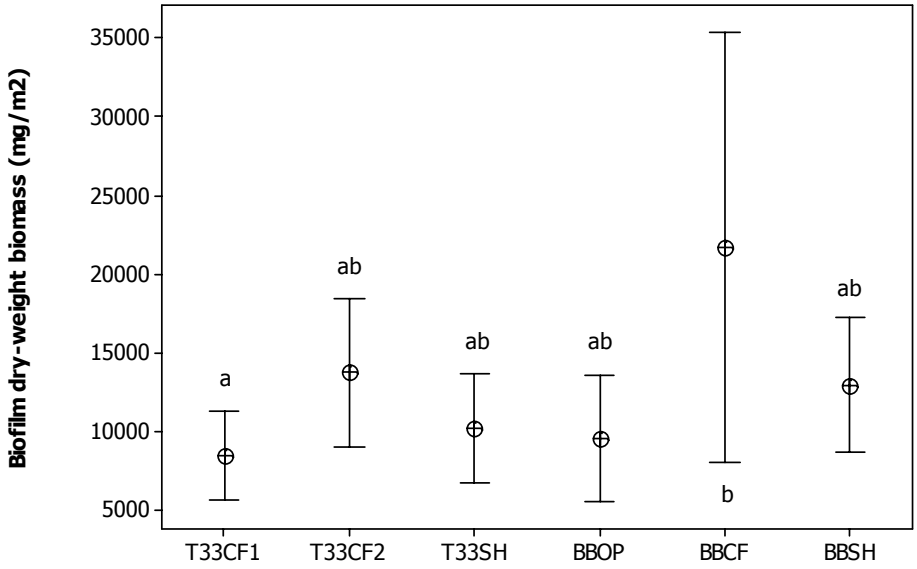

Fig 3.9. T33 and Black Burn mean dry-weight biomass (± 95% confidence interval) for all samples collected in 2005. Tukey test revealed a significant difference (ANOVA, P = 0.050) between T33CF1 and BBCF, as indicated by differential letters (a and b). There is no significant difference between any other sites in biomass production.

However all other sites have comparable biofilm standing crop, despite the variation in corridor characteristics and land uses of the specific riparian zone. To explore this result more fully, data from each sample visit is analysed separately to discern any spatial inter- or intra-site differences occurring over the sampling season.

Table 3-6. Significant differences (ANOVA P values indicated) in biofilm dry-weight biomass between sites, identified through Tukey analysis (95% confidence). Analysis is for sites on each sample date and comparisons are not made between dates. Sites with letter(s) in common are not significantly different on the date of sampling.

	BBOP	BBCO/CF2005	BBSH	T33 CF1	T33CF2	T33SH	P Value
23/12/2003	a	a	a	/	/	/	0.457
20/03/2004	a	b	a	/	/	/	0.009
24/04/2004	a	a	a	/	/	/	0.306
29/05/2004	ab	a	b	/	/	/	0.042
26/06/2004	a	a	a	/	/	/	0.258
02/08/2004	a	b	ab	/	/	/	0.002
31/03/2005	a	a	a	a	a	a	0.295
01/06/2005	ab	ab	ab	ab	a	b	0.032
15/07/2005	ab	a	ab	b	b	b	0.013
07/08/2005	ab	a	ab	b	ab	ab	0.035
16/09/2005	a	a	a	a	b	b	< 0.001
18/10/2005	a	a	a	a	a	a	0.552

Table 3-6 indicates that from June until mid September 2005, one or more of the T33 sites produced significantly less biomass than equivalent sites within the Black Burn. Of the Black Burn sites, BBCF generally produced significantly higher biomass levels.

3.6.3 Chlorophyll Analysis

From the sum of all BB2005 chlorophyll *a* (Chl *a*) concentrations measured in 2005, a mean of 1.08 ± 0.25mg/m^2 (± SE) Chl *a* was found. This was significantly lower (ANOVA, P = 0.012) than the mean of Chl *a* measured at the same sites in 2004 (2.23 mg/m^2 ± 0.31 SE). However, when compared with T33 biofilm Chl *a* (measurements only taken in 2005), there is a significantly greater weight of chlorophyll produced from T33 biofilms (4.04 ± 0.58 mg/m^2 SE) compared with BB2005 (ANOVA, P < 0.001).

Chl *a* concentrations measured here are similar to those found by other comparable periphyton studies. For example, Cushing et al. (1983) investigated chlorophyll production from periphyton of varying stream orders as part of River Continuum Concept (RCC) research. This study sought to determine the relationship between primary productivity and stream order. Although measurements were published in µg/cm^2 (as opposed to mg/m^2 here), conversion of my results indicates that the range of Chl *a* concentration measures are comparable between the two studies. Mean concentrations for the Cushing et al (1983) study, range from 1.2 µg/cm^2 (± 0.1 SE) to 107.2 µg/cm^2 (± 36.8 SE), with periphyton Chl *a* concentrations in low order systems of 7.2 µg/cm^2 (± 2.3 SE). Here BB2004 mean Chl *a* mean concentration was 22.3µg/cm^2 (± 3.1 SE), BB2005 was 10.8 µm/cm^2 (± 2.5 SE) and T33 mean concentration was highest at 40.4 µg/cm^2 (± 5.8 SE).

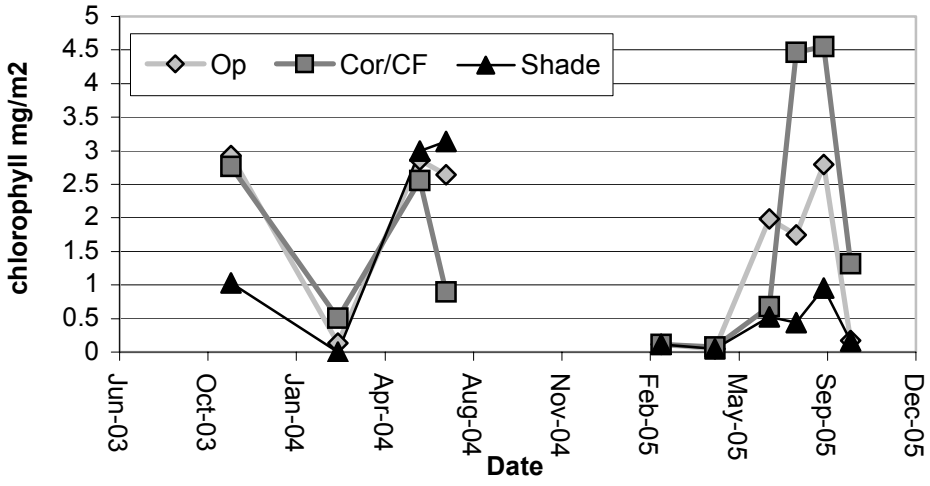

Fig 3.10. Chl *a* at from Black Burn biofilms collected during 2004 and 2005 (mean ± SE)

Temporal and spatial variation of the Black Burn Chl *a* content is shown in Fig 3.10. In both 2004 and 2005, chlorophyll growth patterns indicate midsummer bloom events. However, chlorophyll production was still occurring in winter/spring 2003/4, whereas in 2005, chlorophyll production was almost nil until the June sampling.

There was no significant difference in chlorophyll *a* production between sites in 2004 (ANOVA, P = 0.866). There was a comparative delay in the commencement of Chl *a* production in the 2005 biofilm samples, compared to that of BB 2004. However, in addition, there was also a greater spatial variation in Chl *a* production in 2005 BB samples. For example, there was no significant difference in chlorophyll *a* production between sites in 2004 (ANOVA, P = 0.866). However, chlorophyll concentration at BBSH 2005 was significantly (ANOVA, P = 0.030) lower then other BB sites in 2005 (Fig 3.11).

There was significant temporal variation between sites. BB2005 chlorophyll biomass from the shaded site (BBSH) was lower than that of BBSH 2004 (ANOVA, P = 0.006). In fact, maximum concentrations of BB2005SH were only comparable with late autumn production of 2003. BBOP 2005 was comparable with production from BBOP 2004 (ANOVA, P = 0.233). The expected seasonal increase in production, as observed in other studies of stream algal chlorophyll production (e.g. Rosenfeld and Roff, 1990), was delayed until the June sampling. Chl *a* production in BBCF is also

comparable with concentrations found in 2004 from BBCO. On average, variation between 2004 and 2005 chlorophyll biomass was only significant at the shaded site.

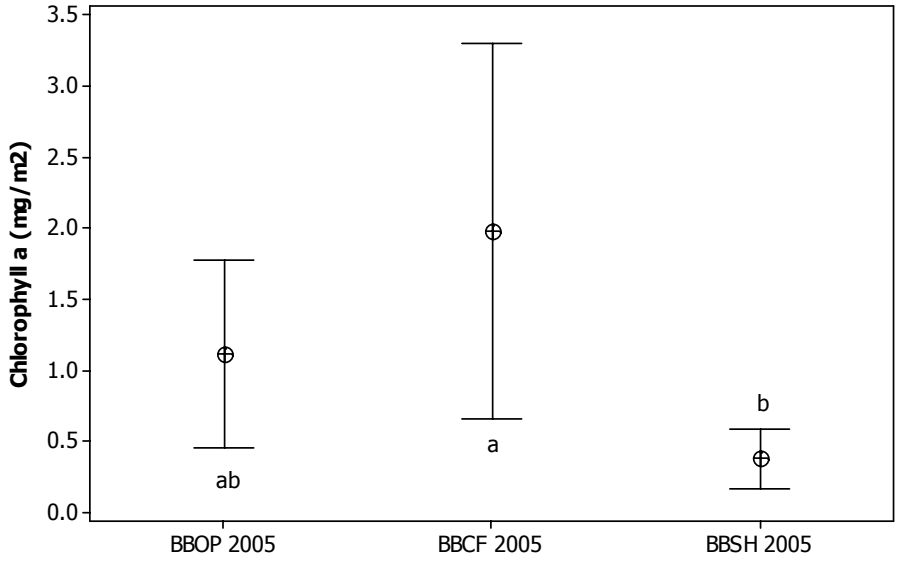

Fig 3.11. Mean Black Burn biofilm Chl *a* concentrations (± 95% confidence interval) from each sample site in 2005. Tukey test reveals significant differences (indicated with differing letters: a or b) between BBCF and BBSH (P = 0.030, n = 157); where chlorophyll production is reduced.

Comparisons of chlorophyll biomass between streams and sites indicate greater chlorophyll *a* concentrations in either one or both of two T33 clearfelled sites (T33CF1 and T33CF2) than many of the Black Burn samples (Fig 3.12). Specifically, the Black Burn corridor and shade site in 2004 (BBCO and BBSH) and the open and shaded sites in 2005 (BBOP and BBSH) have significantly (ANOVA, $P < 0.001$) lower chlorophyll *a* concentrations (mg/m^2). Thus, there is an indication that within the Black Burn, under normal circumstances (i.e. pre felling), chlorophyll *a* production was limited within both the shaded and corridor sites. Under impacted circumstances, the open site at Back Burn was also negatively influenced while the clear-felled site remained comparatively productive within 2005. Within T33, light limitation at the shaded site (T33SH) may be influencing chlorophyll *a* production.

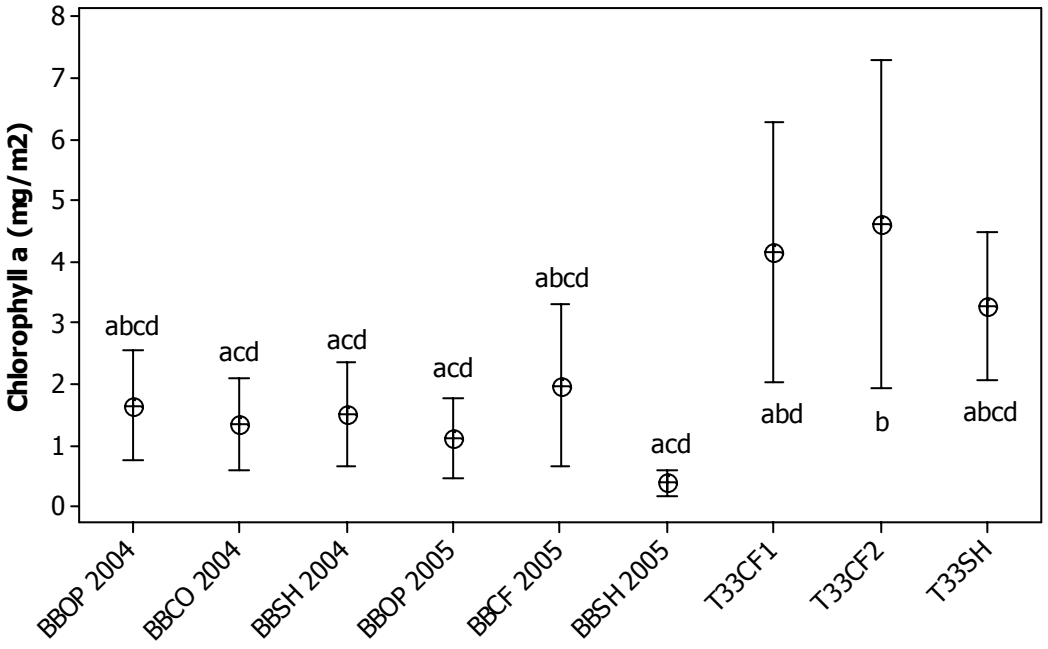

Fig 3.12. Mean chlorophyll a concentrations (± 95% confidence interval) at both Black burn and T33 sites over 2004 (BB only) and 2005. Tukey test reveals significant differences (indicated with differing letters: a, b, c or d) (P < 0.001, n = 227).

3.6.4 Determining biofilm autotrophic status with C:Chl

By combining both Chl *a* and biofilm carbon content data, there is an opportunity to analyse the biofilm in terms of proportional contribution from autotrophic material. Chl *a* concentration has been used in many previous studies as a proxy for algal biomass (e.g. Romani and Sabater, 2000; Frost et al., 2005; Rosenfeld and Roff, 1990). Chl *a* serves as an indicator of the algal content because it is rapidly degraded outside living cells and comprises a negligible fraction of detrital organic carbon (Furlong and Carpenter 1988). Here, it is being used to determine the proportional autochthonous autotroph content of the biofilm. The C:Chl ratio in live algal biomass, which has been investigated mostly in phytoplankton, is known to vary with taxonomic composition, light availability, and other factors (Reynolds 1984).

Using this measure should provide an indication of the primary productivity of the biofilm and also a comparative indication of the hetrotrophic and detrital content. C: Chl ratios <100 are a value indicative of relatively high algal cellular content in

natural organic matter (Geider, 1987). For example, C:Chl mass ratios of phytoplankton in culture and in pelagic environments commonly range between 25 and 100 (Ahlgren 1983; Geider 1987; Riemann et al. 1989), but can be 150 or more in natural algal assemblages (Gieskes and Kraay 1989; Lefevre et al. 2003). Higher C:Chl in biofilms can reflect slower growth rates and consequent secretion of mucilaginous materials (de Jonge 1980). Thus the C:Chl measurements should provide some indication of baseline characteristics of food availability as well as the functionality of the biofilm.

In my dataset, C: Chl *a* (C:Chl) ranged from 74 to 249,181. However, there is high temporal and spatial variation in C:Chl (Kruskal-Wallis, $P < 0.001$). The lowest mean C:Chl ratio (therefore, the biofilms with the highest proportion of carbon as chlorophyll), was found within the Black Burn in 2004, with a mean C:Chl of 393 (± 49.7 S.E). This result is comparable with other studies such as Frost et al.(2005) (with a mean C:Chl of 405). However, in 2005, Black Burn biofilms had on average a 70 fold increase in non-chlorophyll derived carbon to the biofilm biomass reflected by a mean C:Chl of approximately 26,600 (± 5023 S.E), indicating a minimal algal contribution to the biofilm material.

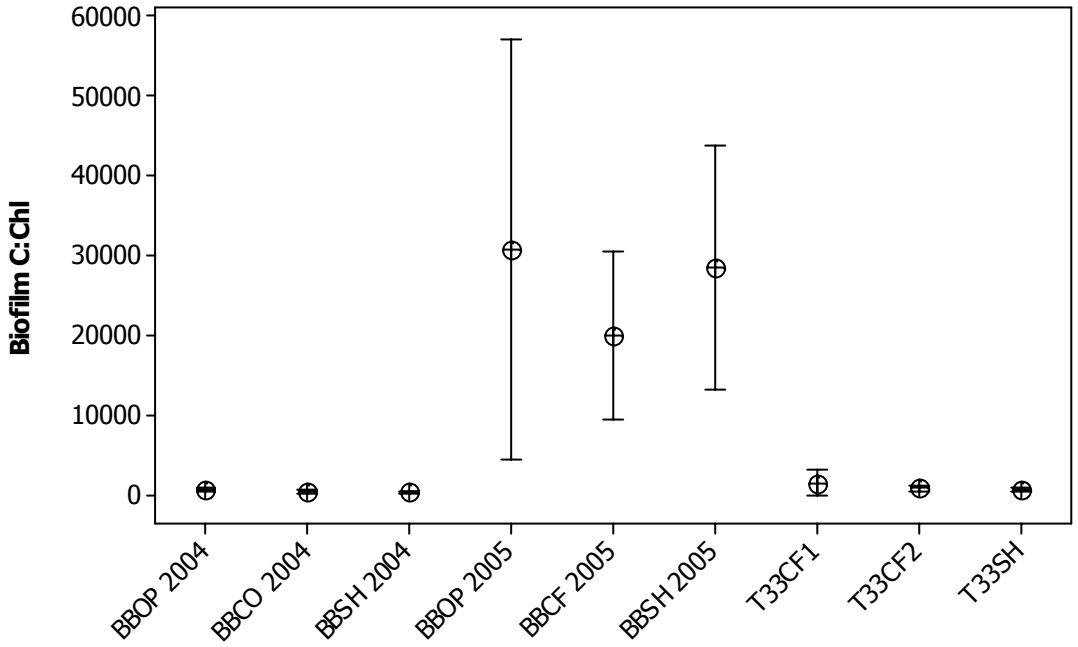

Fig 3.13. Comparison of site specific C:Chl (± 95% confidence interval). Significant differences (Kruskal-Wallis < 0.001) between C:Chl of Black Burn

(2005, 2004) and T33. Distribution of means suggests significantly greater C:Chl at BB 2005.

T33 biofilms were comparable to biofilms of BB in 2004, but C:Chl was significantly lower then BB 2005 biofilms (Fig 3.13); with a mean C:Chl of 741 (± 76.12 S.E).

3.6.5 Prevalence of algae in periphyton

Chlorophyll *a* was employed as an index of epiphytic algal biomass (as standing crop), to estimate total algal carbon since it allows algal material to be distinguished from the mixture of microbes and detritus which make up the biofilm matrix (Fig 3.14). Algal cell carbon/Chl *a* mass ratios vary from about 25 to 80 depending on species and physiological state of organisms (Parsons et al., 1977). Estimating algal biomass from Chl *a* can be problematic, mainly because the Chl *a* to carbon conversion factor is not constant and varies among species, growth conditions (Banse, 1977) and radiation intensity and nutrient availability (nitrogen particularly appears to be a major factor affecting the chlorophyll content of algal cells) (Vollenweider and Kerekes, 1982). However, as a photosynthetic pigment, Chl *a* is directly related to the potential for autotrophic growth and has been employed as an algal biomass index in many limnological studies (Wetzel 1983), I have used it here as a proxy of autotrophic biomass.

The transformation of Algal biomass (algal C mg/m^2) from Chl *a* density primarily used the conversion factor C:Chl of 60 following Romani and Sabater (2000). In their study, biofilm algal composition, similarly to here, was often characterised by diatom-dominated communities. This conversion factor lies in the middle of the range of 20–100 suggested for benthic algae by Margalef (1983), and also well within the range described by Pearson et al. (1977) (25 to 80).

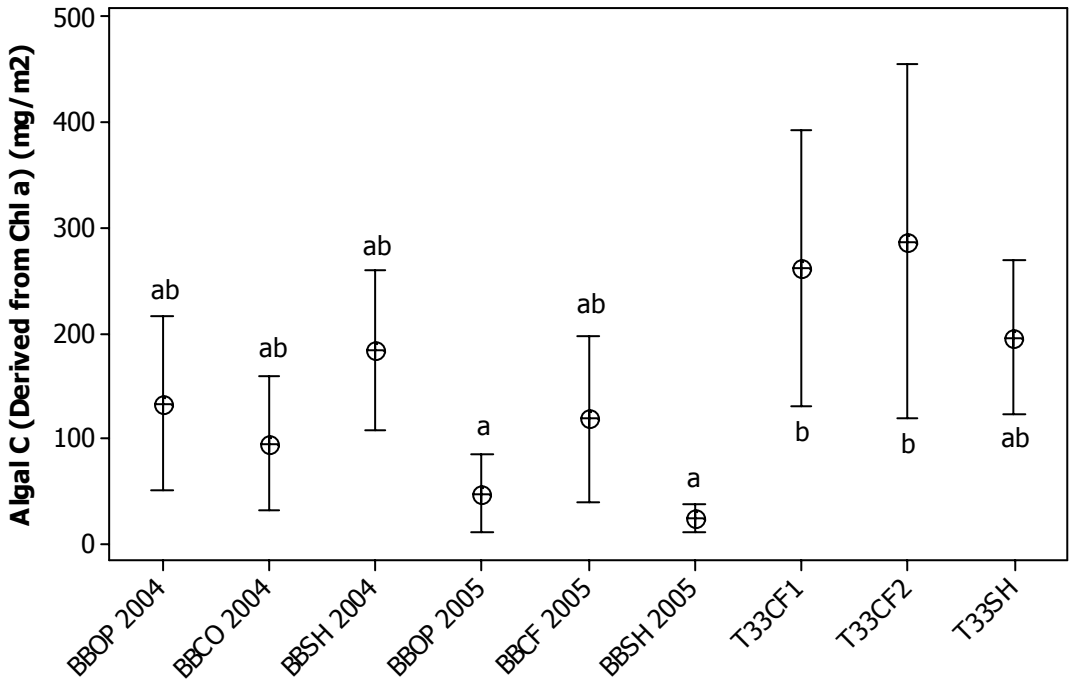

Fig 3.14. Estimates of algal C using an average conversion factor of 60 mg. Algal C/m^2 to mg Chl *a*/m^2 (± 95% confidence interval), which provides an estimate of algal C from Chl *a* calculations, as carried out by Romani and Sabater (2000). Tukey test indicates significant differences (P <0.001) between algal carbon in both clear-felled sites of T33 and open and shaded sites in the Black Burn (BBOP and BBSH), as indicated by different letterings (a and b). There is significantly reduced algal biomass at the lower two BB sites of 2005 compared with the uppermost sites of the control stream (T33, 2005).

From Fig 3.14, the calculations of biofilm algal carbon content suggest that despite both temporal and spatial variation, BB 2004 and T33 are comparable in total algal production. However, significant differences occur between the two more exposed sites of T33 (CF1 and CF2) and the two downstream sites of BB 2005.

Consideration of the comparable proportions of algal carbon derived from allochthonous or heterotrophic carbon source origins, suggests that on average, 5% of biofilm carbon was estimated to be of algal origin. Approximately 72% of biofilm samples from the Black Burn (both 2004 and 2005) had less than 10% of C in the algal cellular fraction. This is very similar to the results of Frost et al. (2005) who found that algal C averaged 8.4% of the periphyton biofilm samples they collected from a variety of substrata from lake and low-salinity coastal habitats, whilst over 75% of samples had algal carbon concentrations under 10% of total periphyton C.

However, chlorophyll content of algae can range from 0.1 to 9.7 per cent of fresh algal weight (Vollenweider and Kerekes, 1982). A great variability in individual cases can be expected, either seasonally or on an annual basis due to species composition, light conditions and nutrient availability (Banse, 1977; Vollenweider and Kerekes, 1982). Thus, it was decided to explore alternative conversion factors in order to determine the potential variation to algal biomass resulting from variation in the algal chlorophyll content. The alternative conversion factors explored were the extremities (20 – 100) described by Margalef, 1983.

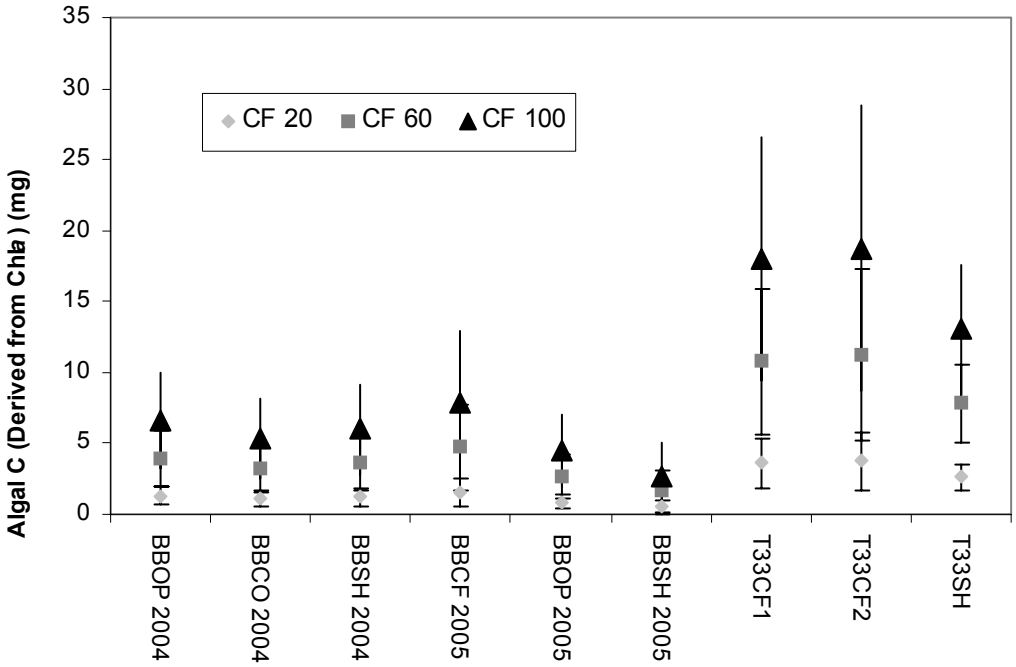

Fig 3.15 The range of algal biomass levels within biofilms at BB and T33 sites as derived from alternative Chl *a* conversion Factors (CF): 20, 60 and 100. Algal carbon content expressed as mean ± 95% confidence interval.

If the conversion factor is a consistent value, changing it does not vary the relative difference in algal biomass between sites. However, Fig 3.15 does illustrate that there is a difference in algal biomass when the conversion factor is varied (particularly significant in T33 samples). As such, I think that with a larger study (in terms of either spatial or temporal scales) one should perhaps caution against using a single conversion factor for Chl *a* measurements and vary it with general species composition, light and nutrient levels. Studies have addressed this problem by measurements of algal bio-volume. Algal biovolume is a useful method for ascribing biomass to individual species. However, the calculations of biovolumes using standard geometric models (Hillebrand et al., 1999) and the conversion from volume

to carbon (Menden-Deuer and Lessard, 2000) are susceptible to microscopic measurement errors and large inter-specific variability (Mullin, Sloan and Eppley, 1966). To assess variation between the alternative approaches, I used microscope assessment of algal content (Fig 3.16) through estimates of proportion of biomass accounted for by algal cells within five random biofilm material fields of view.

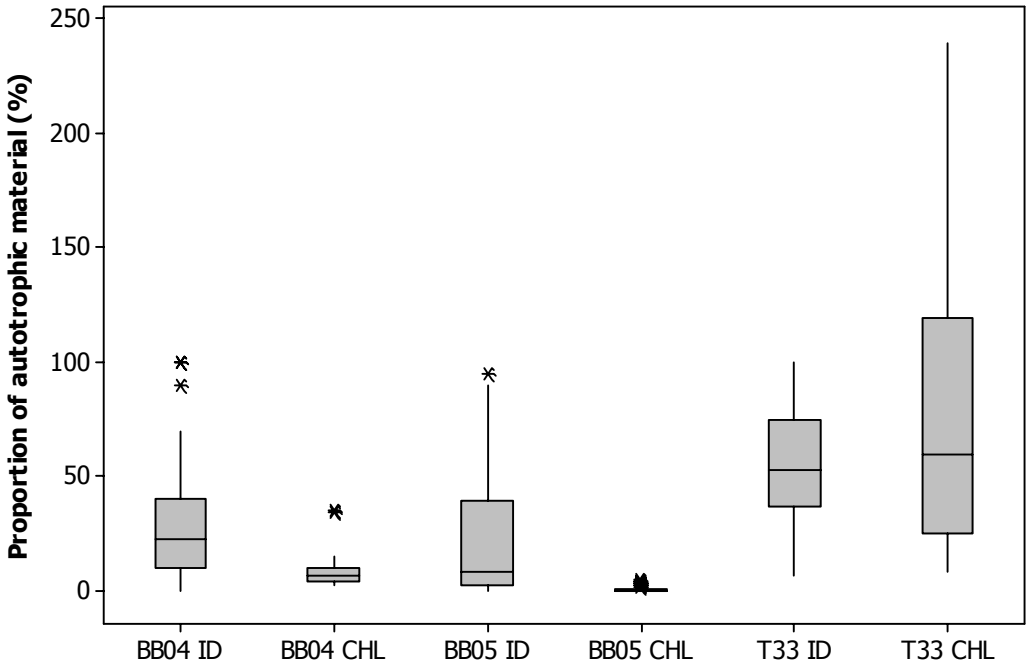

Fig 3.16 Comparison of chlorophyll defined autotrophic contribution and Microscopic identification of algal content. Kruskall-wallis defined significant differences between samples (P < 0.001). Means suggest that microscope analysis consistently overestimated the algal contribution to biofilm material.

There was a consistently greater estimation of algal content from microscopic ID. This could suggest that the conversion factor of 60 was an underestimation, However, a number of studies (e.g. Hamilton et al., 2005), have noted that microscopic examination of the relative proportions of algae and detritus in FPOM samples can be deceptive because large algal cells are conspicuous whereas colloidal detritus is not, often leading to an overestimation of algal cell contribution. Also, microscopic examination lack specificity for definitively separating living from recently dead or partially decomposed cells (Paerl et al., 1976). Therefore, without assessment of representative communities of algal cells in culture, under the differing light and nutrient regimes, the use of an intermediate conversion factor (60), at the very least, allows this study to be consistent with a number of other scientific studies approaches. Additionally, various similar studies have used a single

conversion factor (e.g. Del Giorgio and Gasol, 1995; Romani and Sabater, 2000) or used Chl a directly as an estimation of algal biomass (e.g. Carrick and Lowe, 2007). Further, as this study encompassed a small study area, with similar land-uses, and geologies, and only two different stream systems, the potential for a large amount of variability in the chlorophyll concentration of algal cells was likely to be low. The comparrisons between BB sites and T33, indicates a chlorophyll concentration difference significant enough ($P > 0.005$), to suggest a reflection of increased algal biomass, and not a change in the conversion factor between sites. Therefore, here analysis of autotrophic content is continued on the basis of the middle conversion factor of 60. Yet the potential for this variabillity needs to be considered when discussing results.

The majority of the low algal C estimates came from Black Burn sites in 2005 (Fig 3.17), where only 2.3% of biofilm samples are estimated to have autotrophic carbon contribution of under 10 %. During 2005, the mean algal C contribution to total carbon was only 2.3%. In 2004 this contribution was much greater at 23.7%.

Approximately 53% of T33 biofilms had algal carbon contributions estimated at over 10%, although mean autotrophic algal C was slightly lower than BB 2004 autotrophic standing crop, with approximately 16% of biofilm carbon derived from algal cells at T33 as opposed to 23.7% in BB 2004 biofilms.

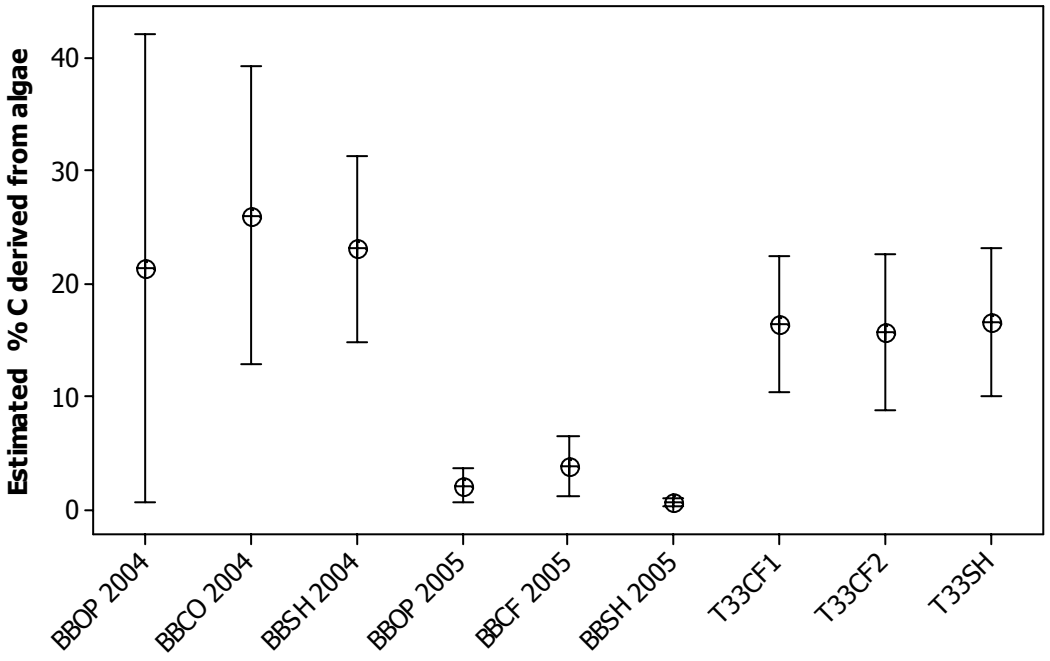

Fig 3.17. Per cent carbon derived from autochthonous algae using conversion factor of 60 from Chl *a* data. Kruskall-Wallis analysis indicates significant differences between groups (P < 0.001). Distributions of means (± 95% confidence interval) suggest that BB 2005 sites had lowest proportion of total carbon derived from algal sources.

The high C:Chl ratios and low biovolume-derived algal cellular C content of Black Burn biofilms indicate a low contribution of algae to organic matter in periphyton/biofilms. Here, only two biofilm samples had C: Chl ratios <100, a value indicative of relatively high algal cellular content in natural organic matter (Geider, 1987). The low prevalence of such values (<100) is a further indication that algal cells are not often a major component of biofilms of low order streams from this study. The results also suggest that the prevalence of biofilms with the high autotrophic contributions indicative of 'healthy' biofilms, with high enzymatic activity (Romani and Sabater, 2000), may be relatively rare at the sites studied here. However, without the hetrotrophic comparative measures, it is not possible to determine definitively if this is so.

There was no significant difference in algal C contribution among any of the sites within 2004 (Kruskal-Wallis, P = 0.54) despite significant differences in light intensity between sites (Table 3-2 and Table 3-4). This consistency among undisturbed, but habitat-variable sites, suggests that with undisturbed conditions, changes in corridor

light regimes have had no significant difference in the biofilm algal C content. Similarily, within the single disturbed stream, (BB 2005), there was no significant difference (Kruskal-Wallis, P = 0.243) in algal biomass between sites. There is a suggestion in the data that the BB clearfelled site (BBCF) had a greater algal standing crop, but this is still significantly lower (P = 0.001) than the majority of BB2004 and T33 biofilm samples (Fig 3.17). Additionally, there was no intra-site difference in T33 algal biomass (ANOVA, P = 0.976). This similarity in estimated algal carbon for sites within the reference stream is unsuprising as differences between mean % light at T33 sites were less pronounced than those at Black Burn (Table 3-2 and Table 3-4).

3.6.6 Benthic algal diversity as an indicator of biofilm biointegrity ("ecological health")

Chemical analyses of water provide a good indication of the chemical quality of the aquatic systems, but do not integrate ecological factors such as altered riparian vegetation or altered flow regime and therefore, do not necessarily reflect the net ecological health of the system (Karr et al., 2000). Biological assessment is a useful addition for assessing the biointegrity of aquatic ecosystems since biological communities integrate the environmental effects of water chemistry, over time, in addition to the physical and geomorphological characteristics of rivers and lakes (Stevenson and Pan, 1999).

Because of their nutritional needs and their position at the base of aquatic foodwebs, algal indicators provide base-line information concerning ecosystem condition. Algae respond rapidly and predictably to a wide range of pollutants and, thus, provide potentially useful early warning signals of deteriorating conditions.

Evaluations of algal production often focus on estimates of quantity, such as primary productivity and standing crop, and ignore the strong influence that changes in the quality of algal production can have on food web interactions. Algal taxa vary greatly in their edibility, and shifts in species composition can affect feeding relationships, population growth, and guild structure at higher trophic levels in aquatic food webs (Porter, 1976; DeMott and Moxter, 1991; Allan, 1995). While functional measures (e.g. productivity) may prove useful as monitoring tools, consideration of shifts in the taxonomic composition, as well as the productivity of the algal assemblage in

response to anthropogenic disturbances is often also required, in order to predict accurately the effects on other ecosystem trophic compartments. Thus, in year two of the study, identification of algal and abundance estimates were undertaken (Fig 3.7). The data were used to determine diversity of algal taxa within biofilms, using the Shannon-Weiner Index. The Shannon-Weiner Index is affected by both the number of species and their equitability ("evenness") of population abundance. A greater number of species and a more even distribution both increase diversity as measured by H. The maximum diversity (Hmax) of a sample is found when all species are equally abundant.

Table 3-7. Taxa assemblages of BB and T33 (2005). Mean taxa abundance (mean algal units across microscopic fields of view (n = 3), at x 10 magnification) (± S.E). Mean Shannon-Weiner Index and overall mean species richness also included.

	BBOP	BBCF	BBSH	T33CF1	T33CF2	T33SH
Amphipleura	0.17 +/- 0.17	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	0.29 +/- 0.18	0.00 +/- 0.00
Anebaena	0.17 +/- 0.17	0.00 +/- 0.00	0.00 +/- 0.00	0.33 +/- 0.21	0.57 +/- 0.30	0.14 +/- 0.14
Ankistrodesmus	0.17 +/- 0.17	0.17 +/- 0.17	0.00 +/- 0.00	0.67 +/- 0.21	0.57 +/- 0.20	0.57 +/- 0.30
Aphanochaete	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.14 +/- 0.14	0.00 +/- 0.00
Batrachopermum	0.33 +/- 0.21	0.00 +/- 0.00	0.00 +/- 0.00	0.50 +/- 0.22	0.00 +/- 0.00	0.14 +/- 0.14
Ceratium	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	0.00 +/- 0.00
Characium	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.14 +/- 0.14
Chlamydomonas	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.14 +/- 0.14
Chlorella	0.50 +/- 0.22	1.33 +/- 0.61	0.71 +/- 0.42	15.50 +/- 15.10	13.57 +/- 9.22	4.43 +/- 2.91
Cladophora	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	0.33 +/- 0.21	0.29 +/- 0.18	0.29 +/- 0.18
Closterium	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	1.00 +/- 0.31	0.57 +/- 0.20
Cocconeis	0.00 +/- 0.00	0.67 +/- 0.49	0.00 +/- 0.00	0.83 +/- 0.17	1.43 +/- 0.69	0.71 +/- 0.18
Cocystis	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.14 +/- 0.14
Coelastrum	0.00 +/- 0.00	2.00 +/- 2.00	0.00 +/- 0.00	1.50 +/- 0.96	0.43 +/- 0.20	0.43 +/- 0.20
Cosmarium	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.50 +/- 0.22	0.29 +/- 0.18	0.29 +/- 0.18
Cyclotella	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	0.14 +/- 0.14	0.29 +/- 0.18
Cymbella	0.33 +/- 0.21	0.50 +/- 0.34	0.00 +/- 0.00	0.83 +/- 0.65	0.29 +/- 0.29	0.29 +/- 0.18
Diatoma	0.33 +/- 0.21	0.33 +/- 0.21	0.29 +/- 0.18	1.50 +/- 0.92	2.14 +/- 1.65	1.14 +/- 0.83
Draparnaldia	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.33 +/- 0.33	0.86 +/- 0.70	0.00 +/- 0.00
Eugena	0.33 +/- 0.21	0.50 +/- 0.34	0.43 +/- 0.20	0.17 +/- 0.17	0.14 +/- 0.14	0.14 +/- 0.14
Fragularia	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	1.83 +/- 1.05	2.57 +/- 1.04	1.29 +/- 0.36
Frustullia	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.14 +/- 0.14	0.00 +/- 0.00
Gloeocystis	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	0.14 +/- 0.14
Gomphonema	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	0.83 +/- 0.48	1.00 +/- 0.31	0.71 +/- 0.42
Gonyostomum	0.17 +/- 0.17	0.00 +/- 0.00	0.14 +/- 0.14	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
Gyrosigma	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.14 +/- 0.14
Melosira	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.33 +/- 0.21	0.14 +/- 0.14	0.29 +/- 0.18
Meridion	0.17 +/- 0.17	0.00 +/- 0.00	0.00 +/- 0.00	2.50 +/- 0.72	2.43 +/- 0.75	3.43 +/- 2.27
Mesotaenium	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.14 +/- 0.14	0.14 +/- 0.14
Microspora	0.50 +/- 0.22	0.33 +/- 0.21	0.14 +/- 0.14	0.83 +/- 0.31	0.86 +/- 0.40	0.71 +/- 0.29
Microthamnion	0.17 +/- 0.17	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
Mougeotia	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	0.43 +/- 0.20	0.29 +/- 0.18
Navicula	0.67 +/- 0.21	0.67 +/- 0.21	0.57 +/- 0.30	15.00 +/- 7.10	12.43 +/- 6.21	5.86 +/- 1.22
Nitzschia	0.17 +/- 0.17	0.00 +/- 0.00	0.00 +/- 0.00	0.67 +/- 0.33	0.14 +/- 0.14	0.29 +/- 0.18
Oedogonium	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.50 +/- 0.22	0.43 +/- 0.20	0.29 +/- 0.18
Palmodictyon	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.50 +/- 0.34	0.00 +/- 0.00	0.00 +/- 0.00
Pediastrum	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	0.00 +/- 0.00
Peridinium	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
Pinnularia	1.00 +/- 0.63	0.50 +/- 0.22	0.43 +/- 0.20	1.33 +/- 0.61	1.29 +/- 0.68	0.71 +/- 0.29
Sphaerotilus	13.67 +/- 7.14	11.83 +/- 10.64	3.00 +/- 1.13	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
Staurastrum	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	0.14 +/- 0.14
Stephanodiscus	0.00 +/- 0.00	0.17 +/- 0.17	0.14 +/- 0.14	0.17 +/- 0.17	0.14 +/- 0.14	0.43 +/- 0.20
Stichococcus	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.17 +/- 0.17	0.29 +/- 0.18	0.00 +/- 0.00
Stigeoclonium	0.67 +/- 0.33	1.00 +/- 0.63	0.00 +/- 0.00	0.50 +/- 0.22	0.14 +/- 0.14	0.29 +/- 0.18
Surirella	0.00 +/- 0.00	0.17 +/- 0.17	0.00 +/- 0.00	0.17 +/- 0.17	0.43 +/- 0.20	0.43 +/- 0.20
Synedra	0.67 +/- 0.21	0.67 +/- 0.33	0.29 +/- 0.18	1.33 +/- 0.42	1.14 +/- 0.34	1.14 +/- 0.40
Tabellaria	0.33 +/- 0.21	0.00 +/- 0.00	0.29 +/- 0.18	1.83 +/- 0.40	1.43 +/- 0.20	1.29 +/- 0.29
Trebonema	0.17 +/- 0.17	0.17 +/- 0.17	0.14 +/- 0.14	0.50 +/- 0.22	0.29 +/- 0.18	0.29 +/- 0.18
Trentopohia	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.50 +/- 0.34	0.29 +/- 0.29	2.57 +/- 2.25
Ulthrix	0.17 +/- 0.17	0.17 +/- 0.17	0.14 +/- 0.14	0.67 +/- 0.21	0.29 +/- 0.18	0.43 +/- 0.20
Uroglena	0.33 +/- 0.21	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.14 +/- 0.14	0.00 +/- 0.00
Shannon-Weiner	1.38 +/- 0.51	1.34 +/- 0.44	0.91 +/- 0.36	2.34 +/- 0.28	2.18 +/- 0.25	2.34 +/- 0.17
Species Richness	7.67 +/- 2.50	6.67 +/- 2.03	4.00 +/- 1.29	17.50 +/- 1.26	15.29 +/- 1.90	15.14 +/- 1.53

In total, 51 taxa were identified within biofilm sub-samples of 2005. Diversity (Shannon-Weiner Index: H) of biofilm algae in spring/early summer (March – July) was significantly lower at all the BB sites compared with T33 sites (ANOVA, $P < 0.001$). However, diversity increased from spring to summer 2005 at both sites, creating a diversity peak at both streams during late summer (Fig 3.18), with significantly greater species richness scores in August – September rather then April – July (ANOVA, $P < 0.001$ for both streams).

T33 represents a stream undisturbed in its recent history. Black Burn had clear felling disturbance during winter 2004/2005. The diversity index scores for BB sites during spring and early summer are close to zero (Fig 3.18), indicating both low diversity and unevenness and consequently suggesting that the clear-felling event had significant impact on the biofilm algae community composition. However, there is also evidence of some recovery in diversity (H) post felling from August onwards; diversity indices from Black Burn show no significant difference from T33 sites (ANOVA, $P = 0.093$).

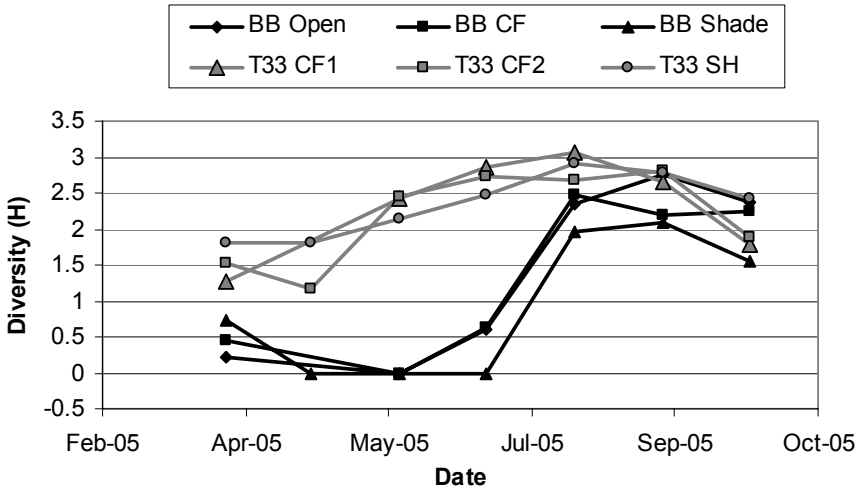

Fig 3.18. Temporal and site variation in biofilm algal Shannon-Weiner Diversity Index scores throughout 2005, at both BB and T33 sites.

Water chemistry and other environmental variables (e.g. PAR, pH, conductivity and corridor characteristics) were not measured at each algal sampling trip and thus, there are insufficient measurements to assess the importance of these directly to species composition. Consequently, species assemblages were assessed independently of environmental variables using two-way indicator species analysis: TWINSPAN (Hill, 1979) (Fig 3.19), a divisive classification method. This approach

was used in conjunction with DCA (Detrended Correspondence Analysis) (Hill and Gauch, 1980), an indirect-gradient ordination procedure (Fig 3.20 and Fig 3.21): the two approaches together permitting definition of species assemblages and the fidelity of species to an assemblage.

3.6.7 TWINSPAN analysis

Although the TWINSPAN groups could be sub-divided further, the five end-groups identified were the result of divisions with relatively high eigenvalues, suggesting good within-group similarity. Additionally, further division had the disadvantage that sample size is further reduced for each group, which would have reduced more groups to $n < 3$.

The results of the TWINSPAN analysis are shown in Fig 3.19. Due to the nature of the taxa associations, uneven separations formed three small groups within the first three iterations of the analysis (Groups 1 -3). However in iteration 4, the main group was divided to form the two main taxa groups (groups 4 and 5). The strongest separation of groups is at iteration 1 (Eigenvalue = 0.401) and separates the main group of taxa from *Chlorella, Cocconeis* and *Sphaerotilus* (group 1). *Sphaerotilus* is a colonial bacterium species and so cannot be strictly grouped with the other algal taxa. However, the widespread presence of this taxon in a number of samples meant that its presence and relative abundance was recorded. *Cocconeis* and *Chlorella* are both unicellular algae. The division of this small but significant group early on in the ordination suggests that this assemblage group is very specific and significant to a number of sites. This assemblage is also common in the low diversity samples of BB from spring 2005.

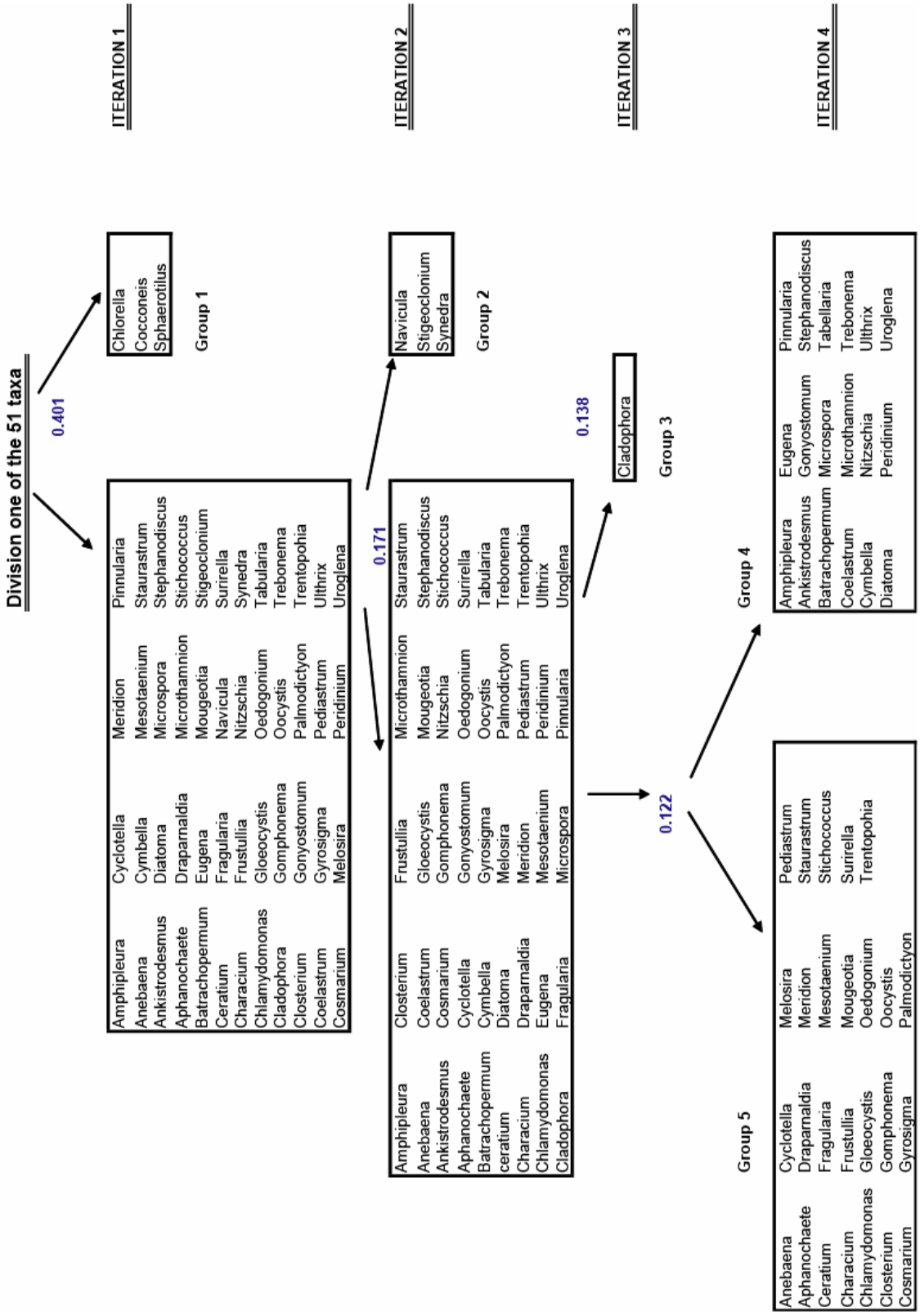

Fig 3.19. Division of biofilm taxa by TWINSPAN (Hill, 1979). Strength of divisions indicated by eigenvalues (shown in blue).

3.6.8 DCA (Detrended Correspondence Analysis)

DCA ordination of the species assemblage data was used to indicate the relative positions of the TWINSPAN groups as described in Fig 3.19. Axis units are standard deviations of species turnover (as a rule of thumb, 2 SD of species turnover along a major axis of a sample ordination corresponds to approximately a complete change in taxonomic content of the assemblage present: Gauch 19nn). There is lateral separation of the whole assemblage across group one. Separation of groups and associations with axis one were extremely strong, resulting in an eigenvalue of 0.9089. The largest TWINSPAN species group (group 5) has formed a gradient following axis 2 (vertical). However the strength of the relationship of species with axis 2 was weaker (eigenvalue of 0.4237), explaining why the species are stretched along this axis and not heavily constrained as with axis one. TWINSPAN group 4 was also quite constrained along axis one. The separation of the assemblage groups across both axes by approximately 5.5 SD, suggest that across each axis, DCA has divided the assemblage into approximately 2-3 separate communities; suggesting substantial differences within this single group.

The late separation of species groups 4 and 5 by TWINSPAN manifests in Fig 3.20 as the substantial mixing of the two groups within the ordination, further suggesting that both these relatively diverse groups are not significantly distinct within biofilm samples (and associated sites).

The green alga *Cladophora* has often been associated with eutrophication events (Hynes, 1961) and is often highly abundant in nutrient- rich flowing waters (Dodds and Gudder, 1992), with large streamers developing under nutrient-rich conditions. These streamers potentially lead to low oxygen conditions at night, alter the community structure, slow water flow in canals, and clog industrial and domestic water intakes (e.g. Dodds and Gudder, 1992). Here, this taxon has been separated within the ordination at iteration 3, to form an individual group (group 3). Reasoning for this specific separation is not clear as within the DCA (Fig 3.20), it is fairly centralized and not separated within the ordination.

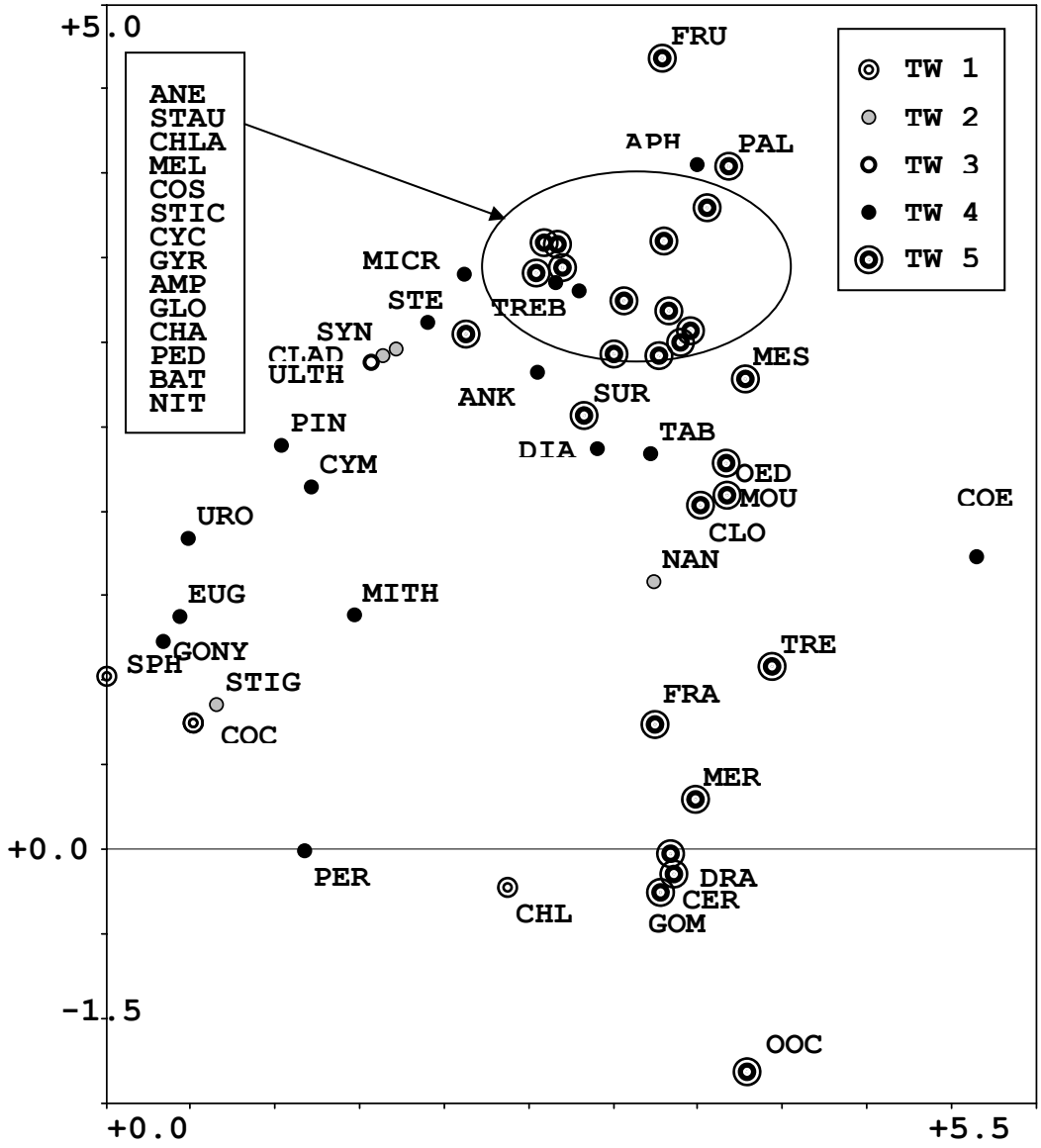

Fig 3.20. Detrended Correspondence Analysis (DCA) of algal taxa collected from the Black Burn and T33 in 2005. Axis units are standard deviations of species turnover. Taxa full names located in Table 10-1, in the appendix. TWINSPAN groups (Fig 3.19) indicated by different dots (labelling at top right corner of ordination). Ordination significant (Monte Carlo Test, P = 0.05). Strength of sample associations with axis one and two signified by eigenvalues (0.9089 and 0.4237 respectively).

Using DCA to separate sites (i.e. assemblages within samples) provides an indication of similarities both spatially and temporally amongst samples. For example, Fig 3.21 indicates separation of samples temporally (following axis 2). Late summer samples tend to be positioned towards the top of the DCA, whereas spring samples are more towards the bottom of the ordination and as generally two standard deviations corresponds to a complete change in species composition of the samples, it appears that there are approximately three communities separated by seasonal variation.

This separation provides evidence of seasonal succession of species at T33. Seasonal separation in BB species assemblages is less obvious, as BB samples were much more strongly constrained to axis two.

In addition, there is substantial spatial variation evidenced in this ordination: the majority of BB samples are located to the left of the ordination, on the low end of axis 1. T33 sites have been positioned towards the right of the ordination towards the higher end of axis 1. However, there is an indication of a seasonal gradient of BB sites along axis 1 with samples from spring and early summer situated towards the left and samples from late summer and autumn closer to the right of axis 1. This gradient may be evidence for seasonal recovery of biofilm algae species assemblage structure (as the community composition more closely resembles that of the undisturbed T33 sites).

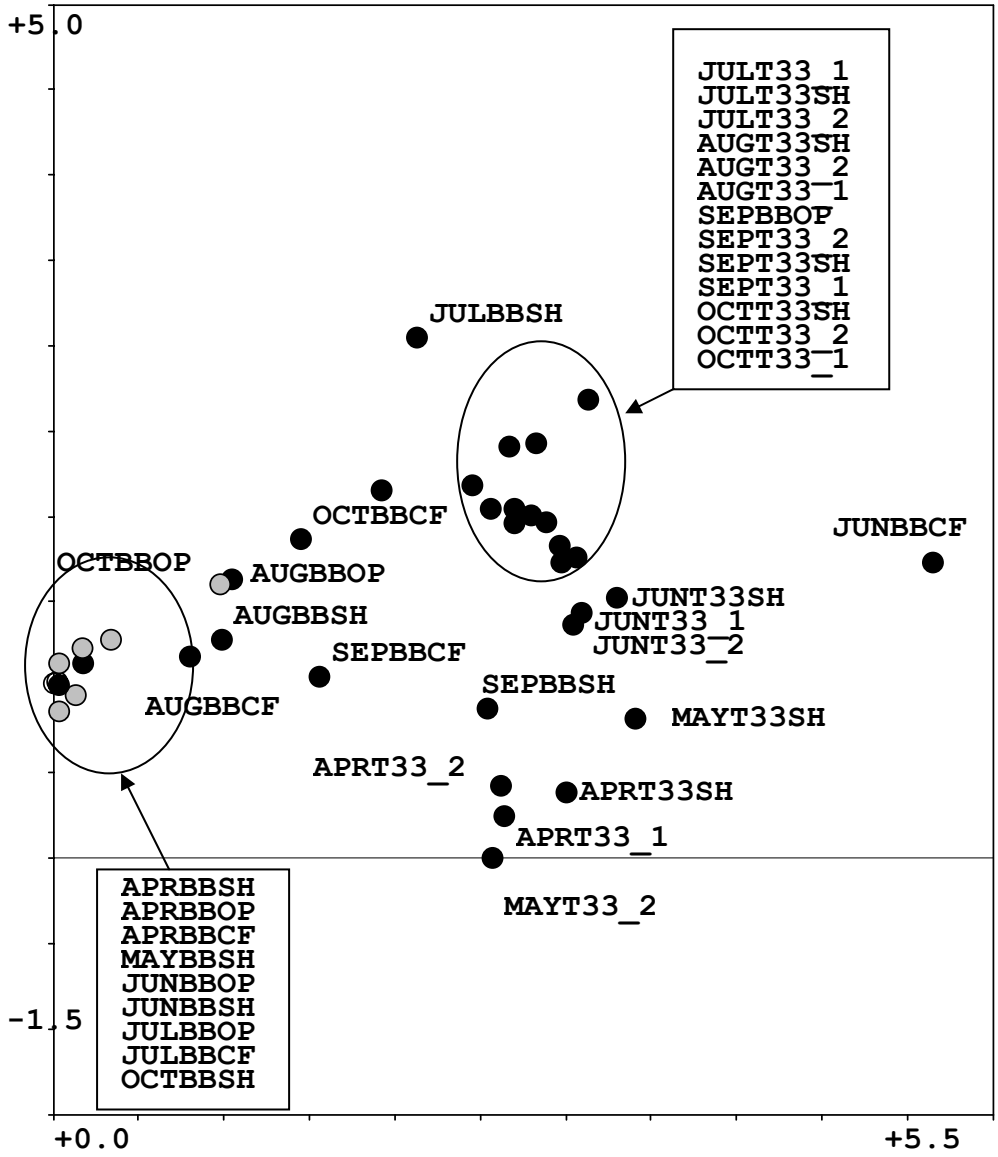

Fig 3.21. Detrended Correspondence Analysis (DCA) of sites (i.e. taxa assemblage found within each site) collected from the Black Burn and T33 in 2005. Axis units are standard deviations of species turnover. Ordination significant (Monte Carlo Test, P = 0.05). Sites are defined though abbreviated month sampled in 2005 (i.e. BB = Black Burn – OP (Open) CF (Clear-felled) and SH (Shade). T33 1 and 2 correspond with T33 CF1 andCF2, T33SH = Shaded). TWINSPAN groups one and two (Table 3-8) indicated through differential shading: grey = Group 2, Black = Group 1. Strength of sample associations with axis one and two signified by eigenvalues (0.9089 and 0.4237 respectively).

Table 3-8. TWINSPAN sample classification. Group one and two separation yielded an eigenvalue of 0.588.

Group 1				Group 2
APRT33_1	AUGT33SH	JUNT33SH	OCT T33SH	APRBBOP
APRT33_2	JULBBSH	MAYT33_2	SEPBBCF	JUNBBOP
APRT33SH	JULT33_1	MAYT33SH	SEPBBOP	JULBBOP
AUGBBCF	JULT33_2	OCTBBCF	SEPBBSH	APRBBCF
AUGBBOP	JULT33SH	OCTBBOP	SEPT33_1	JULBBCF
AUGBBSH	JUNBBCF	OCTBBSH	SEPT33_2	APRBBSH
AUGT33_1	JUNT33_1	OCTT33_1	SEPT33SH	MAYBBSH
AUGT33_2	JUNT33_2	OCTT33_2		JUNBBSH

A TWINSPAN of sample sites (Table 3-8) indicates that there was no specific separation of site type (e.g. SH, CF or OP), and that the specific riparian characteristics of sample sites do not significantly impact the specific assemblage structure of the biofilm algae. Yet, there is separation of spring BB samples (all sub-sites) indicating variable conditions and associated assemblage structure away from the samples assemblage types during this period at the Black Burn sites. This shift in algae community composition may be an indication of significant alteration of site conditions at BB during spring 2005.

Combining both DCA ordinations (Fig 3.20 and Fig 3.21) provides an indication of which taxa were found within various sites and seasons. For example, *Sphaerotilus* and the other members of group 1 are found to be associated with spring/summer biofilms from BB, as are the majority of group 2 taxa. The diverse assemblage of group 5 is almost exclusively associated with T33 biofilms, whereas group 4 can be categorized as common generalists as they are found throughout all of the sites and sample periods. Using the distribution of group 5 also provides some information on the seasonal distribution of species, as there is the indication of a taxa gradient occurring along axis 2 of the ordinations. Spring samples are associated with taxa such as *Oocystis*, *Gomphonema*, *Draparnaldia* and *Ceratium*, whereas, late summer assemblages are dominated by a slightly more diverse collection of taxa, including *Frustula* and *Palmodictyon* at the top of the ordination.

3.7 Discussion

The results of this chapter do not support the original hypothesis that algal biomass would be primarily limited by light intensity. There were no site-specific significant differences in chlorophyll concentration between either of the undisturbed sites, BB 2004 sites or T33, 2005 (Fig 3.12).

This finding contradicts a number of studies viewing light intensity as the primary controlling factor of in-stream algal biomass (e.g. Mosisch et al., 2001). The lack of coupling between these factors may suggest that light levels (PAR) available at all sites (including BBSH and T33SH) were not reduced enough at any point to limit chlorophyll production. However, it is not possible to relate variation in biomass to variation in primary productivity. Specifically, findings in the literature suggest that the correlation between estimates of algal biomass (chlorophyll *a* concentration) and primary production is quite poor (Benke et al., 1984; Mosisch et al., 2001). Specifically, it is important to distinguish the difference between standing crop biomass (independent of turnover rate) and primary production, as algal biomass in streams is as much a function of flow regime (Rounick andGregory, 1981; Tett *et al.,* 1978) and invertebrate grazing (e.g. Steinman, 1996) as it is of growth rate. Thus other factors may influence standing crop more than light availability, reducing and visible coupling between these two factors.

The site-specific differences in the response to the felling event are discussed further in Chapter 7. However, the response of the autotrophic component is severe and has important potential impacts on biofilm character and functioning. Under circumstances of disturbance, it appears that canopy cover and riparian characteristics become increasingly important in controlling biofilm character and specifically autotrophic contribution (as BBCF has the greatest autotrophic production of the BB2005 samples). However, weather this variation in autotrophic biomass is due to light regime or the release of allochthonous-based nutrients was not determined. However, it is worth noting that the level of light required by algae is not only controlled by riparian shading, but is also a function of the turbidity and colouration of the water itself. Repetitions of this study would be improved by including in-stream light measurements combined with calculations of light attenuation (Zeu) in order to determine whether light limitation fell below a critical

point following felling as a consequence of increased organic matter release and sedimentation, and not solely because of the corridor design. Corridor design and management must take into account both the optimum light requirements of the in-stream biota and also water quality, especially light attenuation within the water column.

However, there was significant stream-specific variation in algal biomass as shown in Fig 3.14, with T33 and BB2005 yielding significantly different algal C concentration. Thus T33 provided relatively favourable conditions for algal growth (compared to BB2005). Further, there were detrimental effects to algal standing crop. Conditions were less favourable for autotrophic growth.

As determined by Romani and Sabater (2000), a biofilm complex with an algae component two to three times higher than bacterial biomass results in the highest level of enzymatic activity within the biofilm. They also showed that it is the autotrophic component of the biofilm which performs a top-down control on cellulosic and hemicellulosic degradation in stream biofilms, and thus controls the rate of processing of benthic organic matter within stream systems. Changes in the proportional contribution of carbon to chlorophyll *a* (C:Chl) has the potential to cause significant shifts in biofilm functioning. Although heterotrophic biomass was not determined, variation in C:Chl provides some indication of autotrophic carbon, and the results here, contrary to a large number of studies (e.g. Frost and Elser, 2002; Bowman et al., 2005), suggested that algal cells were a minor component of 'periphyton' (though similar results have been found by others: e.g. Frost et al., 2005; Hamilton et al., 2001).

On average, only 8.5% of biofilm/periphyton carbon was estimated to be from algal cellular origin. However, this proportional contribution was based on a conversion factor from chlorophyll *a* to algal carbon of 60. This value has beeen shown to be variable with species and growth conditions. As indicated by Table 3-2 and Table 3-4, the physical conditions of each site were variable (specifically, the level of radiation). Additionally, the species composition was variable with site and season (Fig 3.20 and Fig 3.21). Thus, variability in the conversion factor was explored in Fig 3.15, with a range of CFs from 20 – 100 (following the range described by Margalef (1983). The range in conversion factors resulted in an up to ~25 mg range in algal

carbon within the tile biofilm biomass (from T33 data). This variability in the potential contribution of autotrophic biomass to the biofilm material has significant potential impacts to any conclusions aiming to determine the proportional contribution of autotrophic and heterotrophic material to the biofilm biomass. However, the potential from variation to the conversion factor to conclusions regarding the potential variability of functioning capacity of the biofilm (due to changes in the autotrophic proportion (following Romani and Sabater, 2000)), are based on the proportion of autotrophic to heterotrophic material. Calculations using alternative conversion factors on the proportion of autotrophic material within a biofilm can be found in Fig 3.22.

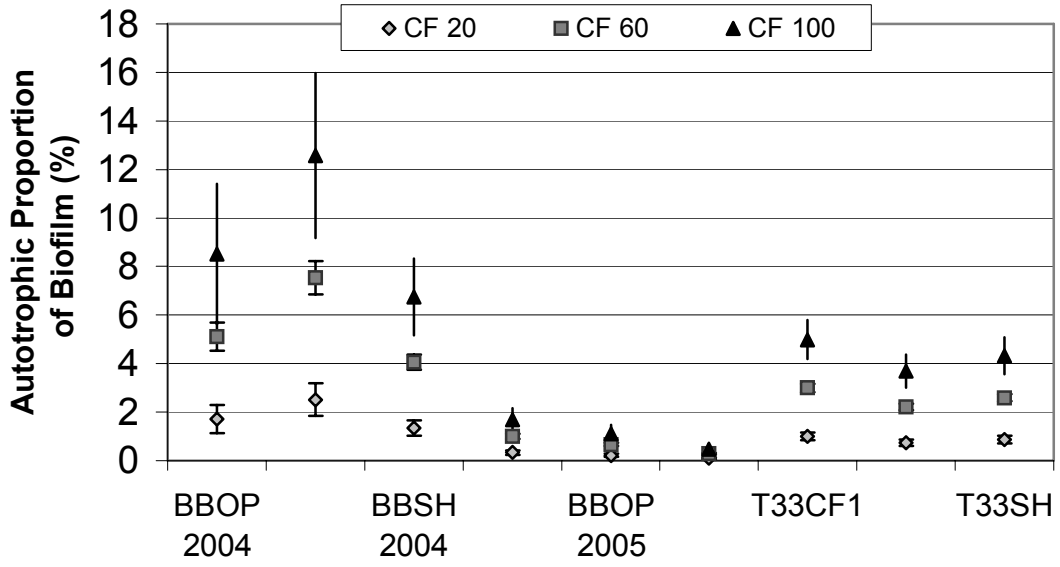

Fig 3.22. Variability in the proportion of autotrophic material to biofilm biomass (mean ± SE) with alterations to the Chlorophyll *a* conversion factor (20, 60 and 100).

Although variation in the conversion factor could potentially influence the proportional contribution of autotrophic material, Fig 3.22 indicates that the autotrophic proportion remains low through all sites. Maximum autotrophic contribution appears to come from BB2004 biofilms. Yet the proportion is still below 15% for all sites. This contradicts findings suggesting that periphyton is primarily composed of algal cells (e.g. Frost and Elser, 2002; Bowman et al., 2005).

With reference to biofilm functioning, without determination of the heterotrophic component it is not possible to estimate the proportion of heterotrophic to autotrophic material. Much of the biofilm biomass is likely to be composed of allochthonous detrital material. In order to make estimates about the relative proportion of heterotrophic material, the allochthonous component must be isolated and removed from these calculations. Consequently, the following chapter (Chapter 4) seeks to determine the source of carbon to biofilm more fully, by exploring methods of determining the allochthonous and autochthonous components of biofilm biomass.

However, a large number of studies have used Chl *a* as a direct proxy for autotrophic biomass, even to the extent where no conversion factor is used at all (e.g. Carrick and Lowe, 2007). Alternatively a large number of studies utilise C:Chl ratios as a measure of algal cellular fraction (e.g. frost et al., 2005; Hamilton et al., 2005), thus the approach of using Chl as a proxy for algal biomass (either directly, or indirectly through conversion) appears to be widespread. As the conversion factor of 60 fell within the centre of the range suggested in literature, it was felt that it was possible to use this factor and still remain consistent with other studies.

Therefore, application of this factor for further calculations of carbon partitioning, indicated that approximately 72% of biofilm samples from the Black Burn (both 2004 and 2005) had less than 10% of C in the algal cellular fraction. However, there was evidence of substantial variation between the results from 2004 and 2005 as the majority of these low algal C estimates came from Black Burn sites in 2005 (Fig 3.17), suggesting that the allochthonous contribution to biofilms in 2005 was substantial.

The significant difference in the mean algal C contribution to total carbon in 2005 (only 2.25%, compared to 23.73% in 2005), is evidence to suggest that the relative contribution to the biofilm of autotrophic and hetertrophic material is substantially altered. It is likely that the functioning capacity is also greatly altered, resulting in biofilms with low autotrophic production that are likely to have a far poorer bacterial population, enzyme activity level and associated ability to process the excess allochthonous carbon entering the stream environment (Romani and Sabater, 2000). However, without analysis of the relative heterotrophic component it is not possible

to make proper conclusions. As a biofilm will be made up of allochthonous and autochthonous material, one can assume that within the autochthonous component, biomass can be split into either autotrophic or heterotrophic material. Thus in order to determine the heterotrophic component, I examine approaches to determine the relative proportion all autochthonous material within Chapter 4.

Microscopic analysis was useful in providing information on community composition. However the technique has serious limitations in determining proportional contribution from algal cells, as quantities are often overestimated due to cells being more conspicuous. Additionally, there is no way of determining the origin of unidentifiable detrital material.

However microscopic analysis proved useful in determining the variability of community composition across sites and also with seasonal variation. DCA analysis of the species assemblage revealed information on species associations and community composition of sites (Fig 3.20), as well as temporal and spatial variation in the community composition of sites (Fig 3.21). However there was no distinction between site type and community composition, suggestion that the degree of canopy cover and corridor design does not significantly impact on the in-stream biofilm algae community composition. There was evidence of both seasonal separation (through gradients along axis 2), and site separation (with BB and T33 sites separated and constrained in their distribution along axis 1).

Species indicators were also identified within the ordinations. Diatoms are a siliceous class of algae reputed for being very sensitive to chemical conditions. They usually account for the highest number of species (up to 80%) among the primary producers in aquatic systems (Pan et al., 1999). As a consequence, they have frequently been used as biological indicators of water quality (Kelly et al., 1998, Prygiel et al., 2002; Leira and Sabater, 2005). For example, Sabater, (2000) showed that diatom indices successfully indicated the effect of a catastrophic heavy metal spill on a river system, although, this approach failed to reliably detect the recovery. Diatom analysis has therefore been considered an important contribution to the European Water Framework Directive (European Commission, 2000), which aims to achieve a "good status" for all waters in the EU before 2015. Fig 3.20 indicates that few of the diatom species have been located towards the left hand side of the

ordination, close to the BB samples, indicating evidence for low water quality at BB sites. Further the sewage fungus, *Sphaerotilus* was closely correlated with BB samples following the felling event in winter 2004/5.

Therefore, a combined approach of autotrophic determination and species composition analysis has indicated that the key findings of this chapter were:

- Seasonal variation in biofilm biomass at BB for both 2004 and 2005, yet biomass settlement was delayed in 2005 until mid summer, but was significantly increased overall (particularly at the clearfelled site). This increase in biofilm biomass was not reflected in T33, suggesting a less seasonally affected site.
- Net chlorophyll concentration was comparable between sites during 2004. Similarities in standing crop were consistent despite seasonal variation. However, site-specific variation occurred in 2005, following felling, where chlorophyll production was greatest at the site of closest proximity to the clearfelling, suggesting a positive correlation between disturbance and production.
- Greatest net chlorophyll mass was found at T33 sites, indicating the most favourable growth conditions for benthic periphyton.
- C:Chl concentrations were highest at BB2005 sites indicating low contribution from algal carbon, and high inputs from external carbon contributions, leading to the need to determine source of carbon not of autotrophic origin.
- The potential variation in the conversion factor (CF) of chlorophyll *a* to algal carbon indicated the potential for a 25mg increase in algal biomass per tile in association with CF error. This was reflected in around a 15% variation in the proportion of autotrophic material to biofilm biomass. It was determined that without estimates of heterotrophic component biomass, it was not possible to determine if this variation would significantly influence biofilm functioning.
- However, consideration of study size and length and the comparative similarities between streams, a conversion factor of 60 was deemed comparable to a number of literature observations, and the approach comparable to the many studies that use chlorophyll as a proxy for algal biomass.

- Consideration of all potential conversion factors still indicate that biofilms are not autotrophic dominated. This contradicts assumptions that periphyton is primarily algal in origin.
- Microscope analysis was not comparable to estimations of algal content using chlorophyll. Results were consistent with literature suggesting visual estimations result in overestimations of algal proportion.
- However, visual ID of algal species indicated variation in algal community composition with season and stream, but not within stream site-specific variation. There was also indication of a Diatom response to disturbance events.

4 Characterising energy sources within benthic biofilms of upland streams

4.1 Abstract

This chapter expands on work done in the previous chapter (Chapter 3), in order to determine:

- temporal and spatial variation in the elemental composition, general characteristics and overall production of the baseline resource
- variation in autochthonous production and diversity with changing corridor characteristics
- potential physical factors which can affect biofilm elemental composition
- the variability in the functioning capacity of the biofilm with changes in compositional characteristics.

Isotopic and stoichiometric analyses of biofilm samples were used to define sources of carbon available at the base of the food chain. Using this elemental composition analysis, a two-source mixing model was applied to compositional data in order to define allochthonous and autochthonous contributions to biofilms. Analysis of intra/inter-site differences is used to consider temporal and spatial responses from biofilms growing under different riparian conditions.

Variation in the molar C:N ratio is discussed with consideration of food quality variation. The results indicated that biofilms from BB 2005 were found to have both increased allochthonous proportion and likely low protein content and as such, lower nutritional value.

There was no difference in the proportion of autochthonous production within the biofilms under different intra-site conditions despite variations in percent light and canopy cover. However, T33 analysis indicated a reduction in autochthonous contribution within the shaded site, suggesting light limitation to primary productivity (PP). Using the autotrophic proportional data analysed in chapter 3, I compare the results with the autochthonous content derived using the approach outlined within this chapter in order to determine the proportion of algal-derived material within the autochthonous proportion of the biofilm biomass. Although there is error surrounding

integration of the results from different approaches, autotrophic biomass was significantly (P value < 0.001) lower than the values derived for autochthonous contribution. Further, this allowed for estimates of the heterotrophic proportion of the biofilm material to be estimated. Results indicated that a high proportion of the autochthonous material from BB biofilms was of heterotrophic, and not of autotrophic origin. The relative proportion of heterotrophic to autotrophic and allochthonous to autochthonous varied with stream and season. However site-specific variation was minimal suggesting that increasing 'openness' of corridor may not contribute to an enhanced autotrophic proportion of basal resources, nor substantially influence the biofilm resource characteristics. However, the high autochthonous contribution at all sites suggested significant 'self sufficiency' of the biofilm at all sites.

4.2 Introduction

Food quality (edibility, digestibility, nutritional sufficiency) is determined to a large part by the relative contributions from allochthonous sources (terrestrial inputs) and autochthonous production in stream systems (Findlay et al., 2001; Sobczak et al., 2005). The former is detrital material of low nutritive value, whereas the latter (algal fraction) is enriched in mineral nutrients and important biochemicals (fatty acids, amino acids, etc.) whose concentrations vary with algal species composition and nutritional status (Brett and Muller-Navarra, 1997; DeMott et al., 1998; Von Elert and Wolffrom, 2001).

Isotope analysis offers an important contribution to our understanding of ecological systems and the approach is becoming increasingly popular in characterising ecological systems (e.g. Rundel et al., 1988; Zah et al., 2001; Melville and Connolly, 2003).

The elemental composition (C:N:P) of organic matter at the base of food webs potentially plays a key role in food-web dynamics in the benthos (Frost et al., 2005; Bowman et al., 2005). Stoichiometry is the measure of that balance of the elemental composition and can be used to characterise organic matter according to the ratios of the three nutrients.

Here, these approaches are taken to characterise base-line resources available within streams of afforested catchments through analysis of benthic biofilm growths in order to characterise basal resources available to higher consumer groups within the stream environment.

4.3 Identification of carbon source in aquatic ecosystems

4.3.1 Background

Many elements exist whose atoms can have alternative atomic weights. The alternative atomic forms are termed isotopes. Stable isotopes are those that are not radioactive. For example, the two common isotopes of carbon are ^{12}C (carbon with atomic weight 12, natural abundance 98.89%) and ^{13}C (atomic weight 13, natural

abundance 1.11%). The relative abundance of these two isotopes is modified by physical processes, biochemical incorporation into living systems and inorganic chemical reactions. For example diffusion constraints, source effects, enzyme selectivity and/or interactions between compounds (Rundel et al., 1988) can modify composition and this is termed isotopic fractionation.

Isotopic compositions are expressed as the ratio of the two species of the element (e.g. ^{13}C to ^{12}C) compared with that of a standard. This standard will have a known value relative to the international working standard. The differences between most samples and the standard are very small, thus the results are expressed as parts per thousand (per mil or ‰). Samples are expressed as follows (Craig, 1953) in Equation 2.

Equation 2

$$\delta X = \left[\left(\frac{R\text{sample}}{R\text{standard}} \right) - 1 \right] x1000$$

δ is delta, *i.e.* the change in ratio from the standard value. Isotope ratios values are expressed as parts per thousand (‰).

For $\delta^{13}C$, CO_2 produced from fossil Belemnite $CaCO_3$, from a strata of marine sediment called "The Peedee Formation", (VPDB standard Chicago, *Belemntella americana*, Peedee Formation, Cretaceous, South Carolina) is the international standard. For $\delta^{15}N$ atmospheric air (AIR) is the standard (Ehleringer and Rundel 1989). Repeat analysis of international and internal laboratory standards shows that $\delta^{13}C$ and $\delta^{15}N$ can usually be measured with precision and accuracy of ≤ ± 0.1‰ and ≤ ± 0.3‰ respectively.

4.3.2 Carbon Isotopes

The first data on carbon isotopes (Nier and Gulbransen, 1939) showed differences in $^{13}C/^{12}C$ between limestone, atmospheric CO_2, marine plants and terrestrial plants. Improvements in the techniques and the development of better equipment have

made the technique widespread and much more accessible. In the mid 1960s interest in isotopic fractionation was extended with the discovery that the C_4 metabolic pathway in plants produced a more isotopically enriched tissue signature (-9 to –14‰) than tissue from C_3 plants (-20 to -35‰) (Bender, 1968, 1971; Smith and Epstein 1971). Aquatic plant tissues have a larger carbon isotopic range (between –8 and -30‰), reflecting both the metabolic pathways of the plant, and the mechanism by which they gain carbon - atmospheric CO_2 for emergent plants and dissolved carbon dioxide or bicarbonate for fully submerged species.

$\delta^{13}C$ of the dissolved inorganic carbon used by aquatic plants tends to be more depleted in ^{13}C than atmospheric CO_2. Thus, species of plants which are fully submerged and which through their roots, use one of the dissolved carbon species drawn from interstitial hydro-soil water (e.g*. Littorella uniflora*) are likely to be more ^{13}C depleted than plants which are emergent and have access to atmospheric CO_2 (e.g. *Persicaria amphibia*). Similar are aquatic plants use dissolved atmospheric CO_2, drawn from the water for photosynthesis (e.g. algae, *Elodea canadensis*). Dissolved CO_2 and bicarbonate can also both be used by certain species of submerged macrophytes (e.g. *Potamogeton pusillus*) depending on pH. Table 4-1 provides examples of dominant sources of carbon for different plant photosynthetic pathways.

Table 4-1. Carbon form, sources and photosynthetic pathways for freshwater plants (species shown are examples) (- = no known examples). *Potamogeton pusillus* shifts carbon source depending on water pH. SAM = Submerged Aquatic Macrophyte photosynthesis (Spencer and Bowes, 1990)

C Form	Source	SAM	C4	C3
HCO_3^- (dissolved)	open water	*Potamogeton pusillus*	-	-
CO_2 (gaseous)	air	-	*Echinochloa crus-galli, Paspalum repens*	*Persicaria amphibia, Typha latifolia*
CO_2 (dissolved)	open water	*Potamogeton pusillus; Elodea canadensis*		Charophytes; Benthic algae
CO_2 (dissolved)	interstitial hydrosoil water	-	-	*Littorella uniflora*

4.3.3 Nitrogen Isotopes

The two stable isotopes of nitrogen (^{15}N and ^{14}N) had until the late 1960s received little attention within the biological world, but now measurements of the ratio of these isotopes is routine; as information can be provided on food web structure or N source. For example, it is possible to determine the source of soil nitrates from the isotopic composition of the soil water; i.e. soil-water isotopic signature would be different if the nitrates were of a natural origin rather than from nitrate based fertilisers (Kohl et al., 1971).

Diet studies using stable isotopes have determined that within a food chain, enrichment of the isotopic signature occurs within the consumer. A consumer's isotopic signature reflects that of its food, after biochemical fractionation with increasing trophic level is accommodated (Peterson and Fry, 1987). Generally, $\delta^{13}C$ becomes 0.0–1.0‰ enriched between trophic levels, while $\delta^{15}N$ show a greater enrichment of 3–5‰ (Peterson and Fry, 1987). This makes $\delta^{15}N$ an important parameter for the assessment of trophic status. If food types differ in their signatures, the proportional contribution of each food type to the consumer's diet can be inferred from its isotopic ratio, assuming that all food types are included in the analysis, all foods are assimilated equally and homogeneously, and trophic fractionation values are known (Phillips and Koch, 2002).

The relative contribution of specific food sources can be assessed using a dual stable isotope method (e.g. $\delta^{13}C$ and $\delta^{15}N$: Fry, 1991). Modelling food web structure with the use of stable isotopes has now become an important part of freshwater research (e.g. Fry and Sherr, 1984). Isotopic signatures of consumers generally reflect the organic matter assimilated (e.g. Zah et al., 2001). This method, works on the assumption that the food source is isotopically distinct.

However, the majority of nitrogen cycling studies based on isotope analysis use enriched isotope tracers released within aquatic ecosystem studies in order to determine cycling of nitrogen within systems (e.g. Robinson, 2001). This makes references to comparative studies within the literature which utilize natural $\delta^{15}N$ signatures difficult. However, deviation of $\delta^{15}N$ signatures from 0‰ provides indications of nitrogen sources (0‰ being, by definition atmospheric nitrogen signature) and quantity and quality of N derived by fixation (via the nitrogenase enzyme). Nitrogen fixers commonly produce $\delta^{15}N$ values around or slightly below

0 ‰ (Handley and Raven, 1992; Gu and Alexander, 1993; France et al., 1998). Thus, pure algal samples displaying nitrogen isotopic signatures below zero are obviously subject to alternative nitrogen species sources.

4.3.4 Stoichiometry

At the beginning of the 20th century, scientific interest was focussed on nitrogen cycling in the oceans and the role of marine organisms in this process. This work is now responsible for an understanding in the relationships between the chemistry of the environment and of the associated organisms. The Redfield ratio (after the marine chemist: Alfred Redfield) was defined after Redfield found that the bulk of marine particulate organic matter is extremely constrained in its elemental composition and that this composition was remarkably similar to the composition of the seawater (Redfield, 1934). This elemental composition is commonly taken to be 106C: 16N: 1P (by atoms). The Redfield ratio is generally regarded as the average composition of marine phytoplankton. This equates to a C:N ratio of 106:16 (i.e. 6.6).

Variations in the Redfield ratio in algae are generally due to cell-wall composition of different taxa. For example, dinoflagellates with a cellulose wall generally have a molar C:N of around 8-10, while cyanobacteria contain relatively large volumes of protein which means that their C: N is closer to 5-6 (Sterner et al., 1993).

The elemental composition of organic material at the base of the food-chain potentially plays a key role in determining community composition and food-web dynamics and food quality (e.g. Frost and Elser, 2002; Frost et al., 2005; Bowman et al., 2005; Sobczak et al., 2005). Here, the parameter of molar C:N is investigated alongside isotopic measures for inferring changes in the proportion of allochthonous and autochthonous derived material within the biofilm.

High molar C:N signatures are a reflection of increased input of organic compounds low in nitrogen. Organic material of allochthonous origin contains a low proportion of nitrogen resulting in a relatively high molar C:N. For example Wetzel, (1975) reports allochthonous organic material C:N of about 50:1, while material produced

autochthonously had a much higher initial N concentration, resulting in a molar C:N of around 12:1.

4.4 Aims

The relative roles of various sources of organic matter as the basis of stream food webs are difficult to ascertain. Streams commonly receive substantial allochthonous inputs of detrital organic matter, but they also vary greatly in their primary productivity, as a consequence of differing habitat conditions (e.g. light intensity, Allan, 1995). Therefore, this chapter aims to:

- Demonstrate the utility of using stable isotope analysis (SIA) and stoichiometry measurements in order to determine the relative proportion of carbon derived from autochthonous and allochthonous production sources within stream biofilms.

- Integrate analysis performed in the previous chapter (quantification of the autotrophic proportion) in order to ascertain estimates of the relative proportions of autotrophic to heterotrophic material.

- Explore how biofilm character varies with habitat conditions and with references to previous studies, explore possible variability in biofilm functioning, and level of self-sufficiency.

4.5 Methodology

Biofilm samples were measured through the placement of artificial substrates (linoleum tiles, as illustrated in chapter 3, methods section) within two streams of the study; Black Burn (Cree) and T33 (Bladnoch). For a full description of sites and sub-sites (BBOP, BBCO, BBCF and BBSH and T33CF1, T33CF2 and T33SH) and artificial substrate settlement methodology within the study see Chapter 3.

4.5.1 Stable Isotope Analysis (SIA)

For each sample, approximately 2 mg (weighed out to 0.01mg precision) of the dried material was loaded into an 8x5mm tin capsule and crimped closed. Using

continuous-flow isotope-ratio mass-spectrometry (CF-IRMS), the crimped capsules were processed for measurement of the $\delta^{13}C$ and $\delta^{15}N$, molar C:N and wt% C and wt% N by combusting in a Carlo Erba C/N/S analyser interfaced with a Finnigan Tracer Matt continuous flow isotope ratio mass spectrometer (CF-IRMS). These analyses were carried out by Dr. Susan Waldron at the Scottish Universities Environmental Research Centre (SUERC) in East Kilbride.

4.5.2 Mixing Models

The difference in composition of allochthonous and autochthonous energy sources can be used to quantify the relative contribution of those components to the biofilm mixture, using mixing models (e.g. Phillips 2001).

As a biofilm is a complex mixture of detrital matter (of both allochthonous and autochthonous origins), algae, bacteria and fungi, the ideal analysis would be the application of a mixing model which addressed separation of *all* these sources and thus providing the greatest amount of information on biofilm content and functioning. However, this approach relies on delineation of each individual source component by identification of associated end-member signatures. Due to the nature of the biofilm signatures measured in this study (which will be addressed further, in latter sections), a more practical approach, using a two-source mixing model was applied (Equation 3). This model uses the major organic matter sources (allochthonous and autochthonous inputs) as the two end-members of the proportionate mix (biofilm).

Equation 3. Two-source mixing model

$$\delta_T M_T = \delta_1 M_1 + \delta_2 M_2$$

Where;
δ = isotopic/stoichiometric signature
M = mass (the fractional contribution of each organic matter source to the biofilm)
T = Total
1 = Source 1
2 = Source 2

The equation product is a linear relationship between the two potential source components, indicating the potential mixes of sources along the gradient. However a perfect linear relationship is only observed when there is no variation in the two end-

member source signatures or contamination from alternative source material signatures. If there is variation and/or contamination of source material, then this can potentially result in either a different gradient in the model observed, a curvature to the relationship, or outliers from the linear relationship as mix signatures deviate from the gradient defined by either of the end-member extremes (e.g. Fig 4.1).

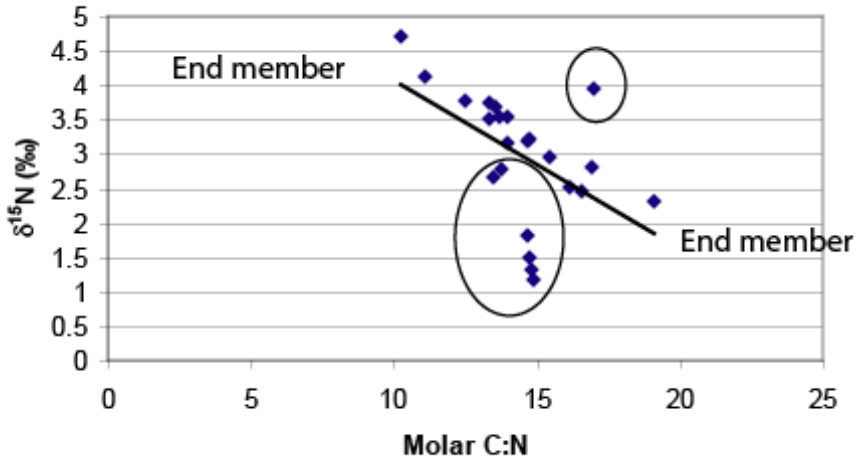

Fig 4.1. Sample $\delta^{15}N$ and stoichiometric data (BB April, 2004). Circled data points cannot be formed from any possible combination of the two end-member source signatures. Therefore, there must be variation in source signatures (from a source with both high molar C:N and low $\delta^{15}N$ properties).

Variation and, specifically, multiple allochthonous and/or autochthonous sources produce difficulties in defining the characteristics of heterogeneous biofilm material. However, this chapter aims to explore the use of multiple measures (e.g. ^{13}C, ^{15}N and molar C:N), to define a proxy able to delineate carbon source without being influenced by specific changes in intra-source composition (e.g. within allochthonous classification there are potential species separations such as pine needles, moss, deciduous leaves etc, or different algal species within the autochthonous classification).

4.5.3 Allochthonous detritus estimations made by microscopic visual inspections

See 'Tile composition analysis (species / detrital measurements)' section: Chapter Three, Methods.

4.6 Results

4.6.1 Temporal variation in elemental composition

Biofilm $\delta^{13}C$ signatures ranged from –25.1‰ to –32.6‰, whilst $\delta^{15}N$ ranged from -2.6‰ to 7.1‰. Molar C:N values also showed a large signature range of 6.4 to 34.1.

Black Burn (BB)

As discussed in the previous chapter and repeated here in Fig 4.2, there were significant increases in biomass yielded from the artificial substrates (dry weight biomass (g)) through the summer period (May to Aug) of 2004, compared with winter values (Dec to April 2004) of that same year (ANOVA, $P < 0.001$) of biofilm material from the Black Burn (BB). However, in addition, there was significant temporal variation in mean monthly BB biofilm biomass production in 2005 (ANOVA, $P < 0.001$) and this biomass production was significantly ($P < 0.001$) greater than that of 2004.

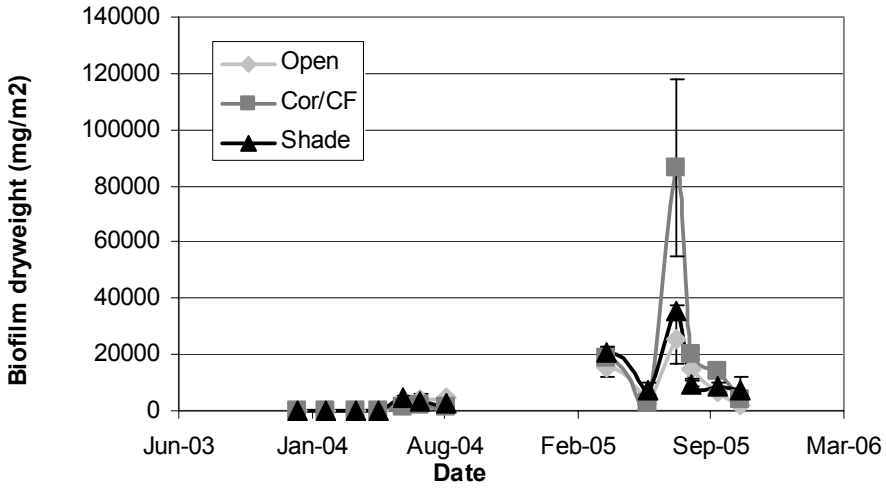

Fig 4.2. Black Burn temporal variation in years one and two (2004 – 2005) of dry-weight biomass per m^2 of tiles (mean ± standard error).

Such variation provoked questions regarding the possible comparable variability of the other measured characteristics of the biofilm material: the proportional contribution of allochthonous and autochthonous material over seasonal and temporal variation. Fig 4.4 to Fig 4.8 illustrates the temporal variability in the biofilm mean isotopic and stoichiometric signatures at the Black Burn for the study period (2003 – 2005). This signature variation is explored in order to define the elemental composition of the biofilm over spatial and temporal scales. Similarly, this variability is also compared with signatures from T33 biofilms (Fig 4.9 - Fig 4.11), which were subject to less dry-weight biomass seasonal variation within 2005 (Fig 4.3).

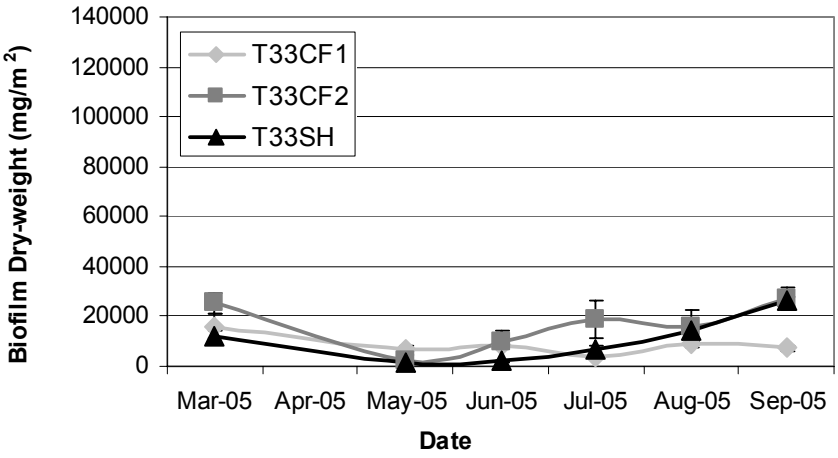

Fig 4.3. T33 biofilm dry-weight biomass (mg/m^2) sampled in 2005 (mean ± standard error) (*n* = 4 samples per site, 12 per stream/ month (72 in total)).

BB 2004

A Tukey test (Fig 4.4) revealed that the $\delta^{15}N$ values of 2003/2004 winter samples were generally more enriched than the summer samples. The data represents means (± 95% confidence interval) for the Black Burn, with all data from sub-sites pooled, and thus, only indicating differences in temporal variation. There was no significant difference (ANOVA with April omitted: $p = 0.109$) in the ^{15}N values of summer biofilms (March - August). Monthly values during summer overlapped considerably, with the exception of April which was significantly more depleted ($P = 0.001$) than the other summer months.

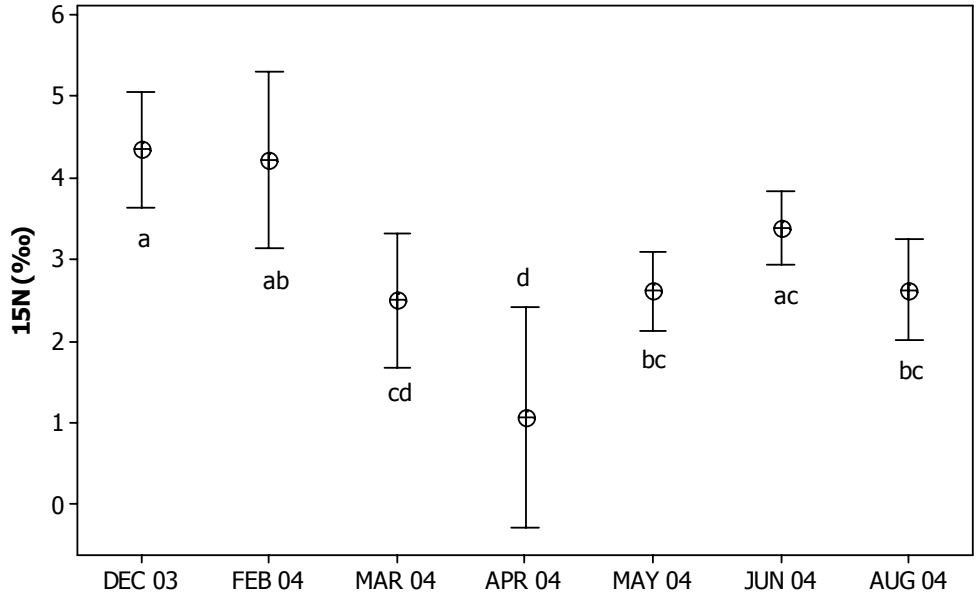

Fig 4.4. Temporal variation of $\delta^{15}N$ (mean ± 95% C.I) at BB within sampling season of 2003/2004. Significant differences between sampling trips defined through Tukey test (95% confidence). Group separations defined through differing letterings (a, b, c and d).

Analysis of $\delta^{13}C$ 2004 data reveals that winter biofilm samples from 2003/2004 were also generally more enriched compared to those of summer 2004 (Fig 4.5). However, samples from May 2004 are the exception, with enriched samples comparable with winter biofilms. Additionally, April samples again, had the most depleted ^{13}C signatures (Fig 4.5).

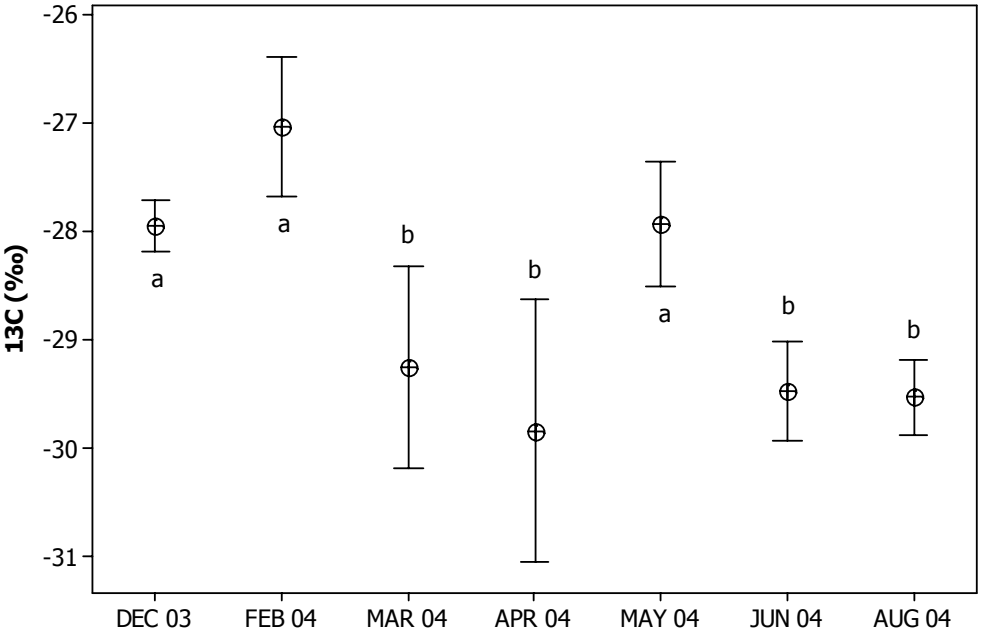

Fig 4.5. Temporal variation of $\delta^{13}C$ (mean ± 95% C.I) of BB biofilms within sampling season of 2003/2004. Significant differences between sampling trips defined through Tukey test (95% confidence). Group separations defined through differing letterings (a, b, c and d).

There was no such significant seasonal variation of molar C:N signatures within the first season of sampling (2003-2004); ANOVA: p = 0.948.

Clearly, there is significant temporal variability in the isotopic parameters measured in year one (2003/2004). There is a suggestion of synchronous temporal changes in the two isotopic measures ($\delta^{15}N$ and $\delta^{13}C$) although performing a regression on the linear relationship between the two measures does not yield a significant correlation (n = 57, r = 0.192, (Spearman Rank Correlation r = 0.085), P > 0.05). However, there was a visual suggestion of synchronous changes between isotopic signatures during this period. This indicates the possibility that the same causative vector is likely to be affecting both measures. During the same period, the values obtained for the stoichiometric measure (molar C:N) show very little temporal variation.

4.6.2 2005 Black Burn

Variation in BB signatures within 2005 are of particular interest as not only is there the potential for seasonally related changes, but also Black Burn was subject to

forest clearance during the winter of 2004/2005 of the riparian conifer plantation immediately adjacent to the uppermost site of the BB site chain (BBCF/CO).

A Tukey test of 2005 BB $\delta^{15}N$ revealed considerable temporal variation (ANOVA: P <0.001) (Fig 4.6). The variation in signatures in 2005 was of a much greater extent, than that of 2004. However, clear spring and summer seasonal trends are not as apparent as within the 2004 BB data. Additionally, as illustrated in Fig 4.6 and Fig 4.7, there was no indication of any relationship (such as synchronicity) in temporal trends of mean signatures of the two isotopic signatures measured (Correlation coefficient of ^{15}N and ^{13}C, $r^2 = 0.0008$, r = 0.028, P >0.05).

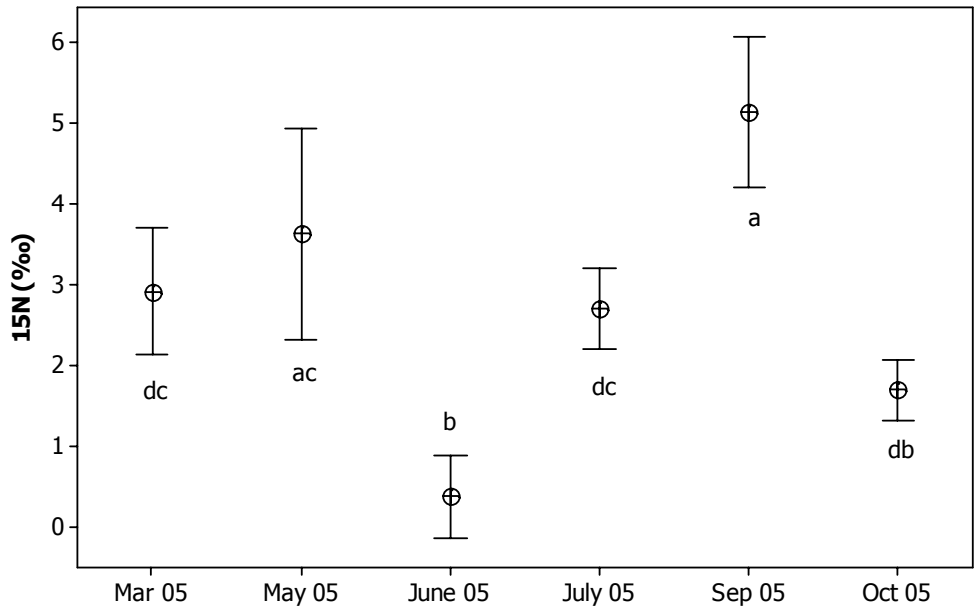

Fig 4.6. Temporal variation of $\delta^{15}N$ (mean ± 95% C.I) at BB within sampling season of 2005. Significant differences between sampling trips defined through Tukey test (95% confidence). Group separations defined through differing letterings (a, b, c and d).

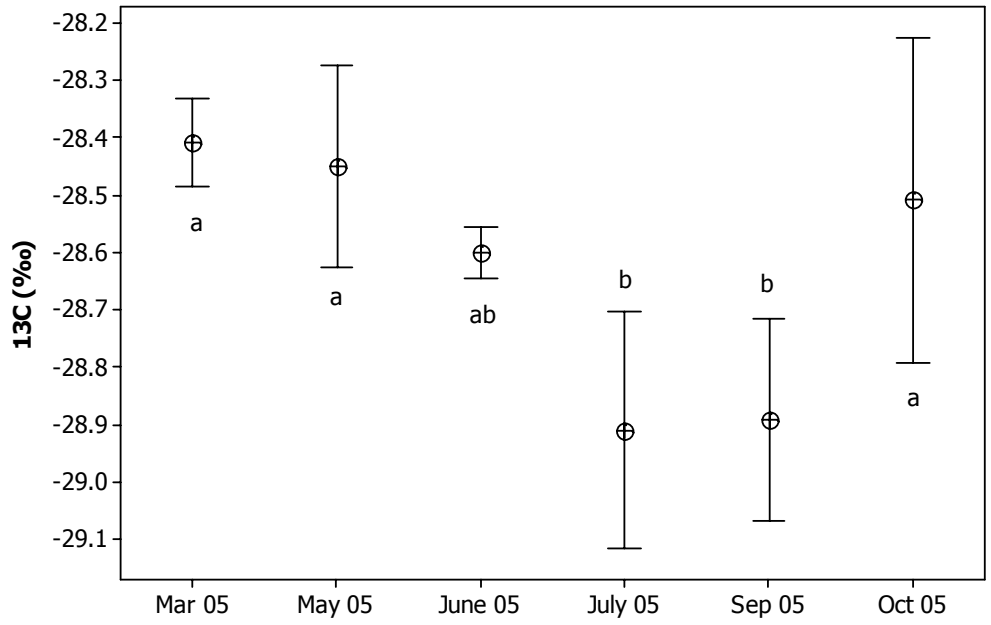

Fig 4.7. Temporal variation of $\delta^{13}C$ (mean ± 95% C.I) at BB within sampling season of 2005. Significant differences between sampling trips defined through Tukey test (95% confidence). Group separations defined through differing letterings (a, b andc).

Variation of $\delta^{13}C$ occurred with depletion of signatures during mid-summer (June - September): from a mean of -28.4‰ in spring to –28.8‰ in mid summer. However compared with the variability in signatures which occurred within 2004 (Fig 4.5), where there was up to a 3‰ shift in mean signatures within sampling months. Such variations, although statistically significant, do not vary enough to infer changes to biofilm composition.

Unlike in 2004, there was also significant variation in the molar C:N signatures of 2005 samples as June biofilm samples had significantly greater C:N ratio ($P = 0.001$) (Fig 4.8).

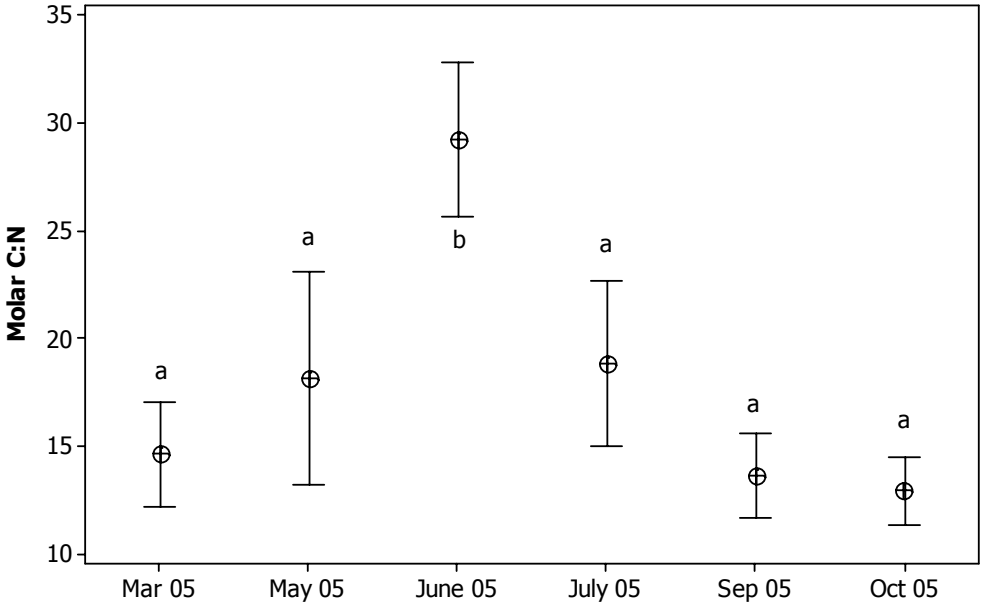

Fig 4.8. Temporal variation of molar C:N (mean ± 95% C.I) at BB within sampling season of 2005. Significant differences between sampling trips defined through Tukey test (95% confidence). Group separations defined through differing letterings (a and b).

4.6.3 2005 T33

Consideration of 2005 T33 samples (Fig 4.9, Fig 4.10 and Fig 4.11) revealed significant temporal differences among sample dates for all three measured parameters ($\delta^{15}N$, $\delta^{13}C$ and molar C:N) (all parameters, $P < 0.001$). There is a strong negative correlation ($r = -0.648$, $r^2 = -0.42$, $n = 63$) between the temporal patterns of $\delta^{15}N$ and molar C:N. By applying knowledge of allochthonous organic matter stoichiometric signatures outlined in the introduction (specifically that allochthonous material reflects a high C:N, autochthonous, a low C:N), it is possible to discern that the increase in molar C:N of the biofilms during mid summer, reflects an increase in allochthonous organic material. Further, that through inference of the negative correlation between $\delta^{15}N$ and molar C:N, allochthonous organic matter also appears to have a more depleted $\delta^{15}N$ signature.

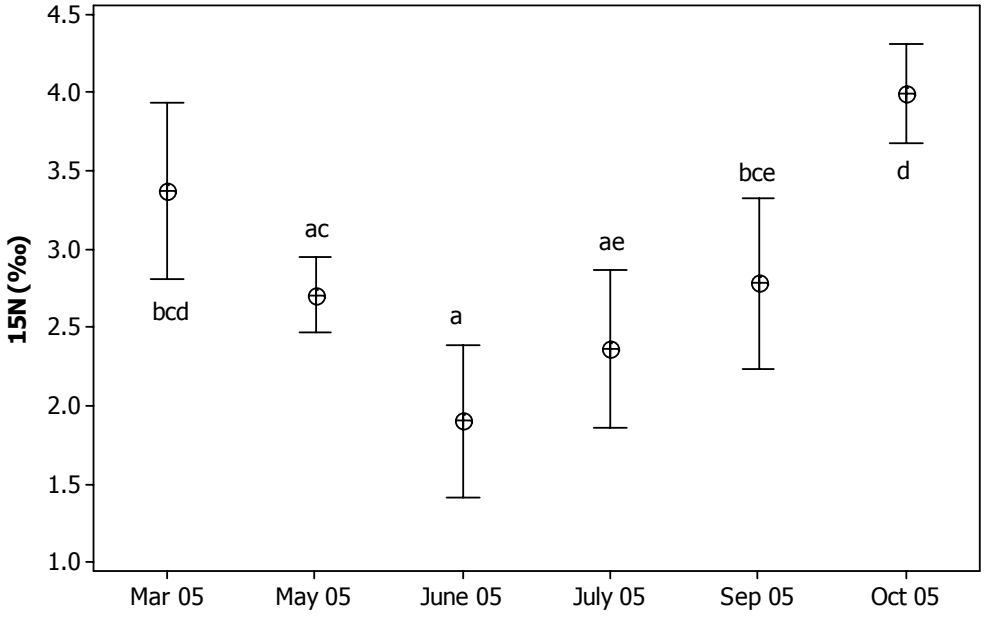

Fig 4.9. Temporal variation of $\delta^{15}N$ (mean ± 95% C.I) at T33 within sampling season of 2005. Significant differences between sampling trips defined through Tukey test (95% confidence). Group separations defined through differing letterings (a, b, c, d and e).

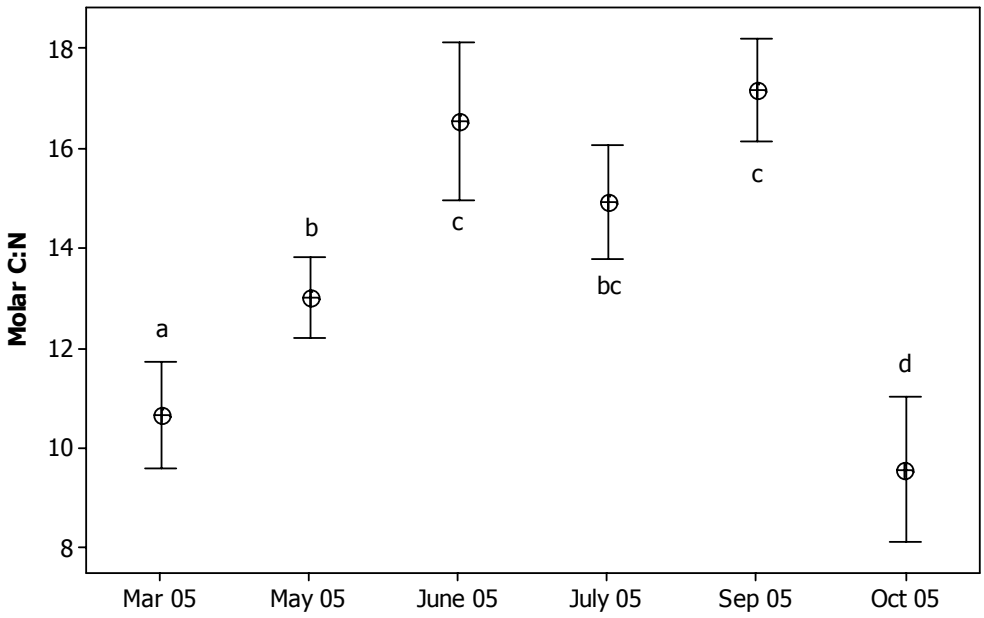

Fig 4.10. Temporal variation of molar C:N (mean ± 95% C.I) at T33 within sampling season of 2005. Significant differences between sampling trips defined through Tukey test (95% confidence). Group separations defined through differing letterings (a, b, c and d).

Although biofilm signatures were significantly more $\delta^{13}C$ enriched in March 2005, T33 biofilms $\delta^{13}C$ mean signatures remained relatively consistent over the course of the 2005 season; showing reduced temporal variability compared to the other elemental measures considered.

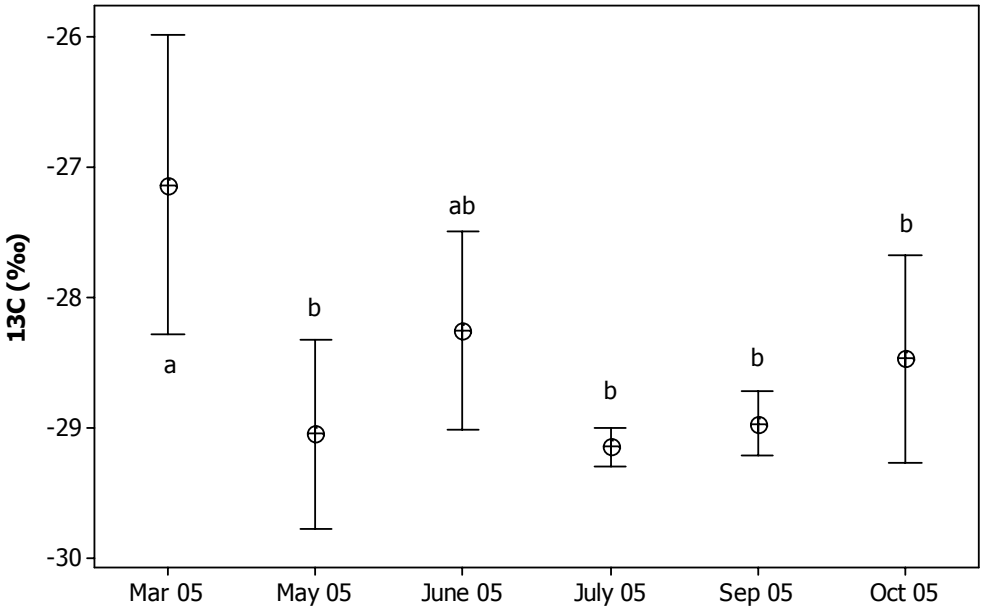

Fig 4.11. Temporal variation of $\delta^{13}C$ (mean ± 95% C.I) at T33 within sampling season of 2005. Significant differences between sampling trips defined through Tukey test (95% confidence). Group separations defined through differing letterings (a and b).

4.6.4 Spatial Variation in elemental composition

This study considered both inter and intra-site spatial variation of the biofilm data through a sampling programme that sought to discern variation both between the two sample streams as a whole (BB and T33), but also the variation among sites of a single stream (BBCF/CO, BBOP and BBSH as well as T33CF1, T33CF2 and T33SH).

These sample sites represent variable riparian conditions which may contribute to changing delivery and production of alternative organic matter resources. This aspect of the experimental design is discussed fully within chapter three (methods section). For ease, the physical variables of BB and T33 are repeated here in Table 4-2 and Table 4-3 respectively.

Table 4-2. Mean values for physical parameters measured for the three Black Burn sites over sampling periods in 2004 and 2005 (± standard error) BBCOR and BBCF are the same location but physical changes to the characteristics of the sites occurred post felling activities in 2004/2005 winter.

Physical Parameters	BBCOR (2004)	BBMCF (2005)	BBMOP	BBMSH
Light (%)	56.50 +/- 3.54	90.18 +/- 6.41	82.61 +/- 2.06	8.83 +/- 2.80
Stream wet width (m)	1.05 +/- 0.07	1.40 +/- 0.19	1.71 +/- 0.08	2.20 +/- 0.15
Stream depth (cm)	24.50 +/- 6.36	22.00 +/- 6.91	17.42 +/- 1.19	16.42 +/- 1.02
Bedrock (%)	20.00 +/-7.01	21.33 +/- 5.67	25.87 +/- 3.17	10.50 +/- 2.31
Boulders/cobbles (%)	45.00 +/- 7.01	36.67 +/- 12.02	42.50 +/- 4.79	35.00 +/- 2.89
Pebbles (%)	30.00 +/- 14.14	16.67 +/- 8.82	24.25 +/- 9.44	32.50 +/- 4.79
Sand (%)	5.00 +/- 0.00	3.33 +/- 3.33	3.75 +/- 2.39	12.50 +/- 6.29
Silt/Clay (%)	0.00 +/- 0.00	23.33 +/- 14.53	7.50 +/- 7.50	10.00 +/- 7.07
Riparian tree diversity	1.00 +/- 0.00	1.50 +/- 0.29	1.50 +/- 0.29	1.50 +/- 0.29
Overhanging vegetation (%)	20.00 +/- 0.00	20.00 +/- 0.00	27.75 +/- 9.22	2.75 +/- 2.43
Corridor width (m)	32.5 +/- 3.54	90.33 +/- 14.95	30.48 +/- 1.45	8.85 +/- 0.28
Corridor tree height (m)	22.50 +/- 3.54	2.56 +/- 0.59	21.67 +/- 2.04	19.17 +/- 1.44
Site altitude (m)	220.00 +/- 0.00	220.00 +/- 0.00	200.00 +/- 0.00	210.00 +/- 0.00

Table 4-3. Mean measurements for physical parameters measured for the three T33 sites over sampling periods in 2005 (± standard error).

Physical Parameters	T33CF1	T33CF2	T33SH
Light (%)	38.18 +/- 9.49	61.78 +/- 2.22	41.95 +/- 10.12
Stream wet width (m)	0.94 +/- 0.14	1.12 +/- 0.28	1.10 +/- 0.24
Stream depth (cm)	3.33 +/- 0.38	5.78 +/- 0.40	10.89 +/- 2.26
Bedrock (%)	0.00 +/- 0.00	0.00 +/- 0.00	10.00 +/- 3.33
Boulders/cobbles (%)	5.00 +/- 2.89	13.33 +/- 8.33	30.00 +/- 11.28
Pebbles (%)	83.33 +/- 3.33	46.67 +/- 11.67	43.33 +/- 12.02
Sand (%)	5.00 +/- 2.89	10.00 +/- 5.77	10.00 +/- 5.77
Silt/Clay (%)	6.67 +/- 3.33	31.67 +/- 9.28	6.67 +/- 3.33
Riparian tree diversity	0.67 +/- 0.67	1.33 +/- 0.33	1.00 +/- 0.00
Overhanging vegetation (%)	44.00 +/- 21.20	24.00 +/- 11.37	2.00 +/- 1.53
Corridor width (m)	88.89 +/- 5.88	66.67 +/- 10.18	46.56 +/- 15.91
Corridor tree height (m)	1.56 +/- 0.78	22.44 +/- 3.20	30.00 +/- 0.96
Site altitude (m)	70.00 +/- 0.00	69.00 +/- 0.00	65.00 +/- 0.00

Fig 4.12 displays the overall differences between the biofilm signatures of the two streams. The initial observations are that T33 had more constrained nitrogen isotopic and stoichiometric signatures, whereas the two years of study of the Black Burn, yielded a much wider range in signatures. However, applying an ANOVA to the data sets revealed no significant difference between sample signatures of the two streams for either $\delta^{15}N$ ($P = 0.704$) or the $\delta^{13}C$ measures ($P = 0.166$) (Fig 4.13). But Black Burn biofilm samples did have significantly more enriched molar C:N signatures than T33 ($P = 0.003$).

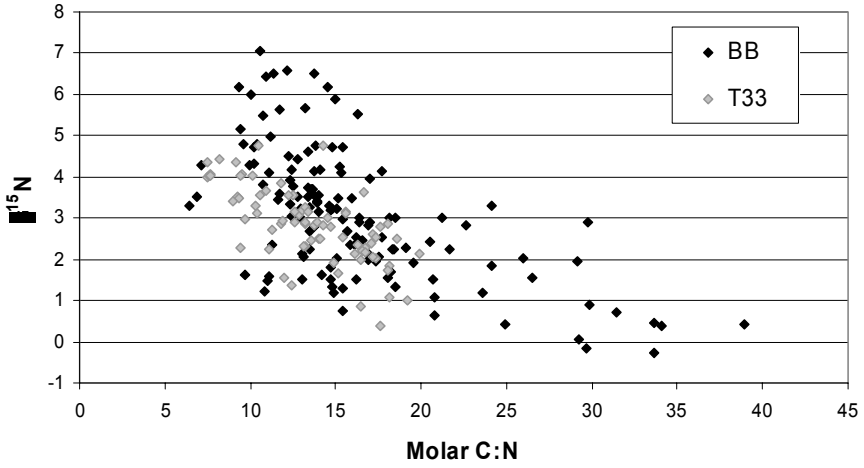

Fig 4.12. Spatial variation of $\delta^{15}N$ and molar C:N signatures of 2004 and 2004 BB (in black) and T33 (in grey) biofilms. April 2004 BB samples removed due to significantly depleted $\delta^{15}N$ signature.

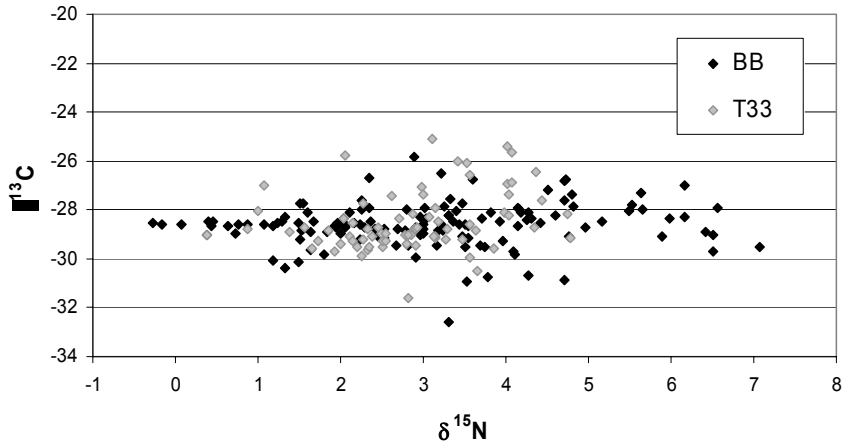

Fig 4.13. Spatial variation of $\delta^{13}C$ with $\delta^{15}N$ signatures of 2004 and 2004 BB (in black) and T33 (in grey) biofilms. April 2004 BB samples removed due to significantly depleted $\delta^{15}N$ signature.

Intra site differences were also explored. An ANOVA determined no significant difference in $\delta^{15}N$ between all BB and T33 sites pooled for both 2004 and 2005 sampling seasons. However there was spatial intra-site variation of the $\delta^{13}C$ signatures (ANOVA, $P < 0.001$) (Fig 4.14).

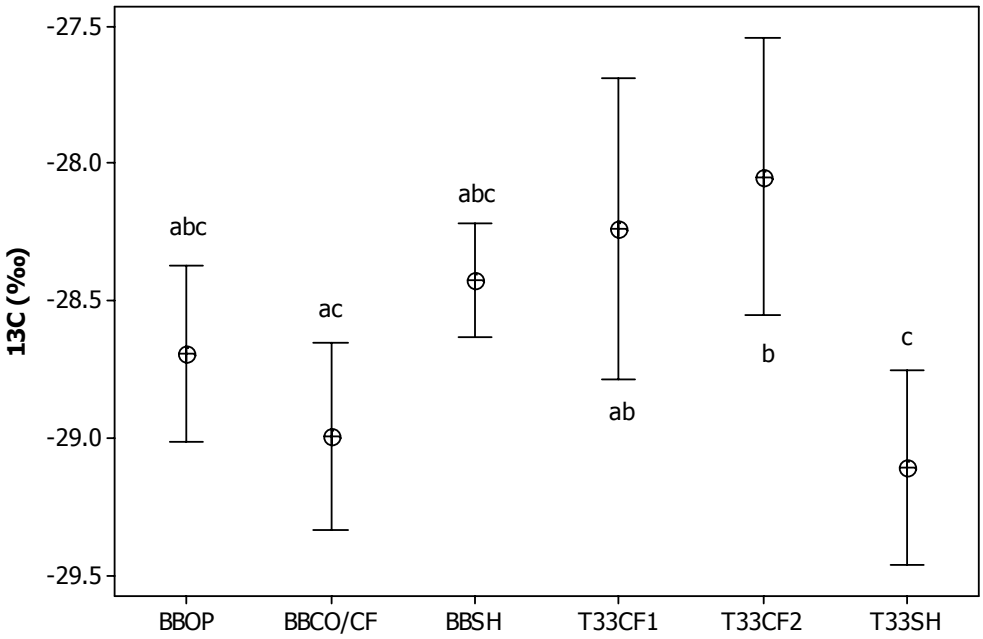

Fig 4.14. Spatial variation of mean $\delta^{13}C$ signatures of pooled 2004 and 2005 from BB and 2005 only from T33 sites. Data illustrates mean (± 95% C.I). Significant differences between sites defined through Tukey test (95% confidence). Group separations defined through differing letterings (a, band c).

Additionally, consideration of intra-site variation of molar C:N indicates that there were significant differences amongst both T33 and BB sites (P = 0.012). BBSH was significantly more C:N enriched compared with BBCO 2004, T33CF1 and T33CF2. Generally, mean data indicated that BB sites tended to be more molar C:N enriched and in addition, there appeared to be an increase in molar C:N with reduced light levels (open to corridor/CF to shade) (Fig 4.15).

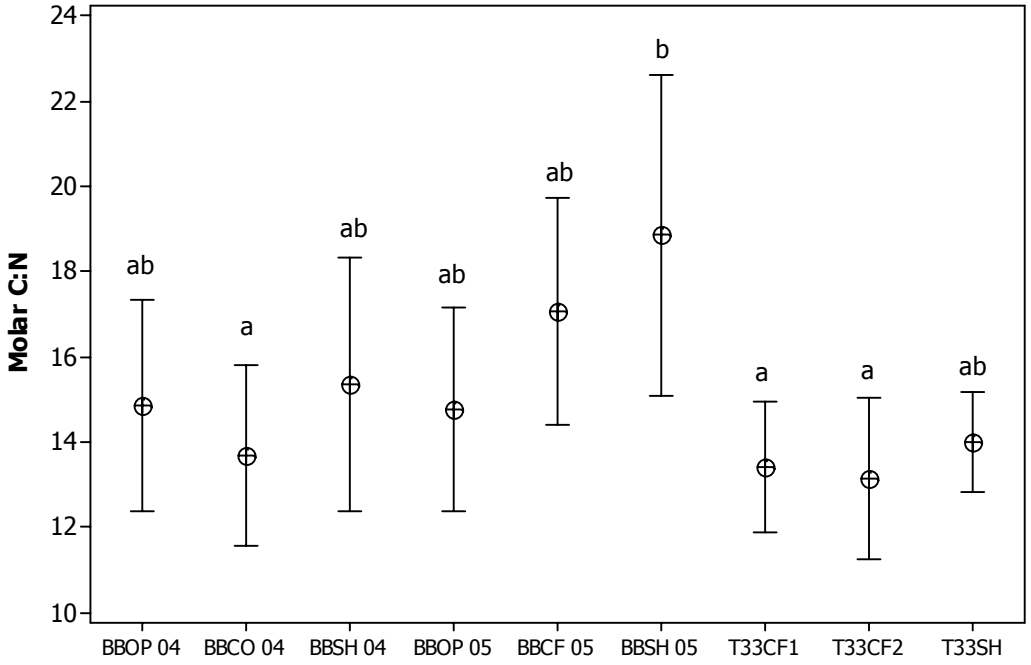

Fig 4.15. Spatial variation of mean molar C:N signatures of pooled 2004 and 2004 from BB and T33 sites (2005 only). Data illustrates mean (± 95% C.I). Significant difference in sites (P = 0.012). Differences between groups, defined through Tukey test (95% confidence) and indicated through differing lettering (a and b).

4.6.5 Effects of flow on $\delta^{13}C$ signatures

Although the majority of studies have used carbon isotopes for the delineation of organic carbon sources within the resource base of aquatic food webs, both the high spatial and temporal variation often associated with the $\delta^{13}C$ signatures of autochthonous organic matter (Rosenfield and Roff, 1992, Zah et al., 2001; Winterbourn et al., 1986; Boon and Bunn, 1994), has limited the applicability of this approach to a number of studies. Within the present study, site or date variation is explored in relation to flow to determine if the spate nature of the system can be related to $\delta^{13}C$ signature.

A twofold fractionation process with both the solution of gaseous CO_2 in the water and discrimination by carbon fixation (mediated by the RubisCo enzyme) (Hecky and Hesslein, 1995) results in the organic carbon derived by aquatic plants having a theoretical isotopic signature close to -37‰ (if a full equilibrium between water and atmospheric CO_2 exists and CO_2, as a source of carbon is not limited). However, even in fast flowing systems, equilibrium between atmospheric and isotopic carbon is

rarely achieved (Raven et al., 1982, Hecky and Hesslein, 1995). In addition, when CO_2 does become a limiting factor, some aquatic primary producers can actively uptake HCO_3^- (a dissolved inorganic carbon species with a higher $\delta^{13}C$ signature than that of dissolved CO_2: Mook et al., 1974).

In a study by Singer et al., (2005), small-scale variation in carbon isotopic signatures was explored and, specifically, the source and availability of carbon to autochthonous periphyton/biofilms during variations in flow conditions investigated. This study concentrated on the fact that mass transfer across a diffuse aquatic boundary is affected by flow velocity and, as a consequence, water velocity has the potential to control both CO_2 supply and isotopic discrimination by primary producers (Keely and Sandquist, 1992). In addition, this effect can be more pronounced in situations of high productivity and carbon demand (and thus carbon limitation) (Findlay et al., 1999).

Here, samples were analysed along-side site discharge using back calculations from data collected by downstream SEPA gauging stations. This was done in order to determine whether variation in $\delta^{13}C$ measurements was correlated with the mean flow conditions occurring within each of the biofilm artificial substrate settlement periods. The mean daily flow in the ungauged sub-catchments (BB and T33) was estimated from mean daily gauged flow at Minnoch Bridge (25 (NX) 352 746 for BB) and at Low Malzie (25 (NX) 382 545) for T33, weighted by the topographic area contributing to flow using Equation 4, following Wade et al. (1999):

Equation 4

$$Qu = Qg(Au / Ag)$$

Where Q is mean daily flow ($m^3\ s^{-1}$), A is the topographic area contributing to flow [m^2] and the subscripts u and g refer to ungauged and gauged catchments, respectively.

The mean calculated discharge rates of the Black Burn and T33 were correlated with the associated mean $\delta^{13}C$ measurements for the settlement period, which produced a positive linear relationship. However, neither were found to be significant (BB Correlation coefficient of r = 0.301, P = 0.369, and T33, r = 0.372, P = 0.467) (see

appendix: Fig 10.2 and Fig 10.3). My results were not found to be consistent with those of Singer et al. (2005) in respect to the overall relationship between $\delta^{13}C$ signatures and velocity. Singer et al. (2005) describe an inverse relationship of $\delta^{13}C$ with flow velocity (mean from 35 days settlement). Here there is no relationship at all, which may be due to the comparatively small size of the data set. For this reason the relationship between $\delta^{13}C$ and flow will no longer be considered.

However, as it is not possible to assign correlation of elemental signature variation to flow patterns, I suggest that the majority of the isotopic and stoichiometric signature variation (over both spatial and temporal scales) is due primarily to compositional changes of the biofilm biomass and specifically variation in the proportional contribution of material which have differing C and N sequestering and/or fractionation pathways. I have used this rationale to explore approaches designed to delineate biofilm composition, and provide information on basal organic resource composition and source in order to provide information contributing to the understanding of biofilm functioning and characteristics.

4.6.6 Approach

Using this rationale, the chapter now explores the variability of the biofilm composition and seeks to determine whether the variation in the isotope and/or stoichiometric signatures are a response to either:

1. Variation in the proportional contribution of organic material from either external and internal sources (i.e. allochthonous or autochthonous) or,

2. Changing signatures of the autochthonous material (for example as a consequence of changing autotrophic contribution).

These options are explored first by combining isotopic measures with stoichiometry to determine a measure of autochthonous material and second, by combining elemental composition with chlorophyll *a* measures utilised in the previous chapter to compare with alternative approaches of quantifying autotrophic material.

4.7 1. Development of a mixing model to define allochthonous and autochthonous contribution using isotopic and stoichiometric measures.

Linear regression relationships between molar C:N and $\delta^{13}C$ (Fig 4.16) or $\delta^{15}N$ (Fig 4.17) can be used to indicate the extent to which the isotopic signatures deviate from the C:N ratio which is known to reflect, in a broad sense, the relative contribution of allochthonous and autochthonous organic matter. Combining the two measures may provide information on biofilm composition and characteristics which are not revealed using stoichiometric measures alone. Thus, I assess the potential use of both $\delta^{13}C$ and $\delta^{15}N$ as potential secondary measures of biofilm composition and characteristics.

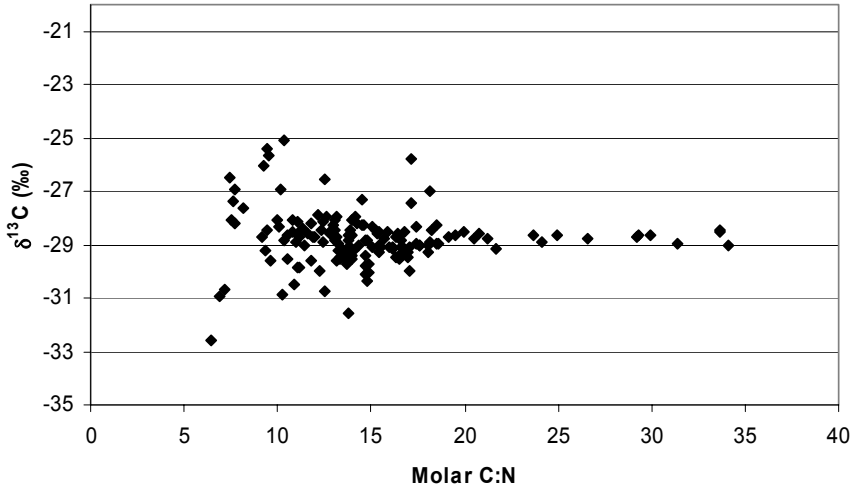

Fig 4.16. BB and T33 2003 – 2005 $\delta^{13}C$ and molar C:N showing no relationship (r^2 = 0.001). Figure also illustrates how constrained the $\delta^{13}C$ signatures are compared with that of molar C:N.

There is no relationship between molar C:N and $\delta^{13}C$ (Fig 4.16). I hypothesise that the likely cause of this inconsistency between these two measures is related to changing isotopic signature of the autochthonous component (e.g. caused by algal species change). However, the significance of the variability of the autochthonous signature contribution is thus dependent on the relative proportional contribution of autochthonous material to the biofilm biomass. If a biofilm is primarily composed of allochthonous material, then any extent of change to algal species composition and

associated isotopic signature are unlikely to significantly impact on overall biofilm signature dominated by allochthonous organic matter.

Further, apparent from Fig 4.16, the carbon isotopic signature of the biofilm is very similar in nature to that of the generally regarded common terrestrial signature; ~ -27‰ (e.g. Fry and Sherr, 1984). Specifically, autochthonous material has a large carbon isotopic range (between –8 and -30‰); reflecting both the metabolic pathways of the plant, the mechanism by which they gain carbon, and any fractionation of autotrophic material by heterotrophic primary consumers. However, the majority of biofilm $\delta^{13}C$ signatures here happen to be close to the top of the range described above (–27 to -28‰). Unfortunately, the upper limits of the autochthonous $\delta^{13}C$ signature range, overlaps with the values described in the literature for allochthonous material. The fact that the $\delta^{13}C$ is so closely reflecting -27‰ significantly impedes the potential of using $\delta^{13}C$ for defining differences in allochthonous and autochthonous proportional organic material contribution, as most signatures are indistinguishable, and would be masked by an allochthonous signature.

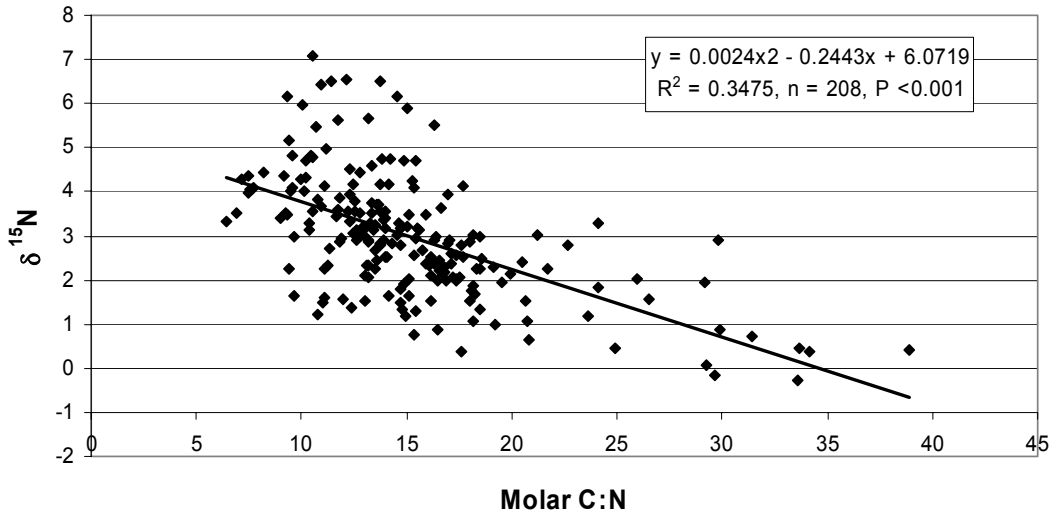

Fig 4.17. Negative linear relationship between $\delta^{15}N$ and molar C:N ($P < 0.001$) for both BB and T33 data over both years of study (2003 – 2005), minus April 2004 data (as explained in following text).

Fig 4.17 shows a significant ($r = 0.6$, $P < 0.001$) negative linear relationship between $\delta^{15}N$ and molar C:N. From the r^2 value, approximately 35% of the variation in the data is explained by this linear regression. Due to this significant correlation between the two measures of $\delta^{15}N$ and molar C:N, it was felt that the $\delta^{15}N$ could also be

explored in relation to discerning autochthonous contribution. From the relationship (Fig 4.17), it appears that autochthonous biofilm production can also be characterised by the higher δ^{15}N signatures. Similarly, low δ^{15}N signatures can be related to an increasingly allochthonous-derived biofilm.

The potential difficulties in differentiating and distinguishing the δ^{13}C signatures of algae (which are often subject to species and growth pattern changes) from allochthonous material has been well documented (Rosenfeld and Roff, 1992, Zah et al., 2001; Winterbourn et al., 1986; Boon and Bunn, 1994). Because carbon isotopic signatures from the biofilms displayed similar values and were generally indistinguishable from potential allochthonous sources (Fig 4.18 Table 4-4), it was decided to use alternative attributes to delineate the two potential sources of carbon to the biofilm material.

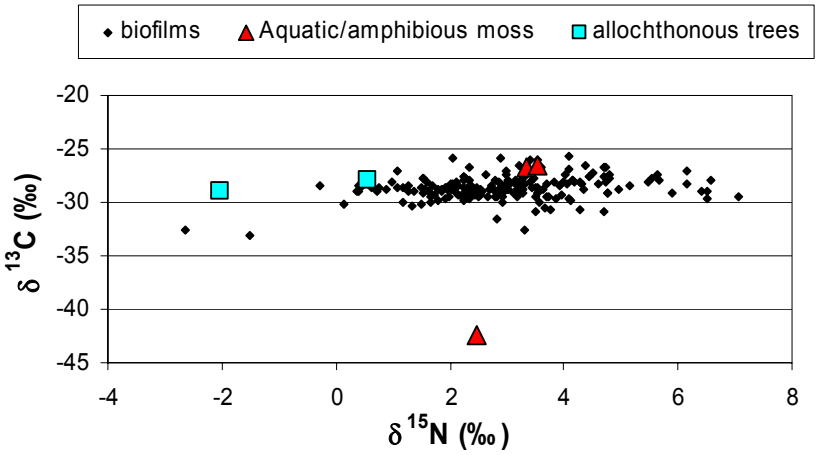

Fig 4.18. Comparisons of δ^{13}C and δ^{15}N signatures of the biofilms with the potential source materials which will be used to construct the mixing model. The figure indicates that allochthonous source δ^{13}C signatures are often indistinguishable from the bulk of biofilm material. This makes this measure impossible for application to a model which is dependent on calculations based on the proportional contribution of identifiable distinguishable end-members.

Thus δ^{13}C will be rejected in this respect, and both δ^{15}N and molar C:N are explored in the production of a two-source mixing model to derive the autochthonous content of the biofilm material.

4.7.1 Designing the mixing model

Biofilm signatures were significantly different in both $\delta^{15}N$ and molar C:N values compared with potential allochthonous end-member source materials (deciduous and coniferous tree species) as source values were far out with the 95% confidence limits of the biofilm sample signature means. This confirms the rationale for an approach based on using $\delta^{15}N$ and molar C: N as the measures of interest. The mixing model was produced using either molar C:N or $\delta^{15}N$ following Equation 3. The proportional contribution of each source is determined through equating the source of the mix (biofilm) to the source of the associated two end-member values.

Five potential non-algal source materials were analysed (Table 4-4). The particular materials were chosen as potential sources due to their abundance and proximity to the river course.

Table 4-4. Mean isotopic and stoichiometric signatures for the biofilm and potential source materials.

Material Type	Taxa	∂13C	∂15N	Molar C:N	wt% N	wt% C
Potential source material						
Amphibious moss	*Eurhynchium* n 1	-26.8	3.34	19.4	1.5	24.3
Amphibious moss	*Eurhynchium* n 2	-26.63	3.54	34.4	1.4	39.8
Aquatic moss	*Fontinalis*	-42.37	2.47	25.7	2	43.8
Deciduous tree	alder	-27.93	0.57	48.3	1.3	52.3
Coniferous tree	spruce	-28.95	-2.02	44.1	1.4	51.3
Biofilm Material						
Biofilm (all)		-28.67 ± 0.08	2.94 ± 0.01	14.87 ± 0.38	2.3 ± 0.09	25.92 ± 0.61
Biofilm (Black Burn)		-28.71 ± 0.1	3.01 ± 0.15	15.5 ± 0.53	2.7 ± 0.12	30.8 ± 0.47
Biofilm (T33)		-28.58 ± 0.14	2.81 ± 0.11	13.73 ± 0.41	1.57 ± 0.09	16.9 ± 0.61

Fig 4.19 indicates that using either molar C:N or $\delta^{15}N$ creates separation of allochthonous end-member source signatures from the biofilm signatures.

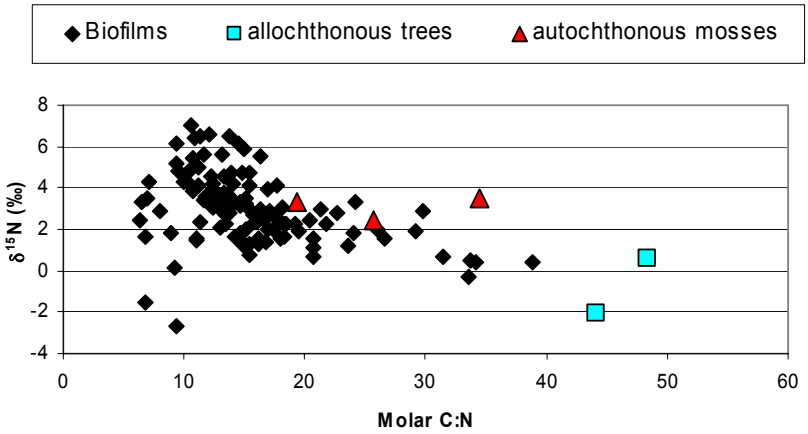

Fig 4.19. Biofilm signatures and potential source materials showing separabillity of the allochthonous signatures in terms of both molar C:N and $\delta^{15}N$ signatures.

Identifying the appropriate end-member signatures has resulted in various difficulties. The range of allochthonous material available for use as the end-member signature means that I have chosen to combine the two commonest riparian tree species (Alder and Spruce - Table 4-4). However when the autochthonous end-member is considered, there is not a 'pure' autochthonous signature available, through in-situ sampling methods, to be utilised as the end-member source signature. Instead, the most enriched $\delta^{15}N$ signature was utilised for the ^{15}N model, and the most depleted molar C:N signature was used as a proxy to an autochthonous signature.

4.7.2 April 2004 BB data

The biofilms growths in April 2004 had a mean $\delta^{15}N$ signature significantly different ($P < 0.001$), and significantly depleted (mean = 1.06, max = 3.18 and min = -2.65), compared with the rest of the 2004 data set. By including this depleted data set, the autochthonous end-member would be reduced by approximately 3‰ (Table 4-5). As April 2004 biofilm samples were both depleted in $\delta^{15}N$ and molar C:N, they did not fall within the normal negative linear relationship which this model is based (Fig 4.20). Therefore, it was decided to remove these outliers from the model as it was not felt that they accurately represented the remainder of the biofilm signatures and thus, was inappropriate sample signatures to be utilized as the model end-member. Such a significant depletion of the $\delta^{15}N$ signature would result in significant increase in the estimations of the overall autochthonous contribution of the biofilm.

Although algal quantitative identification was restricted to 2005 sampling methodology, presence / absence observations were made of samples during 2004. This data has not been included in the study as it as difficult to ascertain good estimates of community structure, or make comparisons between year one and year two data, yet consideration of the 2004 species assemblages revealed the abundance of dual colonial brown cellular structures, thought to be Cyanobacteria, *Nostoc* spp. These growths were only present in the majority of BB samples during April 2004. This unusual assemblage structure provides further support for the removal of the data from further analyses.

Table 4-5. $\delta^{15}N$ biofilm signatures, maximum and minimum values used as potential end-member with addition or removal of April 2004 data.

$\delta^{15}N$ end-member contributions		
	Max (‰)	**Min (‰)**
All data	7.06	-2.64
Minus April 04'	7.06	-0.27

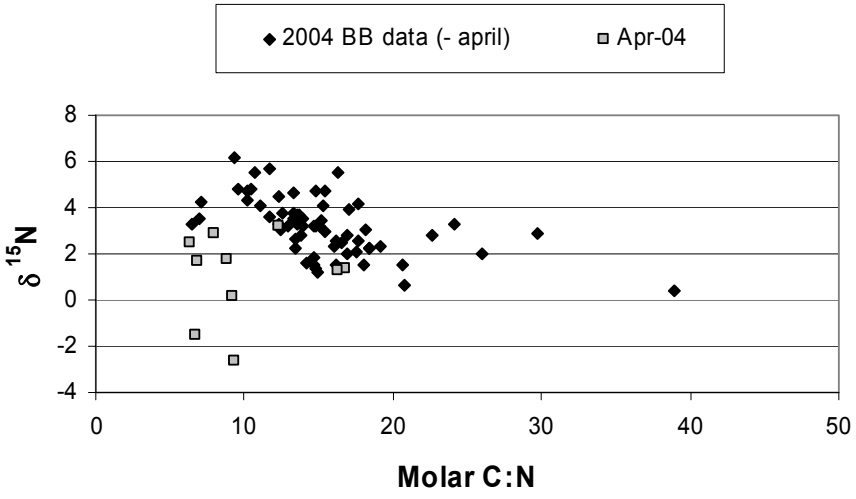

Fig 4.20. 2004 BB biofilm data, highlighting the depleted signatures attained from the April 2004 data. April 2004 samples were significantly more depleted in both δ $\delta^{15}N$ (ANOVA, P <0.001) and molar C:N (ANOVA, P = 0.003).

4.8 Mixing model results

4.8.1 Autochthonous component of Black Burn biofilms

The model derived from $\delta^{15}N$ signatures (Fig 4.21), combines an allochthonous end-member (mean of the spruce and alder signatures) of -0.72‰ and an autochthonous end member (the highest $\delta^{15}N$ signature) of 7.14‰.

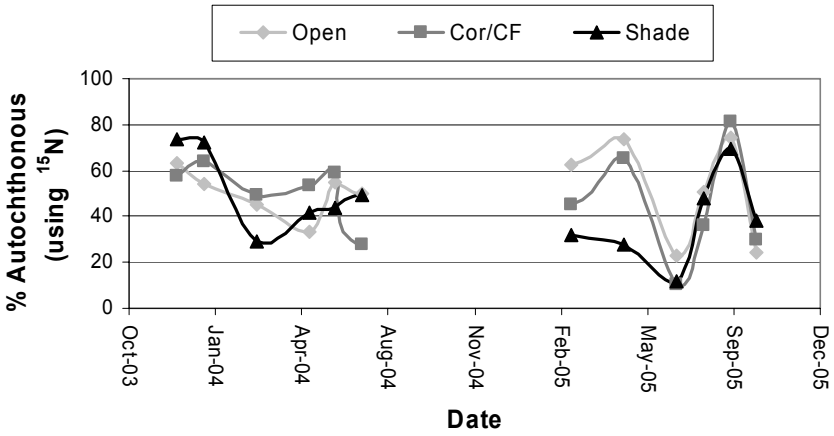

Fig 4.21. Biofilm mixing model of Black Burn sites, based on $\delta^{15}N$ signatures in order to determine proportional contributions of autochthonous inputs

There was little intra-site spatial variation between the autochthonous content of the biofilms of different BB sites, for either years (2004, P = 0.974, 2005 P = 0.158 and pooled P = 0.289)), yet the variation over a temporal scale is much greater. Biofilms in 2004 show a decline in autochthonous content during the summer of 2004. Biofilms are significantly lower in autochthonous proportional content from March 2004 onwards (with the exception of June 2004) (Fig 4.22).

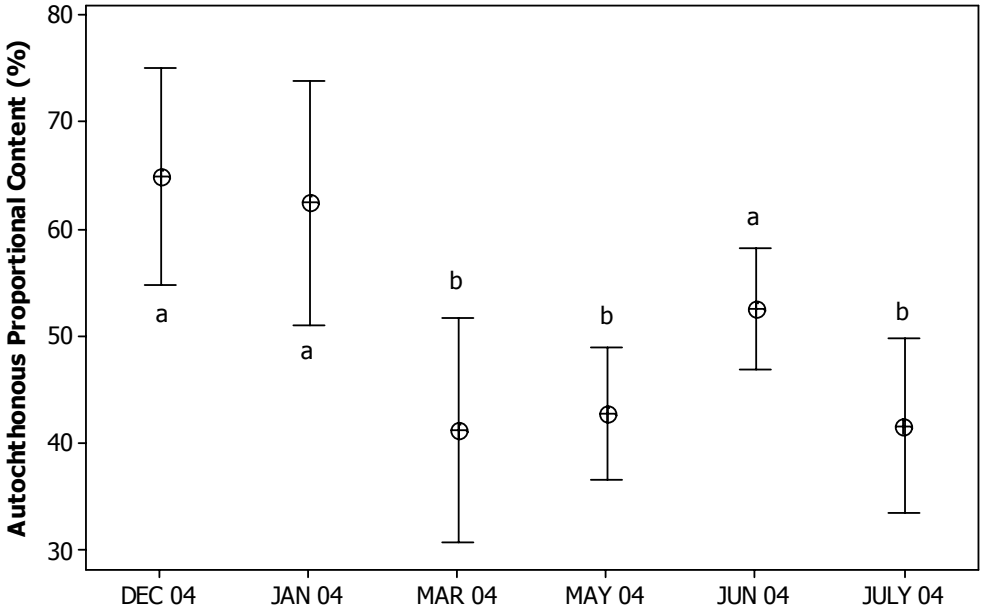

Fig 4.22. Mean (± 95% C.I) autochthonous contribution of pooled biofilm data. 2004 biofilm model results derived from calculations based on $\delta^{15}N$. Significant differences between sample visits derived through arcsine transformation of proportional data, and application of a Tukey test (95% confidence). Different groups indicated through differing letterings (a and b).

However, autochthonous contribution varied to its greatest extent in the 2nd year of study (2005); with contributions to the biofilm ranging between a maximum mean of 81% autochthonous content in BBCF, September 2005, to a minimum of 10.5% (BBCF) in July of that same year with the most depleted mean signatures collected during June 2005.

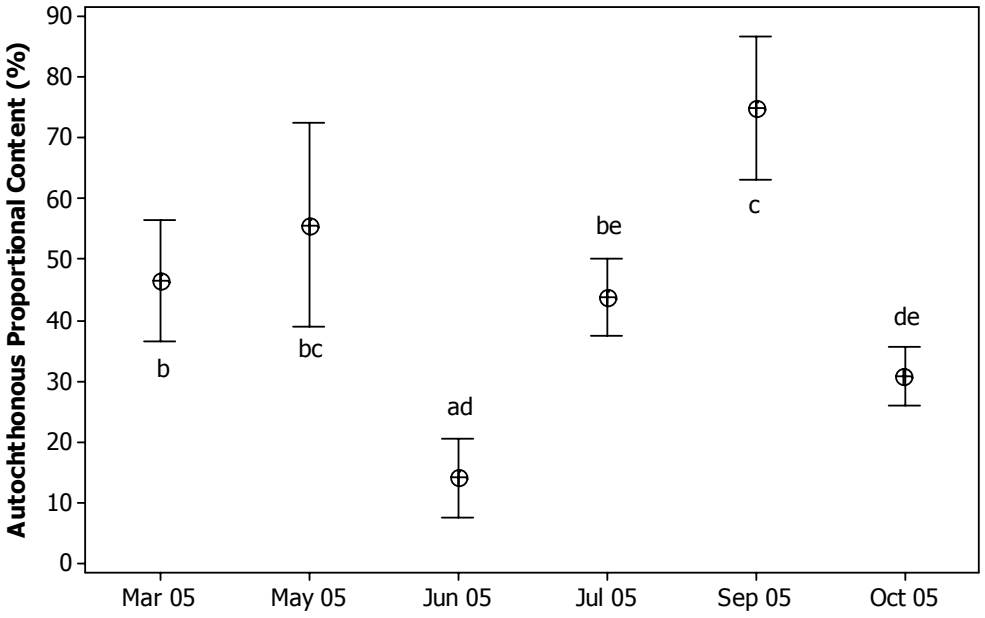

Fig 4.23. Mean (± 95% C.I) autochthonous contribution of pooled biofilm data. 2005 biofilm model results derived from calculations based on $\delta^{15}N$. Significant differences between sample visits derived through arcsine transformation of proportional data and application of a Tukey test (95% confidence). Different groups indicated through differing letterings (a, b, c, d and e).

Using this measure ($\delta^{15}N$) as a proxy for autochthonous contribution produces a relatively low estimation of the autochthonous contribution to biofilm material; with a 2004 mean of 50.1% (± 15.69 St. Dev.) and 2005 mean of 46.7% (± 24.05 St. Dev.). There was no significant difference in overall autochthonous proportional contribution between the two years of study (P = 0.328), despite significant temporal variation in autochthonous production throughout each sampling season.

The alternative model based on molar C:N signatures again utilises an allochthonous end-member sourced from spruce and alder leaves to produce a molar C:N end-member of 46.2 and an autochthonous end-member from lowest of the biofilm molar C:N signatures (6.4). These values were similar to those in the literature as the autochthonous end-member molar C:N (6.4) is comparable to cellulose walled dinoflagellates signatures of 8-10 (Dixon and Holligan, 1989; Holligan et al., 1984) and the bulk of marine phytoplankton (6.6, Redfield, 1934). Additionally, the allochthonous signature of 46.2 is comparable with Wetzel (1975) who measured allochthonous material at 50:1.

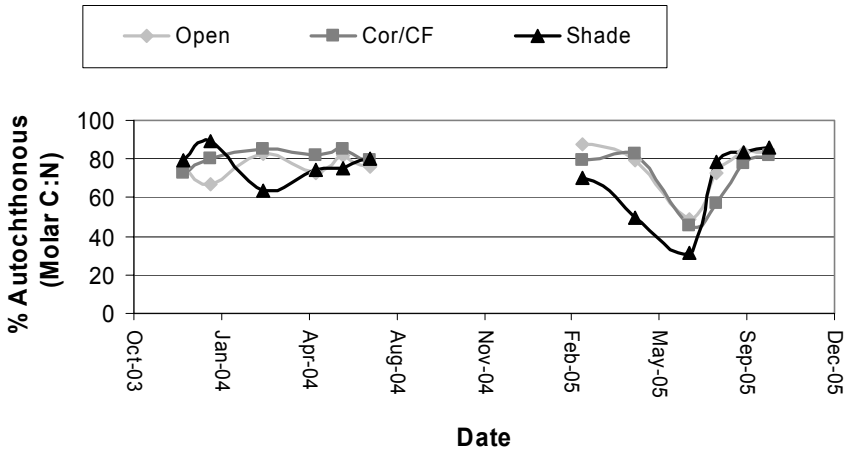

Fig 4.24. Assessment of proportional contribution of autochthonous carbon to the Black Burn biofilms using molar C:N based mixing model.

The model (Fig 4.24) produces a significantly different pattern in the autochthonous biofilm content compared with the previous $\delta^{15}N$ model. Assessing intra-site variation reveals that there was no significant difference in the proportion of autochthonous production within the biofilm over different intra-site conditions (Kruskal-Wallis, 2004 $P = 0.334$ and 2005 $P = 0.177$ (pooled, $P = 0.125$)) despite variations in percent light and canopy cover (Table 4-2 and Table 4-3). Additionally, within the first year, pooled site data had no significant temporal variation (Kruskal-Wallis, $P = 0.77$) of autochthonous contribution.

However in 2005, autochthonous contribution in samples varied significantly over the sampling season (Kruskal-Wallis, $P < 0.001$) with a reduction and then recovery of mean autochthonous content during mid-summer. This shift in biofilm signatures resulted in June samples being significantly depleted in autochthonous material (Fig 4.25). This result will be explored further in Chapter 7, where the affects of felling are addressed as during the winter of 2004/2004 an extensive area of land (which encompassed the most upstream site (BBCO) was clear-felled (producing BBCF).

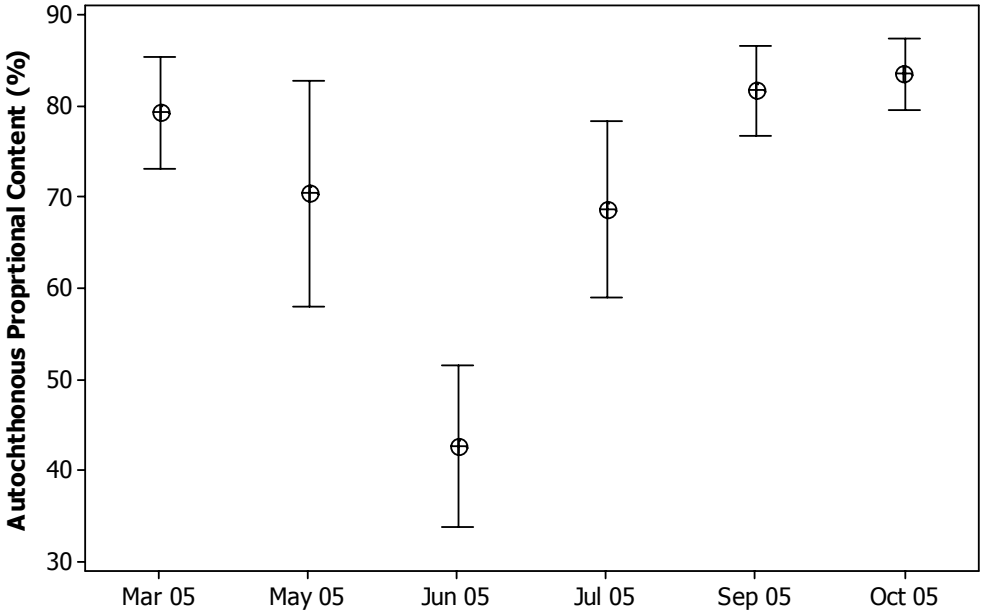

Fig 4.25. Mean (± 95% C.I) autochthonous contribution of pooled BB biofilm data. 2005 biofilm model results derived from calculations based on molar C:N. Significant differences between sample visits derived through application Kruskal-Wallis (P < 0.001). From the figure, it appears that June 2005 samples were significantly depleted in autochthonous content.

Comparisons between the two approaches (molar C:N and $\delta^{15}N$) reveals that the most obvious observation which can be made is that the $\delta^{15}N$ model predicts less (Kruskal-Wallis, $P < 0.001$) autochthonous carbon in the biofilm material. From the molar C:N model, the mean autochthonous contribution is 75.3% (± 1.31 St. errors), whereas the $\delta^{15}N$ model derives the mean autochthonous contribution at 48.0 % (± 1.73 S.E) for 2003-2005 pooled.

The difference between the autochthonous contribution derived from the $\delta^{15}N$ measurements and that of the molar C:N measures could be due, most likely to misidentification of end-member signatures (i.e. the autochthonous end-member may only constitute an 80 – 90% autochthonous biofilm) or natural variation of the end-member signature in the $\delta^{15}N$ model (from species variation or flow regime).

Variation in the natural isotopic signature of the autochthonous component of the biofilm material was illustrated with the rejected April 2004 data set and consideration of this and the wide range of $\delta^{15}N$ measures of algae taken from the literature, confirm that different algal/cyanobacteria can naturally vary in their $\delta^{15}N$ isotopic signatures (either within-species variation (e.g. Korb et al., 1996) or with

species community shifts (e.g. Brett and Muller-Navarra 1997; DeMott et al., 1998; Von Elert and Wolffrom, 2001). Natural variation can also occur through changing growth conditions and the species of carbon used for photosynthesis (e.g. Singer et al., 2005). However, this model, as based on a set end-member signature cannot account for the variability of signatures independent of the variation of proportional contribution.

Using an overestimated autochthonous end-member would result in the underestimation in the autochthonous contribution of carbon. This scenario is consistent with the depleted results gained thought the $\delta^{15}N$ model (as shown in Fig 4.21 compared with Fig 4.24). Further, long term increase in the abundance of algal species which had a relatively reduced signature (e.g. during summer months) would account for the variation between the two models and cause the $\delta^{15}N$ signature to overestimate the % allochthonous input to the biofilm.

I suggest that the more reliable approach is to use molar C:N which is consistent despite variations in algal assemblage and independent of changes in an algal $\delta^{15}N$ signature.

4.8.2 Autochthonous component of T33 biofilms

Applying the molar C:N model and end-members to the 2005 T33 data, the autochthonous proportional contribution of autochthonous indicates significant temporal variability (arcsine transformed data, ANOVA, $P < 0.001$) (Fig 4.26).

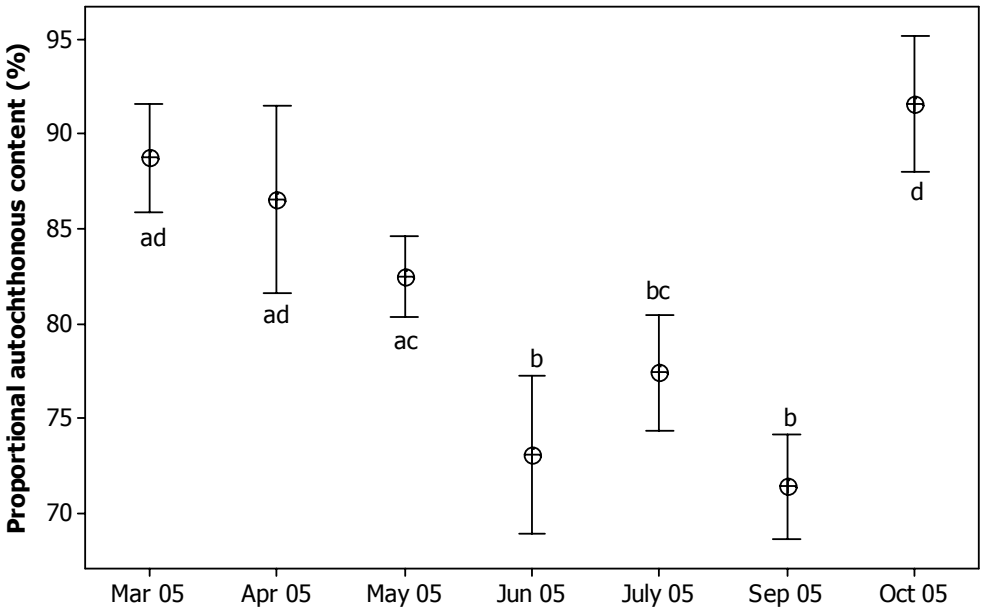

Fig 4.26. Mean (± 95% C.I) autochthonous contribution of pooled T33 biofilm data – indication temporal variation. 2005 biofilm model results derived from calculations based on molar C:N. Significant differences between sample visits derived through application of a Tukey test (95% confidence). Different groups indicated through differing letterings (a, b, c and d).

Additionally, T33 data also displays spatially significant variation as the contribution of autochthonous material is significantly (P = 0.026) reduced within the shaded site biofilms (T33SH) (Fig 4.27).

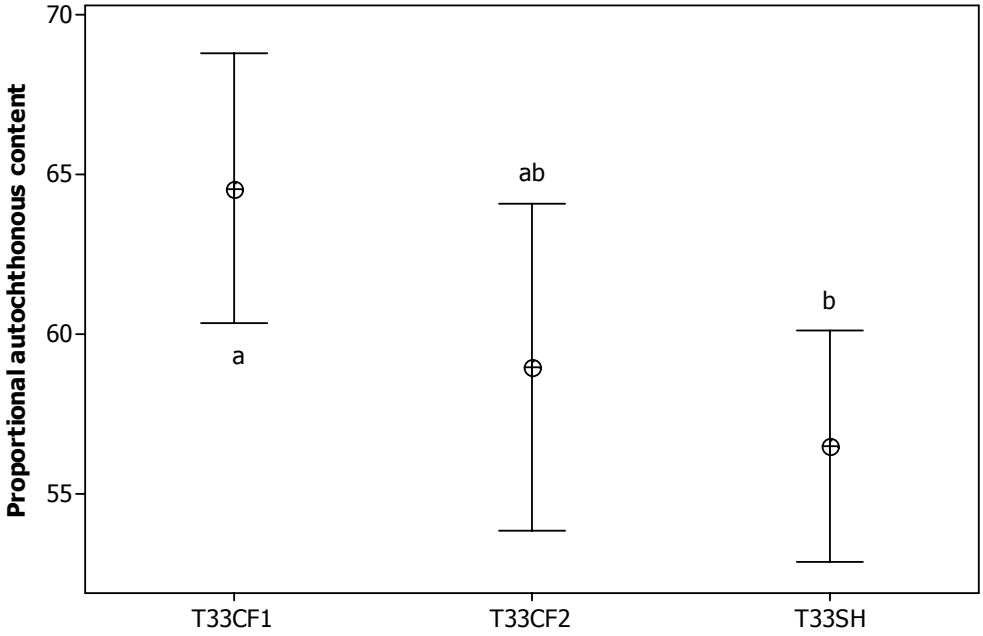

Fig 4.27. Mean (± 95% C.I) autochthonous contribution of pooled T33 biofilm data – indicating spatial variation. 2005 biofilm model results derived from calculations based on molar C:N. Significant differences between sample visits derived through application of a Tukey test (95% confidence). Different groups indicated through differing letterings (a, and b).

However, overall, the availability of autochthonous material remains relatively consistent at all T33 sites within the sampling season of 2005. Indeed, the autochthonous organic matter available from benthic biofilm material constitutes approximately 81% (± 1.02 S.E) of the biofilm biomass (Fig 4.28).

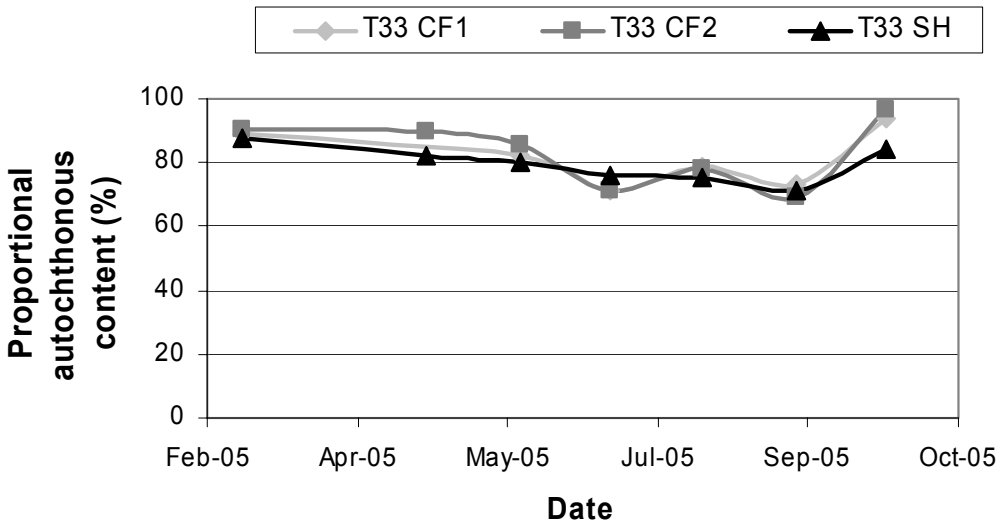

Fig 4.28. Autochthonous content of the T33 Biofilm in 2005. Model produced using Molar C:N signatures.

When this autochthonous production is compared with measurements taken at Black Burn (Fig 4.29), biofilms grown within the Black Burn in 2005 had significantly lower mean autochthonous contributions. However, autochthonous production of T33 was comparable with that of BB in 2004, despite differences in riparian characteristics and conditions (Table 4-2 and Table 4-3).

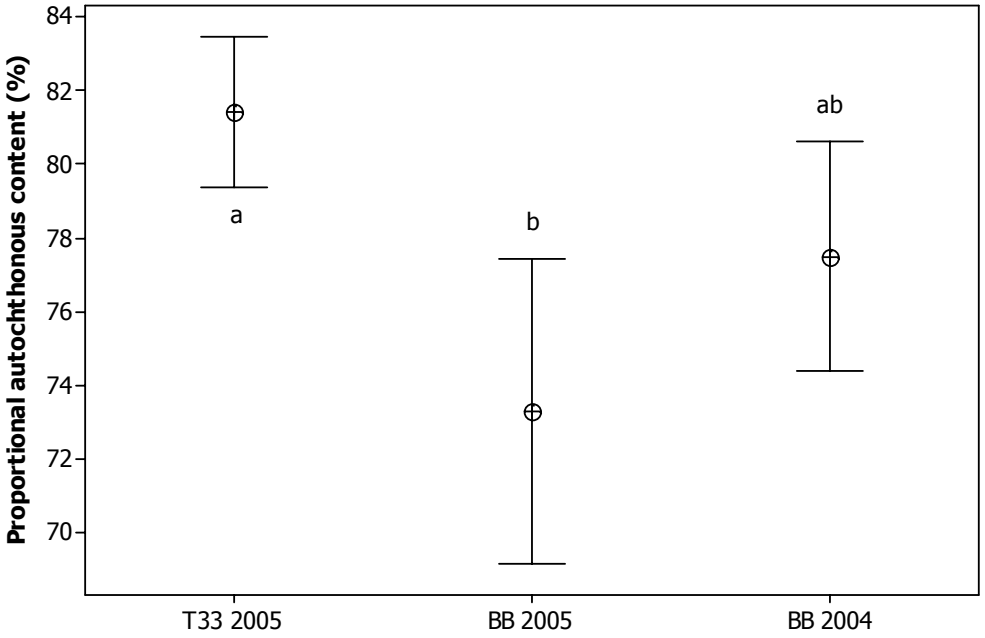

Fig 4.29. Mean (± 95% C.I) autochthonous contribution of pooled T33 2005, BB 2004 and BB 2005 biofilm data – indicating overall spatial/temporal variation. Biofilm model results derived from calculations based on molar C:N. Significant differences between sites derived through application of a Tukey test (95% confidence) on arcsine proportional data. Different groups indicated through differing letterings (a, and b).

Comparison between traditional techniques of deriving % allochthonous detrital content (using a microscope) and the stoichiometric based mixing model.

Here, a comparison is made between estimations of autochthonous contribution using molar C:N (stoichiometric approach), and estimates using microscopy (visual ID) as described in the methods. Percentage detrital content of microscopic slide sub-samples was assessed and material which contained no evidence of algal cell units was defined as allochthonous in origin.

Comparison of this microscope ID approach to characterising the content of biofilm/periphyton material with the stoichiometric mixing model approach detailed

above, indicates that there is a shortfall in the estimations made by microscopic visual ID (Fig 4.30).

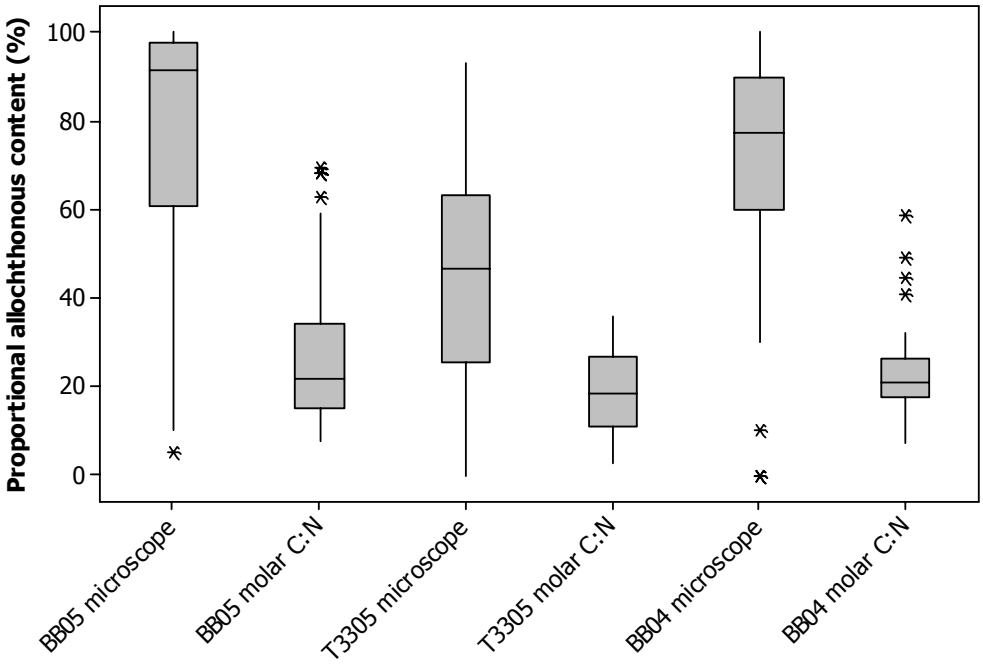

Fig 4.30 Comparative approaches used to define the allochthonous content of benthic biofilms; median allochthonous values (± 1st and 3rd quartiles) microscope and stoichiometric assessment. Significant differences in results defined through arcsine data and using Kruskal-Wallis (P <0.001). Means indicate that microscope ID yields significantly greater estimates of allochthonous material.

Compared with the predominantly allochthonous dominated estimations made by visual inspection, molar C:N measurements (for all three sample groups) indicate that biofilms are dominated by autochthonous material (Kruskal-Wallis, P <0.001). Such variation in the two approaches suggests that the visual inspection of biofilm/periphyton samples made routinely in many studies is inaccurate in estimating the importance of autochthonous material. Comparing these two findings also suggests that the general assumption of allochthonous dominated low-order streams (e.g. Vannote et al., 1980) may in many cases be driven by similar visual inspection inaccuracies.

4.9 Assessing impact of algal species composition and chlorophyll content variability on elemental composition

Within Chapter three, biofilm autotrophic production and algal cellular carbon content was explored by utilizing measures of chlorophyll *a* and carbon content of the biofilm material. Here, I compare that data with the isotopic and stoichiometric measures to determine whether the chlorophyll content and the algal biomass derived from the chlorophyll concentration measurements (using a conversion factor of 60) of biofilm material can be related to the isotopic and stoichiometric signatures. In addition, as the molar C:N mixing model is employed to determine the proportional contribution of autochthonous material, here I compare that measure with data of the algal biomass (autotrophic biomass) to determine firstly any relationships between the two approaches and also what proportion of the autochthonous material is not algal in origin, but rather heterotrophic in-stream production.

Initial consideration of the algal carbon mass compared with the isotopic measurements of the biofilms produces variable results, Firstly; there was no significant relationship between algal carbon and $\delta^{13}C$ ($r^2 = 0.042$, $r = 0.204$, $P > 0.05$). This finding indicated that here, not only was $\delta^{13}C$ an inadequate measure of autochthonous content as it resembled an allochthonous organic matter signature (~ -27 to -28‰), but further, $\delta^{13}C$ variation appeared independent of autotrophic content. Thus, even when the variation of the carbon isotopic signature of the biofilm is considered independently any isotopic resemblance of the signatures to terrestrial resources, $\delta^{13}C$ still does not significantly correlate to patterns in algal carbon content variation as defined through molar C:N. Consequently, this measure could not be used to estimate either allochthony or autochthony within the biofilm biomass of this study.

However, consideration of the $\delta^{15}N$ measurements with algal carbon calculations (Fig 4.31) indicates that there is a highly significant positive linear relationship between the two measures ($P < 0.001$). However, comparing the algal C and $\delta^{15}N$ relationship with that of the algal cellular C and molar C:N (Fig 4.32), the significance of the negative linear relationship of algal C and molar C:N was more significant ($P < 0.0001$) and had a greater r^2, suggesting that more of the variation in the algal

content can be predicted by molar C:N than with $\delta^{15}N$. From this result, it appears that molar C:N is both more accurate at both describing autochthonous content and predicting autotrophic content.

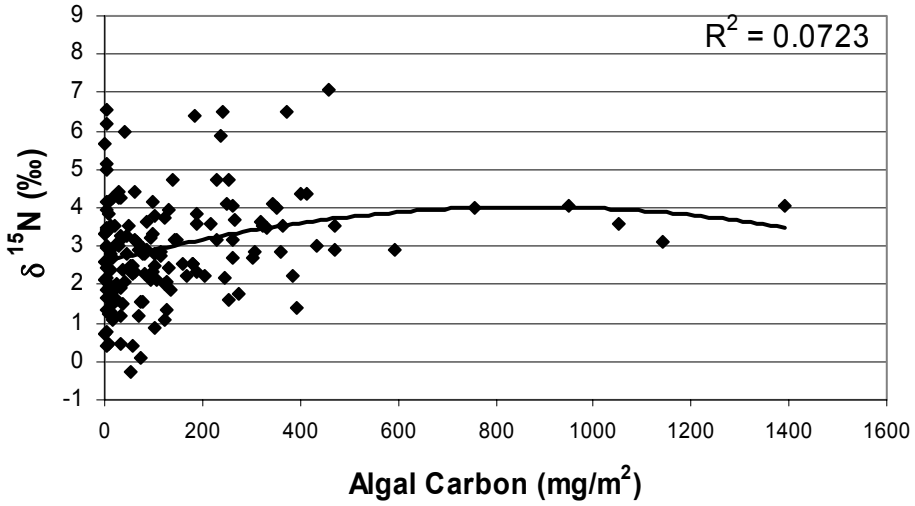

Fig 4.31. Positive polynomial relationship produced from $\delta^{15}N$ and algal carbon measurements. The correlation coefficient or r confirms significance of the relationship of P < 0.001.

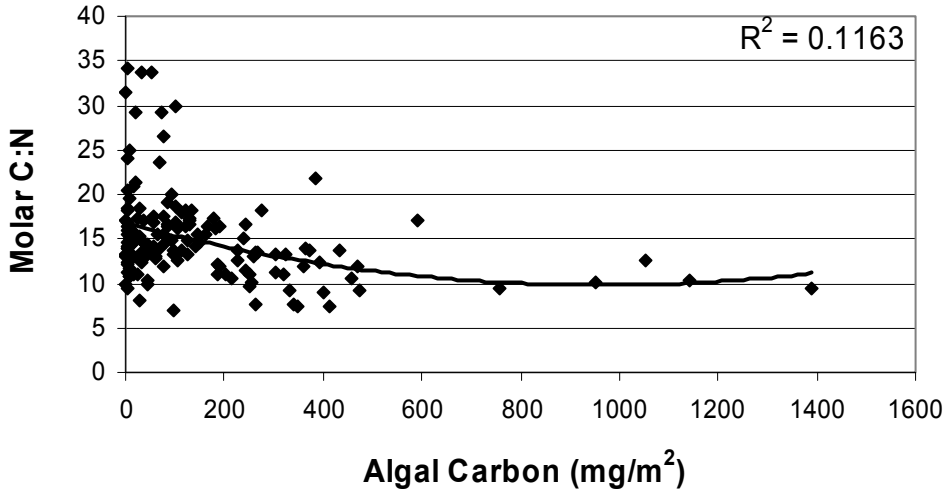

Fig 4.32. Negative polynomial relationship produced from molar C:N and algal carbon measurements. The correlation coefficient or r confirms significance of the relationship of P < 0.0001.

Interestingly, the relationship between autotrophic and autochthonous content of the biofilm, although highly significant (P > 0.001) (Fig 4.33), predicts less than 12% of the variation between the measures. This would suggest that although there is a relationship between autochthonous and autotrophic material, that there is

significant noise in the confidence of using autochthonous measurements to predict autotrophic content.

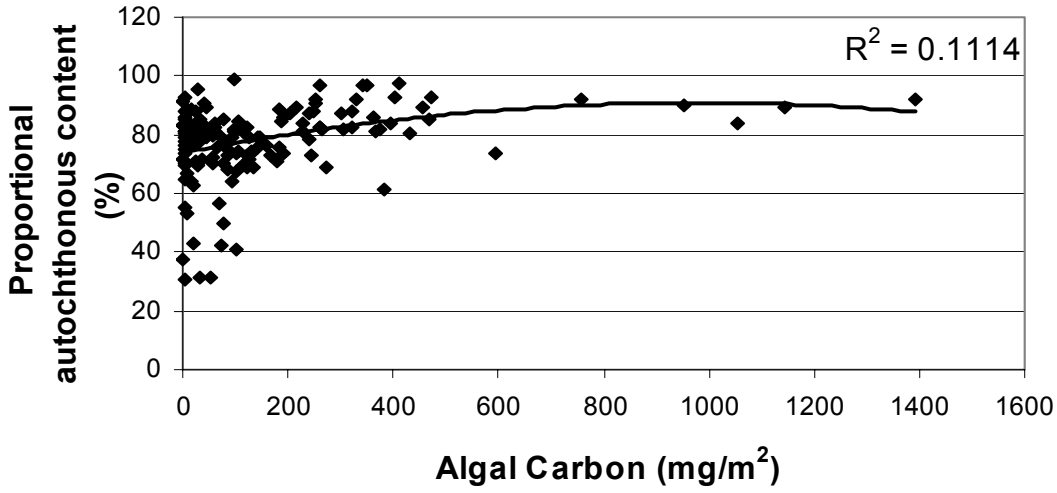

Fig 4.33. Positive polynomial relationship produced from autochthonous proportion and algal carbon measurements. The correlation coefficient or r confirms significance of the relationship of P < 0.0001.

Fig 4.34 integrates the analysis of autochthonous proportion (from the molar C:N mixing model) with the autotrophic proportion (as defined through the three alternative conversions (CF 20, 60, 100) of Chl *a*), and considers these two approaches combined, in order to define the proportion of autotrophic material, within the autochthonous component of the biofilm material.

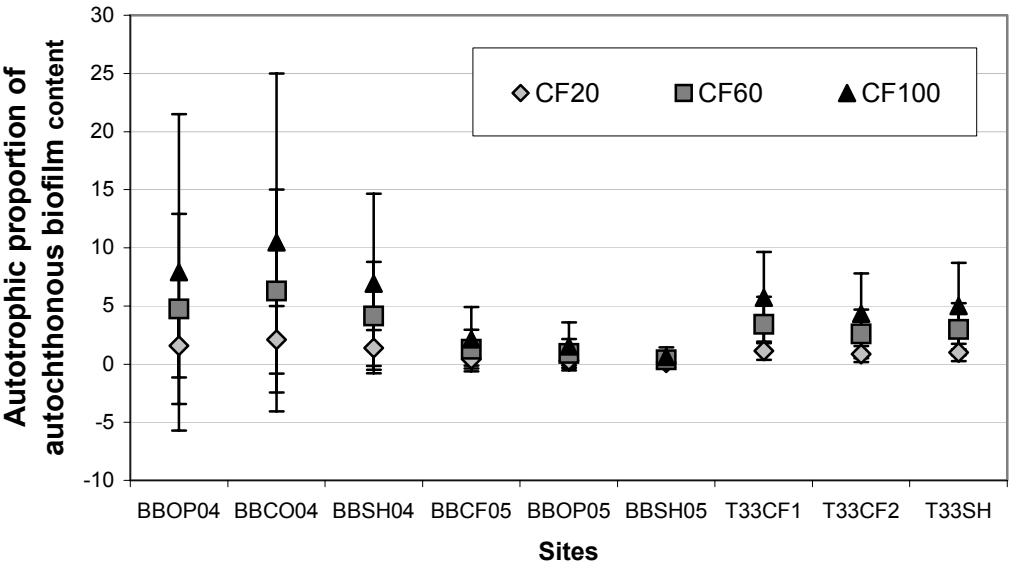

Fig 4.34 proportion of autochthonous material which is autotrophic (mean ± SD). Using the range of chlorophyll conversion factors described in the literature. Autochthonous values obtained using molar C:N derived mixing model. Kruskal-

Wallis analysis indicated significant differences between sites (CF20, P < 0.001, CF60, P <0.001 and CF100, P < 0.001).

Observation of means within Fig 4.34, suggested that Biofilms from T33 and BB2004 had significantly greater proportions of autotrophic material of the autochthonous component than either of the Black Burn sample seasons (CF60, 3.9% ± 4.9 SD for BB2004 and T33 (pooled), compared to 0.8 % ± 1.2 S.D for BB 2005) (Table 4-6). This result suggests that through isolation of the autochthonous component of the biofilms, material which is not of autotrophic origin, must therefore be heterotrophic. Consequently, by combining the results from molar C:N autochthonous modelling with the conversion of chlorophyll *a* concentrations, results suggest that all sites were therefore substantially heterotrophic in character (e.g. CF 60, approximately 95% of BB 2004, 99% of BB 2005 and 97% of T33 could be defined as consumer biomass).

Table 4-6. Mean (± SD), Maximum and Minimum autotrophic proportion of autochthonous component of biofilm biomass, with differing conversion factors (CF) from chlorophyll *a*.

	CF 20			CF 60			CF 100		
	Mean ± SD	Max	Min	Mean ± SD	Max	Min	Mean ± SD	Max	Min
BB2004	1.73 ± 2.3	11.5	0	5.20 ± 6.8	34.5	0	8.67 ± 11.5	57.5	0
BB2005	0.29 ± 0.4	1.56	0.003	0.87 ± 1.2	4.68	0.01	1.46 ± 2.04	7.8	0.016
T33	0.98 ± 0.7	3.13	0.103	2.95 ± 2.1	9.38	0.31	4.93 ± 3.5	15.64	0.51

Using a conversion factor of 20, there was no significant difference between BB2004 sites in autotrophic production (Kruskal-Wallis, P = 0.755, *n* = 46). Further, there was no significant spatial variation within BB2005 (Kruskal-Wallis, P Value = 0.274, *n* = 63). Similarly, there was no variation among T33 sites (Kruskall-Wallis, P = 0.338, *n* = 62). Similarly, using a conversion factor of 60 also indicated little spatial variation of sites within a single stream (BB2004, P = 0.666; BB2005, P = 0.162; T33, P = 0.362), and CF of 100 produces the same significances with Kruskal-Wallis analysis.

4.10 Discussion

The exact composition of the biofilm greatly affects its ability to both purify river systems and perform the role of energy production through primary production and the breakdown of detrital material (e.g. Stevenson, 1996). This role is essential to the functioning of the river ecosystem.

4.10.1 Stoichiometric data and application

The significant temporal and spatial variation in stoichiometric data found within this study has been used within a two-source mixing model to assign proportional contribution of allochthonous and autochthonous carbon to biofilms measured. This was done on the premise that allochthonous organic matter is characterised by C:N of ~ 50, whereas the ratio for autochthonous organic matter is ~ 12 (Wetzel, 1975).

However, it is possible that part of this variability in C:N can also be attributed to internal processes of the biofilm and the subsequent changes in the relative carbon to nitrogen content. Studies have shown that subtle changes in the molar C:N signatures of the biofilms can reflect the conditioning of particulate organic matter (either of allochthonous or autochthonous origin) performed by bacteria and fungi, resulting in the protein content of the biofilm rising and its C:N falling (e.g. Kaushik and Hynes, 1971). Additionally, active polysaccharide matrix development can result in the expression of a higher C:N. Thus, the functioning and growth of a biofilm may have effects on the stoichiometric signature independent of the allochthonous and autochthonous content. However variation in the molar C:N with biofilm matrix formation or increased protein content produce variation on a small-scale compared to the variation between allochthonous and autochthonous resource portioning due to the low nitrogen content of terrestrial organic matter.

Further, studies have used stoichiometry as an indicator of food quality. Generally, a low C:N is taken as an indicator of high protein contained in the organic matter and consequently good availability of the energy (and thus, is used as a food quality indicator: Taylor and Roff, 1984). Consequently, C:N and proteins (as food quality) are negatively correlated and in relation to trophic interactions, organic matter with high protein content is a preferred food for consumers in the riverine food web (see Iversen, 1973).

Thus, the variation identified in C:N within this study suggests that protein levels and associated food quality are greatest during spring and late summer/autumn biofilm growths (Fig 4.8, for BB and Fig 4.10 for T33 data). The significantly lower C:N found in the T33 sites (Fig 4.15) would suggest that food quality was significantly greater. As such, it appears that the higher molar C:N observed at BB during the mid

summer months of both years could reflect low biofilm protein content, high allochthonous contribution and increased polysaccharides matrix formation, and an indication of biofilm self-sufficiency and internal carbon retention (Freeman and Lock, 1995).

As there was a wide range of C:N values (especially at BB), I hypothesise that the influence of allochthonous contribution is likely to be the most influential compositional alteration to biofilm C:N (rather than the protein content of matrix formation). However, the suggestion that increased matrix formation is associated with increased C:N could be evidence to suggest that the significant biomass increase associated with BB 2005 biofilms was related to the maximum nutrient retention by polysaccharide matrix-rich biofilms (Freeman and Lock, 1995; Thompson and Sinsabaugh, 2000) (specifically those limiting autotrophic or heterotrophic growth). The associated high C:N recorded by these biofilms could be evidence for efficient nutrient retention and processing by BB 2005 biofilms. The higher C:N ration would also suggest that due to the increase in C rich organic matter, preferential drawdown and utilization would be towards materials rich in N. However, the increased matrix production can also reduce the diffusion rate of those same compounds into the biofilm (Hamilton, 1987) and as such, would reduce the speed and efficiency of any drawdown or retention.

Romani et al. (2004) suggest that increased autotrophy in light-grown biofilms facilitates bacterial growth, but also internal cycling of algal exudates. In addition, that same study states that, although there is evidence for increased internal cycling of nutrients (and thus hypothetically, a reduced requirement for external drawdown of allochthonous DOC), the uptake of DOC (evidenced by introduction of ^{14}C-glucose) was still higher in light-grown biofilms. Dark-grown biofilms, although entirely dependent on allochthonous DOC (rather than additionally internal cycling of autotrophic by-products), were not as efficient as light-grown biofilms. In addition, dark, heterotophic biofilms were only efficient at uptake of labile C. Therefore, more complex forms of humic substances and polymeric molecules, common in fluvial ecosystems (Volk et al., 1997), require autotrophic-driven extracellular bacterial enzyme activity for heterotrophic uptake (Chrost, 1990). This has significance for forest corridor characteristics, and would suggest that streams should have light levels adequate to ensure no limitation to autotrophic production. As while

autotrophic production is maintained at adequate levels, maximum uptake, retention and processing of excessive nutrients, humic compounds and abiotic compounds released into water-bodies during clear-felling, is ensured.

Using the broader estimations of biofilm allochthony and autochthony, the mixing model indicated little evidence for intra-site variation of autochthonous content within either sample seasons at BB, utilising either the $\delta^{15}N$ or molar C:N mixing model. The lack of intra-site variation would suggest that the site conditions, and interestingly corridor width, tree height and overall light intensity in particular (as outlined in Table 4-2 and Table 4-3) produce no variation in the autochthonous content of the biofilm material. However, intra-site variation did occur at T33. During 2005, the mean autochthonous content of the biofilms was significantly reduced at the shaded site (T33SH), suggesting that biofilm autochthonous production was light limited and thus light dependent within the T33 system.

From the molar C:N results it was clear that both T33 and BB 2004 were dependent on substantial production of autochthonous material. This suggests that biofilm material within these catchments has a high autochthonous content (~80 - 90%) under normal, undisturbed conditions. This is contrary to traditional literature suggestions that such systems have their energetic base dominated by external sources of detritus from bank-side vegetation (Vannote et al., 1980, Webster and Meyer 1997), and a community structure functioning based upon this resource (e.g. Bilby and Likens, 1980; Dobson and Hildrew, 1992). The reliability of this result is high as it is based on comparisons of biofilm stoichiometric signatures with end-members which are influenced minimally by many of the variables which would influence the isotopic signatures (e.g. algal/terrestrial plant species, growth conditions, fractionation processes and flow patterns). Thus, there is confidence that although there may be a small amount of variation occurring due to protein content (Sterner et al., 1993), or biofilm matrix formation (Sabater, 2005), biofilm signatures of molar C:N ~ 20+ are likely to be highly influenced by the addition on allochthonous material (Wetzel, 1975). An increasing number of studies have been indicating similar conclusions from a range of alternative approaches and within different systems (Minshall, 1978), particularly in forested headwater streams (e.g. Hawkins et al., 1982; Mayer and Likens, 1987). Further testing of this conclusion should be done on any future studies. Experimental additions of allochthonous

material to artificial streams may indicate the extent of any natural variation in C:N, not directly influenced by allochthonous contributions.

In order to be able to characterise the autochthonous component of the biofilm, chlorophyll *a* was employed to provide estimates of the autotrophic biomass in order to determine what proportion of the autochthonous compartment was of autotrophic origin. This provides information not only on the reliance on autotrophs within the system, but also on the level of internal recycling brought about by primary production and the associated release of N rich Extracellular Polymeric Substances. Further knowledge of the contribution of heterotrophic biomass provides information on the potential importance of the microbial loop within the stream ecosystem.

Differentiating between autochthonous and autotrophic material is important. Autochthonous material, although of internal origin, cannot be directly linked to the functioning of the biofilm as examined by Romani and Sabater (1999); in terms of autotrophic/heterotrophic ratio. In addition, it is not possible to completely rule out the argument that a proportion of the autochthonous material is actually heavily internally processed and transformed matter of allochthonous origin. Transformation of this material through fractionation and metabolic processes is not detectable by this C:N approach and as a consequence it is not possible to discern the exact external energy requirements from the streams. However, it is possible to state that the biofilms which are not influenced by disturbance events appear to be generally 'self sufficient' in their production. The overall autochthonous signature (~80 %) suggests under normal, undisturbed conditions, there is efficient internal processing of any allochthonous carbon material. As such, the direct demand for allochthonous material at the base of the food chain appears to be significantly reduced compared with autochthonous processing, production and recycling. However, according to a number of studies (e.g. Romani and Sabater, 1999; Freeman and Lock, 1995), self-sufficiency of a biofilm is reliant on a high proportion and production from autotrophic material. Therefore, it was important to determine whether this was the case here.

However, examining the spatial variation in the autochthonous data, one can speculate that if the autochthonous material was based on primary productivity (i.e. autotrophic), then there should be a positive increase in autochthonous content of

biofilms with increased corridor width and light intensity. As there was no site specific variation of autochthonous production at the Black Burn during either 2004 or 2005, one would suggest that the autochthonous component is not light dependent and therefore not photoautotrophic dependent either. However the reduction in autochthonous material at the shaded site of T33, suggests that in this case, light dependent material is present. The inconsistency of the site data illustrates that it is not clear from the autochthonous data alone whether organic material of low molar C:N is formed from:

1. Organic matter actually of allochthonous origin, yet highly processed and transformed by internal cycling and bacterial/fungal activity and thus light independent.

2. Heterotrophic based material (i.e. bacteria and fungi) which are autochthonous, utilising the mobile metabolites and decaying matter from autotrophic production, yet independently, are not light dependent.

3. Or autotrophic material, but not light limited even within relatively shaded, enclosed corridor conditions (as found at BBSH).

The lack of intra-site variation at BB is inconsistent with the first scenario as allochthonous material is independent of the obvious riparian detritus delivery routes associated with increased overhanging trees and reduced corridor width and therefore increased allochthonous material. Thus, assuming no other nutrient limitation, the likelihood is instead that, as the proportion of autochthonous material within a biofilm cannot normally be predicted by the corridor characteristics present, this heterotrophic, light independent material identified is controlled by DOC, whose availability is controlled by wider catchment-scale land uses. Thus, using C:N as a measure of autotrophic production would imply that the need for open corridors which promote high primary production is questionable as the autochthonous production within these streams is consistently high under undisturbed conditions, regardless of corridor characteristics and design.

Temporal variation of pooled site data suggested that allochthonous content of the biofilms was greatest during the mid-summer months of the sampling season, and

BB 2005 was particularly dominated by allochthonous material during June/July 2005. This allochthonous material was associated with a significant dry-weight biomass increase (Fig 4.2) during mid-summer. Additionally, although pooled sites of T33 and BB 2004 were found to be similar in their high autochthonous mass, BB 2005, was significantly more allochthonous dominated. The change of land use surrounding this shift in the composition and characteristic of the biofilm material is discussed in more detail within chapter seven.

4.10.2 Chlorophyll Conversion Factor (CF)

In order to better define the role of the autochthonous proportion of the biofilms, the autotrophic compartment of autochthonous production was quantified. In doing so, comparative information on the heterotrophic compartment was provided. Chlorophyll data was used in conjunction with an algal cellular carbon conversion factor in order to estimate the ratio of mass of algal cellular carbon to autochthonous content. Comparing two separate approaches had limitations as the set conversion factor (60) has been shown in the literature to be subject to variation with species, growth conditions and a number of other biological and physical factors. Therefore, three alternative conversion factors were considered in order to determine the potential extent of influence variation of the CF would have on the comparative proportion of autotrophic and heterotrophic material, which together compiled the autochthonous compartment of the biofilm. This data can be used to explain the lack of correlation between autochthonous content and corridor characteristics at sites. Specifically, despite varying the conversion factor from 20 to 100, the biomass from the autotrophic compartment was negligible compared to that of the remaining proportion (defined to be heterotrophic). At its greatest (BB2004, CF100), the autotrophic proportion only equated to a mean of approximately 9% of the autochthonous material. This is significantly lower than the values provided within Romani and Sabater, (1999), where ratios of up to 3:1 autotroph to heterotroph were quoted. However, some maximum values did reach to a level equating to ~ 1:1, which may indicate more comparable autotrophic production to Romani and Sabater, (1999).

Interestingly, there were no significant differences in the autotrophic proportion among sites of a single stream, despite significant physical (and specifically light)

variation. Therefore, it is not possible to assign light limitation (purely based on corridor design) as the causative factor for the low autotrophic availability within biofilms. One possible limitation may be low light attenuation within the water column during periods of high DOM/DOC release (as illustrated within chapter seven).

The proportional contribution of the alternative resources will obviously influence the contribution to the biofilm matrix, which algae have the potential to make. For example, with reference to gross chlorophyll concentrations from the biofilm material (irrespective of the proportion of other biofilm components), there is site specific variation in the autotrophic biomass, as can be seen in Fig 4.35 (repeated from chapter 3). T33 sites are generally more chlorophyll (and therefore, algal C) enriched. Consideration of streams in isolation indicates that BBCF 2005, had a significantly greater chlorophyll (and therefore algal) biomass, whereas, T33 shaded was significantly lower (than the other T33 sites). Therefore increased canopy or canopy removal significantly influences gross algal content. However, the variation in the other components of the biofilm appear to counter this effect on the overall proportion of autotrophic material.

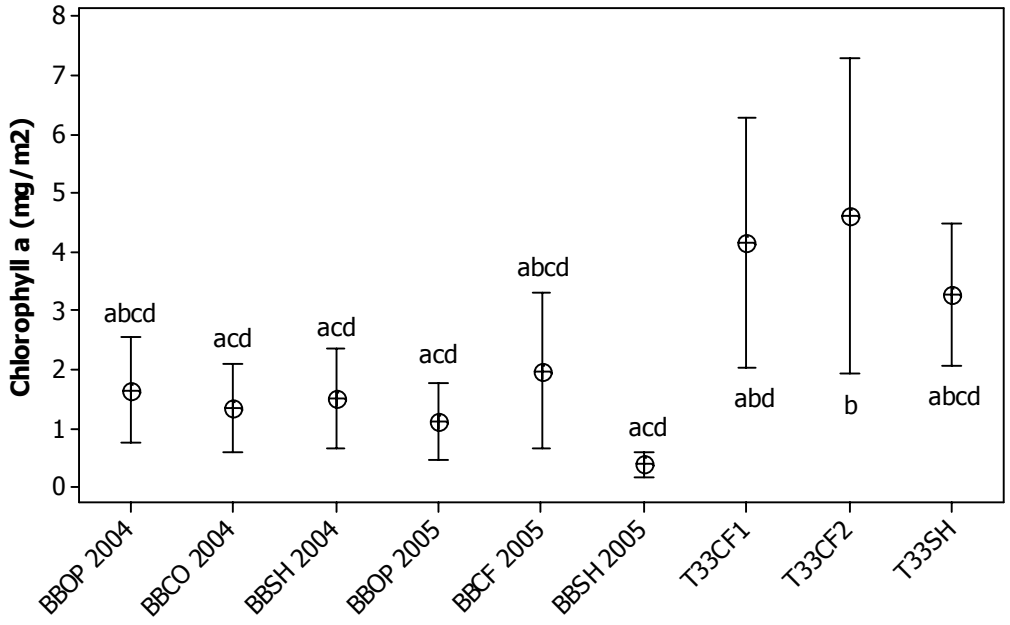

Fig 4.35. Mean chlorophyll *a* concentrations (± 95% confidence interval) at both Black Burn and T33 sites over 2004 (BB only) and 2005. Tukey test reveals significant differences (indicated with differing letters: a, b, c or d) (P < 0.001, n = 227).

The low autotrophic to heterotrophic ratio found in all sites suggests that biofilm functioning may be relatively poor. Romani and Sabater, (2000) showed that benthic heterotrophs within the biofilm preferentially use by-products excreted by the autotrophs (algae). Benthic algae and bacteria actively exude substantial quantities of organic carbon, primarily as exopolymeric substances (EPS) (e.g. Hoagland et al., 1993). EPS can account for up to 50% to 90% of the total organic carbon of biofilms (Flemming et al., 2000). These exudates can constitute a large proportion of the carbon acquired by algae and bacteria (e.g. Goto et al., 1999). This nutrient production from within the biofilm has been found to increase the capacity of biofilms to function as water purifiers (Romani and Sabater, 1999). An ideal ratio of around 3:1 of autotrophs (e.g. algae) to heterotrophs (e.g. bacteria) was found to facilitate the largest increase in the ability of the biofilm to break down allochthonous detritus (Romani and Sabater, 1999). As sites had an average of between 99 and 95% heterotrophic material (using the middle CF, 60) from within the autochthonous compartment (totalling ~75% of the total biofilm content), this would suggest that these biofilms are almost entirely dependant on heterotrophic cycling. As the autotrophic content appears to account for around 4% of the biomass, and the allochthonous material accounts for an average of 25%, the likelihood is that the heterotrophic material (totalling 70 % of the biofilm biomass), having a low molar C:N which resembles an autochthonous signature, is EPS material. This conclusion was similar to that derived by Frost et al. (2005), who also noted the low prevalence of algae within periphyton samples, suggesting the majority of material was mucilage-based. However, the energy base for this production is likely to be dependent on the higher proportion of allochthonous carbon over the smaller proportion of autotrophic material.

Although both algal proportional contribution and autochthonous proportion were estimated, neither provided results comparable with visual ID measurements made alongside (Fig 3.16 and Fig 4.30 respectively). Microscopic field of view counts in general, yielded a significant over estimation of algal material. This inconsistency of results suggests inaccuracy of the visual ID method. A similar off-set has been noted in previous studies (e.g. Hamilton et al., 2005; Frost et al., 2005). Inaccuracy of a method so commonly used could aid in explaining why there is inconsistency between findings here and the general understanding that upland, low-order streams are dominated by allochthonous material (e.g. Vannote et al., 1980, Webster and

Meyer 1997) or that periphyton is generally algal-dominated (e.g. Frost et al., 2001). Such inaccuracy has the potential to drive gross misunderstanding of low order stream energy dynamics and carbon source importance.

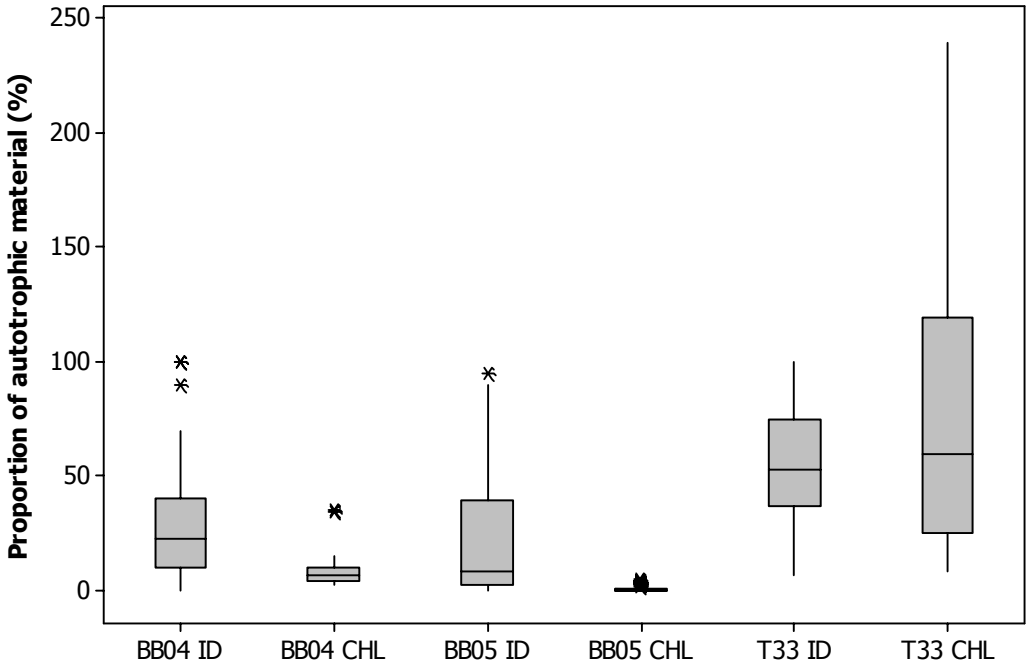

Fig 4.36 Comparison of chlorophyll defined autotrophic contribution and Microscopic identification of algal content. Kruskall-wallis defined significant differences between samples (P < 0.001). Means suggest that microscope analysis consistently overestimated the algal contribution to biofilm material.

4.10.3 Implications of variation of $\delta^{15}N$ signatures

Measurements of isotopic signatures (specifically $\delta^{15}N$) are commonly used in diet studies where consumer diet contributions are assessed using fractional contributions of baseline resource signatures (with the addition of metabolic fractionation factors) (e.g., Peterson and Fry 1987, Kling et al. 1992, France 1995, Vander Zanden et al. 1999). However, many studies make the assumption that the signature of the baseline resources remains relatively constant and thus, set basal resource signatures are used within mixing models (e.g. Cabana and Rasmussen, 1996). Here, baseline resources were found to vary significantly both temporally and spatially over the two-year study period of BB and T33 biofilm signatures (Fig 4.4 Fig 4.11 and Fig 4.14 and Fig 4.15). Knowledge of the possible variability of base-line resources is useful in order to provide information on the general reliability of food-web mixing models based on calculations which assume stable baseline resource signatures.

With $\delta^{15}N$ isotopic measures, biofilm signatures varied from a minimum of -2.6‰ (BB APR 2004) to a maximum of 7.1‰ (BB SEP 2005). This constitutes a temporal range of 9.7‰ of $\delta^{15}N$ baseline resource signatures. As the majority of studies use a trophic fractionation level of 3 – 5‰ (Peterson and Fry 1987), to indicate a change in food chain level, the range in baseline resource $\delta^{15}N$ signatures found within the present study, would equate to up to three trophic enrichments within a comparable food chain study. Additionally, this is of greater significance as the land-use and geography of all sites remains relatively consistent, and the temporal scale is relatively short, encompassing similar seasons. Thus, such variation (or variation of a greater extent) is likely to similarly occur in studies of greater spatial and temporal scales. By not accounting for the potential of variability of baseline resource signatures, diet studies have the potential to assign changes in consumer species signature to diet variability or trophic status and not simply to changes to the signatures of baseline resources (during a consistent diet). Thus this present study confirms the need to account for this variation when undertaking diet studies or using primary consumer invertebrate signatures as proxies for baseline resources.

4.10.4 Wider implications of the technique

This approach demonstrates the variability of baseline resources and therefore, has the potential to be important for any food chain studies wishing to understand the driving forces involved in differential baseline trophic interactions. Without such baseline information gross overestimations of source material are possible as visual inspections are hard to interpret accurately. This approach also provides information on the result of stochastic events such as clear-felling and on stream energy dynamics and any recovery responses by benthic biofilms as will be discussed in Chapter seven.

Although more development of these combined techniques is required, this research has illustrated a potential use for stoichiometry combined with chlorophyll analysis which would have a substantial impact on the ability to characterise river energy inputs. Specifically, this method could have the greatest affect on small order streams where inputs of energy from in-stream production is hard to quantify due to the lack of large primary producers such as aquatic macrophytes. This method also provides information on autotrophic availability and indicates that the possible

utilisation/availability of allochthonous material may be significantly overestimated within conifer-afforested streams.

Understanding of the processing capacity of biofilm growth within small stream systems is a promising approach which could be applied to a wider area in order to better understand the energy availability within different systems and the source material most being utilised at the base of the food chains. This research has demonstrated that biofilms even within heavily afforested catchments are dominated by internal processing of organic matter to such an extent that they appear to be autochthonous in character. This result suggests that, irrespective of reduced levels of autotrophic material, and varying light intensity, biofilm nutrient processing efficiency was relatively high under normal, undisturbed conditions. However, in addition to defining algal cellular carbon content (thus the autotrophic component), chlorophyll data been employed to provide, through elimination, simple estimations of the relative proportion of heterotrophic and autotrophic material. This data also illustrates that the addition of sites of light-intensive areas within afforested riparian zones does not have a significant impact on the relatively poor mean autotrophic content of the biofilm material (between 1 and 10% of autochthonous component). As a consequence, following Romani and Sabater (2000), one can postulate that the ability of biofilms to retain and process organic and inorganic pollutants may be limited by the reduced autotrophic contribution. However, the high overall autochthonous component of the biofilm material in-fact suggests that the reliance of the biofilm on allochthonous material was low and a high content of EPS would aid in nutrient and pollutant drawdown and retention efficiency (Freeman and Lock, 1995).

As such, forestry management practices, which encouraging light intensive areas within afforested catchments, may not significantly influence the autotrophic production, especially if water colouration significantly reduces light attenuation and the depth of the photic zone. Biofilm development was in fact, most seriously effected by the disturbance event of 2004/5, producing a much greater proportional content of allochthonous material and a much lower autotrophic to heterotrophic ratio. This result suggested that the ability of the biofilm to retain organic material and any autochthonous processing and production was significantly reduced.

4.11 Main findings of chapter

- Like biofilm biomass, isotopic and stoichiometric signatures of the biofilms experienced a wide range of spatial and temporal variation. Signatures ranged from –25.1‰ to –32.6‰ ($\delta^{13}C$), -2.6‰ to 7.1‰ ($\delta^{15}N$), whilst molar C:N ranged from 6.4 to 34.1. These measures were considered in respect to providing delineation of allochthonous and autochthonous resources within biofilms.

- $\delta^{13}C$ could not be related any other variable measured. There was also no correlation with flow (as had been suggested in the literature). Additionally as $\delta^{13}C$ signatures overlapped with common terrestrial signatures, it was not possible to use the measure to delineate between autochthonous and allochthonous material. Therefore, this measure was not considered with respect of model development

- Molar C:N and $\delta^{15}N$ displayed a significant negative linear relationship ($P < 0.001$),. Molar C:N was most enriched during mid-summer seasons, while $\delta^{15}N$ was the opposite. Significantly higher molar C:N at BB 2005 sites, suggested increased contribution of C rich organic material to the biofilm material. The opposite was true of T33 sites.

- The high molar C:N values associated with the carbon rich and N low material of terrestrial origin. As molar C:N were highly correlated, both measures were used as a proxy for allochthonous and autochthonous material contribution. The proportional contribution was derived using a two-source mixing model.

- Variation in $\delta^{15}N$ away from the molar C:N values (especially in April, 2004) suggested that species variation of autochthonous material (specifically, the increase in blue-green algae population), resulted in unreliability of the $\delta^{15}N$ as a measure of autochthonous contribution. Therefore, molar C:N was determined to be the most accurate and robust measure.

- Variation $\delta^{15}N$ was so significant as to represent around 2 – 3 food chain trophic levels in terms of the enrichment caused by fractionation through

ingestion. It was suggested that the variation in baseline resource signatures should be explored prior to any isotopic studies of consumer diets, as diet may appear to vary over spatial and temporal scales up to three trophic levels, where in fact, diet is unchained but resource signatures vary considerably.

- Allochthonous contribution increased significantly during mid-summer at all sites in all streams suggesting that terrestrial organic matter release is greatest during this point. This result contradicted studies suggesting that higher temperatures associated with summer, would increase the level of autochthonous production. This result may have been related to increased growth of overhanging riparian vegetation.

- Algal contribution was estimated by converting chlorophyll *a* concentration to algal carbon through the use of a conversion factor (CF). Literature findings suggested that the appropriate CF had the potential to vary from ~ 20 – 100 with species composition and the physical algal growth conditions. Thus, three alternative CFs were explored (20, 60 and 100) in order to determine the extent to which variation of the CF influenced the proportion of autotrophic material within the biofilm biomass.

- The heterotrophic and autotrophic compartment of the biofilm biomass was separated by calculating first the autochthonous proportion, then from within this mass, the autotrophic proportion (using the middle CF of 60). The remaining compartment was defined as heterotrophic.

- From these calculations, variation between the autotrophic proportions was minimal between sites of a single stream. Only BB 2005 displayed significantly reduced autotrophic proportion. However all sites and streams showed low autotrophic proportion. At the undisturbed locations (BB 2004, T33), approximately 5% of biofilm material was autotrophic, 25% was of allochthonous origin, and the remaining 70% was heterotrophic in character. However, with disturbed sites, the allochthonous proportion increased to approximately 40% of the biomass of BB biofilms, and autotrophic contribution rarely reached above 1% of the total mean biofilm biomass.

- Such low autotrophic proportional contribution to biofilm biomass suggested that functioning capability of the biofilm to drawdown and retain organic and inorganic pollutants may be limited. However, the high overall autochthonous proportion suggested that biofilm matrix content was high and as such, the potential for retention was also increased. Further, the increased allochthonous content of the biofilm post-felling suggested that excessive nutrients released post-felling were retained.

- Variation of the chlorophyll CF did not have a significant influence on the results to autotrophic contribution. Variation between streams was still significant regardless of CF and although there was an associated change to the proportional contribution of autotrophic material, CF variation did not result in autotrophic proportion resembling (or not) the 3:1 autotrophic to heterotrophic ratio suggested by Romani and Sabater (1999), for optimal biofilm functioning capacity.

- The lack of correlation between the physical traits of specific sites and the autotrophic contribution to biofilm material was a surprise. The dark colouration and turbidity of the water at BB may have contributed to this result and decreased the photic zone. However, this result indicates that the proportion of autotrophic material within biofilms may be much lower than many have previously suggested. Further, the requirement to 'open up' forest canopies in order to increase autotrophic proportion may not be a worthy approach. However, in terms of gross chlorophyll content there was site-specific variation (with T33 more enriched and intra-site variation at BB 2005 and T33). Consequently, in terms of gross primary productivity, and not just proportional contribution of autotrophic material within the biofilm, results suggest that light levels should be maximised. However, the result to the overall proportional characteristic of biofilm material may be minimal, and not influence the functioning of the biofilm for either retention or processing of nutrients and pollutants.

5 Patterns Stream macro-invertebrate species assemblage structure and the role of forest corridor physical, chemical and biotic factors.

5.1 Abstract

Benthic macro-invertebrates were surveyed in order to provide information on optimal corridor characteristics and design, to maximize in-stream biodiversity evenness and abundance of these organisms. Functional feeding groups and diversity indices were determined for species assemblages at each site. Spatial and temporal variations in total community structure were explored using kick-sample methodology at all streams of the study area. These analyses were used to indicate variation in site diversity and provide information on basal resources influencing the community composition.

Isolated catchment analysis (Cree and Bladnoch) reduced the impact of catchment-specific confounding variables (e.g. altitude and pH), and in doing so, helped to define the role of site-specific corridor characteristics. However, even with this approach, the mechanisms influencing community composition and diversity were more often associated with a number of physiochemical variables of site (e.g. substrate size, algal cover, % overhanging ground vegetation, pH, conductivity and stream temperature), independent of design parameters (e.g. corridor width, overstorey tree diversity and % light).

It was speculated that both algal cover and % overhanging ground vegetation would be closely related to light availability. Thus, although % light itself was not found to be significantly correlated with invertebrate diversity, the increased contribution of both allochthonous and autochthonous carbon resources suggests that light availability is an important control factor. However, the lack of direct correlation appeared to suggest that under maximum light levels, PAR and the two production responses were uncoupled.

Consideration of the Bladnoch in isolation revealed a greater importance of design parameters (e.g. corridor width and tree diversity). Statistically different community assemblages were defined through TWINSPAN. These groups were associated with

environmental variables defined through CCA ordinations. These analyses indicated differential group reliance on either allochthonous detrital resources associated with broadleaf/diverse tree species areas, or a greater supply of algal and overhanging ground vegetation resources (associated with high altitude, light intensive and wide corridors). This provided evidence for alternative strategies to maximise stream invertebrate diversity, which could be applied to future forest design approaches.

5.2 Introduction

The intimate relationship between a stream and its riparian zone strongly influences energy type and availability within upland low-order stream habitats. The balance between heterotrophy and autotrophy plays a key role in determining broad-scale in-stream invertebrate community structure (Wetzel, 1975).

Because the relative dominance of aquatic macro-invertebrate groups within stream communities shifts with differences in the available sources of energy, morpho-behavioural adaptations of food acquisition match both the general resource conditions, and size of the stream habitat. Thus, the differences in adaptations in food acquisition are a major driver of the specific assemblage structure and species composition found at a given site (Cummins and Klug, 1979).

Cummins (1973, 1974) concluded that mouthpart morphology was a good reflection of feeding mechanism, as animals can only be opportunistic within the limitation of their feeding apparatus. However, gut content analysis has been the technique most frequently used to investigate macroinvertebrate feeding, and to assign taxa to functional feeding groups (FFGs) (e.g.; Hawkins 1982; Chessman, 1986). By categorising taxa into functional feeding groups (FFGs) according to feeding preferences and adaptations, and defining relative diversity within each habitat system, the specific community assemblage structure can be used to provide information on food availability, the physical characteristics of the stream and riparian zone, energy cycling within the habitat and the importance of specific allochthonous or autochthonous energy sources to food-web dynamics and the associated community composition.

Here, benthic macro-invertebrate community structure and abundance are quantified. The data are used to identify:

1. Indicator groups of the food resource availability and preferential energy usage with variations in corridor and habitat conditions as well as integrated conditions present over longer periods.

2. Overall diversity and community health according to variation in stream and riparian conditions.

5.2.1 Food resource availability and preferential energy usage with variations in corridor and habitat conditions.

The conceptual model which arguably best describes longitudinal relationships along river systems is the River Continuum Concept (RCC) of Vannote et al. (1980). This suggests that upstream, low order watercourses are dominated by inputs of allochthonous (externally sourced) detritus. As a consequence the invertebrate community composition matches this energy resource and the specific conditions of the upstream environment. This species assemblage structure transits to high order, larger river systems, lower in the catchment, which are dominated by autochthonous (internally produced) production (and a related community composition which matches these resources). In the latter, autochthonous dominated scenario, the riparian zone has less impact on in-stream energy dynamics and the larger river surface area supports a greater proportion of autochthonous primary producers.

The model suggested by Vannote et al., (1980) was based around eastern US stream systems. Streams located in Scotland often have headwaters occupied by moorland, not native woodlands. Additionally, streams located within plantation forests often have a very different riparian habitat than those seen in native woodland. Consequently, the RCC may not be entirely applicable to afforested UK systems and the specific design of the riparian zone and the corridor characteristics, and associated fauna described, may vary significantly from those predicted by the RCC model. However, by matching more natural systems, wide-scale forestry corridor management has the potential to significantly influence community composition and overall in-stream biodiversity (Dobson and Cariss, 1999).

The main energy sources which influence benthic invertebrate community composition and abundance are either autochthonous or allochthonous. The most conspicuous autochthonous component is the primary producers: mainly as algae and macrophytes. However, heterotrophic processing of materials by bacteria, fungi and invertebrates, as discussed in the previous chapter, constitute a significant form

of internal production, and may be responsible for a significant proportion of the available organic biomass within stream systems. External energy inputs come in the form of riparian detritus and riparian heterotrophs. These potential food sources and their relative importance for stream ecosystem functioning are examined below, providing background information for this chapter's main area of investigation.

The overall aim of this chapter was to determine the role of corridor design in influencing the production and availability of food resources, by examination of aquatic invertebrate community response; and to determine the impact of different habitat characteristics on in-stream invertebrate diversity.

5.2.2 Allochthonous Detrital Resources

Decomposition of plant litter is a central ecosystem process in a wide range of both aquatic and terrestrial systems (e.g. Wagener et al., 1998). Headwater streams are comparable with forest floors in their dependence on allochthonous litter inputs (e.g. Wallace et al., 1997). However, within stream systems, litter tends to concentrate into discrete litter patches. Within these areas, resources are concentrated, but they lack the vertical structure often associated with the more complex model of a forest floor system. Further, the community of bacteria and fungi responsible for much of the decompositional processes are often much less complex. However, such 'leaf packs' lend themselves to manipulation by the flowing water and therefore often decompose far more rapidly than their forest floor counterparts (Hieber and Glessner, 2002).

The breakdown of leaf litter (mineralisation and transformation) is dependent on both physical (e.g. physical leaching, abrasion and fragmentation of material by water and objects carried by the current (Hieber and Gessner, 2002)) and also biological processes. The organisms that drive this biological component of the decompositional process include detritivorous macro-invertebrate species - mainly shredders, collector-gatherers and filter feeders (Wallace and Webster 1996), bacteria and filamentous fungi species (e.g. aquatic Hyphomycetes (Suberkropp, 1998)). The quantity, diversity and quality of detritus available within the stream to aquatic detritivore species is often dependent on the type of vegetation growing within the riparian zone and the nature, specifically physical retentiveness, of the stream (e.g. Jones, 1997).

Peterson and Cummins (1974) proposed a 'dietary continuum' of decay of leaf material. The system was based on the differentiation of decay rates of different plant species. Using published decay rates, they classified detritus into 'fast', 'medium' and 'slow' categories and suggested that detritus from each category reached optimal palatability after sequentially longer periods of retention within the stream. Thus, the period during which food was available to detritivore based species was extended long after the initial autumn leaf fall when a diverse range of categories were present within the riparian zone. Therefore, detritivores exhibit dietary plasticity during the winter to accommodate newly available sources of palatable leaf litter.

The relative importance of allochthonous energy sources and indeed, the lack of dependence of stream macro-invertebrate communities on primary production was demonstrated by a number of studies. For example, Hynes (1961) demonstrated that more than two thirds of the Welsh mountain stream species completed their life-cycle in the winter, when primary production would be minimal. Further, Cummins and Klug (1979) found that a large portion of the aquatic insect community in temperate streams was synchronized to the autumnal input of leaf material, having emergence, oviposition and eclosion just prior to the autumn leaf loss.

There are substantial practical difficulties of separating sources and components of detrital biomass. These issues are discussed more fully in Chapters 3 and 4, and in the literature (e.g. Cummins and Klug, 1979). However, the specific separation of the living (heterotrophic biomass) and the non-living components of detrital matter (e.g. the allochthonous component) has as a consequence, often instead been achieved through separating material into broad size categories; Coarse Particulate Organic Matter (CPOM) and Fine Particulate Organic Matter (FPOM). CPOM can be defined as organic matter having particle size larger than 1mm. Particulate material finer then 0.5µm, is considered to be Dissolved Organic Matter (DOM) (Cummins and Klug, 1979).

The complex array of biotic and abiotic interactions that occurs in the processing of detrital matter, combined with the variability in the relative size and composition of the organic matter, determines which food resource 'pool' it is processed into. CPOM entering the stream is often colonised by fungi (primarily aquatic hyphomycetes) and

bacteria. This process is either done in surface waters or within benthic biofilms (see Chapters 3 and 4). Activity from these initial colonisers combined with bacteria and shredder species activity results in the continuous release of FPOM, UPOM (Ultra-fine Particulate Organic Matter) (<50µm, <0.5µm respectively) and DOM (Suberkropp and Klug, 1976). Indeed, this process is often so efficient, that it is estimated that less than 25 % of CPOM is mineralised directly as CPOM; the rest is transformed into other organic pools of detritus (Suberkropp and Klug, 1976).

Differentiation in FPOM and UPOM source material often leads to differences in bacterial colonisation and processing of organic particles. The nature of this heterogeneous colonisation has also been found to stray from the often-generalised relationship described in studies such as Hardgrave (1970a, b, c); where the simple model relating level of bacterial activity directly to particle surface-area has been applied. However, in the case of stream systems, often the residence time of the particle within the aquatic environment has a much greater influence on bacterial activity and processing rate than the size of the particle itself (Suberkropp and Klug, 1976, Suberkropp et al., 1976).

Although the physical, chemical and biological characteristics of the stream dictate the general rate of litter decomposition, the specific differences in breakdown rates between different leaf species are dependent on the physical and chemical characteristics of individual species (e.g. Irons et al., 1988). For example, Gessner and Chauvet (1994) found that oak leaves have tough cuticles as well as high leaf tannin and lignin concentrations which cause slow decay rates, whereas alder has high leaf nitrogen concentrations, and relatively fast associated decay rates. Generally, coniferous species provide litter of low palatability to processing biota (Bärlocher et al., 1978; Sedell et al., 1975). As a consequence, in comparison to deciduous species, coniferous species have slow decay rates and require substantially longer residence times to be processed fully (Friberg and Jacobsen, 1994; Sedell et al., 1975). The slow processing rate has been attributed to a number of different factors. Triska et al. (1975) found that alder leaves, which decomposed most rapidly, had relatively lower concentrations of lignin than Douglas Fir (*Pseudotsuga menziesii*). Berg *et al* (1982) described a negative relationship between leaf lignin concentration and decay rates. Bärlocher et al. (1978) focused on whether needle cuticles were the main limiting factor of decay rate; halving needles

longitudinally caused an increase in decay rates. From this, it was proposed that the cuticle provides a relatively unsuitable substrate for aquatic hyphomycete attachment and in addition, the cuticle locks in inhibitory oils and reduces the effects of the physical processing such as leaching.

5.2.3 Autochthonous Primary producers

Plants often play crucial roles in terms of both physical characteristics of the aquatic environment (O'Hare and Murphy, 1999; Wright, 1995; Marklund et al., 2001) and food availability (e.g. Junk, 1984; Newman, 1991; Sand-Jensen and Madsen, 1989; Jacobsen and Sand-Jensen, 1992; Gross et al., 2001, Elger and Willby, 2003; Elger et al., 2006). As a consequence, their presence and abundance in a system can have significant influence on the in-stream biodiversity and community structure (Carpenter and Lodge, 1986).

Aquatic plants can play a key role in influencing habitat type and structure, both through a simple increase in substrate surface area but also by modification of microhabitats and the architectural diversity produced by plant growth (Murphy and Ali, 1997). A range of microhabitats is formed within the plant beds, producing modifications to flow, substrate and variation of predator/prey exposure preferences (O'Hare and Murphy, 1999). Due to this unique effect, a greater diversity and abundance of invertebrate species are often associated with macrophyte stands than with other substrate types (O'Hare and Murphy, 1999; Wright, 1995; Marklund et al., 2001).

Aquatic plant growth can also influence, both positively and negatively, dissolved oxygen concentration. Significant increases in dissolved oxygen concentrations have been documented during daytime, especially with fully submerged species, as oxygen produced during photosynthesis contributes to oxygenation of the surrounding water column, although at night the situation may be reversed as the balance of photosynthetic oxygen release to respiratory oxygen demand by the plants reverses. Thick mats of floating macrophytes can inhibit oxygenation and mixing at the aquatic boundary layer (Morris and Barker, 1977). In addition, the decay of plant material consumes large amounts of oxygen and thus contributes to further de-oxygenation through an increase in the biological oxygen demand (Godshalk and Wetzel, 1978).

Autotrophic growth also contributes to water nutrient enrichment throughout the water column. Actively growing plants release between 1 and 10 % of photosynthetically produced dissolved organic carbon (DOC) into the water (Hough and Wetzel, 1975). This source of nutrients positively influences both algal growth/productivity and bacterial activity. This increase in activity, results in enhanced processing of detritus and the increase in the overall resources available to higher consumer species (see Chapters 3 and 4). In addition to this, the structural complexity of autochthonous plant matter can be important to controlling allochthonous detritus retention times. Fisher and Carpenter, (1976) showed that the export volume of detrital material from rivers was inversely correlated to the biomass of aquatic macrophytes within the system. Therefore, mechanisms which increase in-stream macrophyte production, may have many benefits to resource availability within the in-stream environment.

In relation to the present study, riparian trees can have significant effects on light availability to primary producers. Trees can reflect and absorb up to 95% of incoming photosynthetically active radiation (PAR) (Godshalk and Wetzel, 1978). The interception of solar radiation by riparian vegetation can often result in streambed irradiances that may average <20 $\mu mol.m^{-2}.s^{-1}$ in undisturbed forests (Hill et al., 1995). Primary producers are directly influenced by availability of PAR. Although photo-acclimation by primary producers can increase the efficiency by which light can be used to fix carbon, long-term light deficiency in many shaded streams cannot be compensated by increased photosynthetic efficiency alone (Hill et al., 1995).

In deciduous forest streams PAR levels change with seasonal leaf growth and senescence. The significantly reduced canopy cover during winter months promotes increased primary (autochthonous) production during what is usually a relatively unproductive season. Coniferous forest stands, with the exception of deciduous conifers such as larch, however, have no such period of reduced canopy cover, resulting in year-round production reduction.

5.2.4 Invertebrate Functional Feeding Groups

Functional Feeding Groups (FFG) are described and classified based on morpho-behavioural mechanisms of food acquisition rather than taxonomic group. The same general morphological and behavioural mechanisms in different species can result in taxa converging in ecological characteristics related to the acquisition of energy, with such convergence often following very different evolutionary pathways (Merritt and Cummins, 1996a).

The benefit of using the FFG approach is that instead of needing to consider potentially hundreds of different taxa individually, a small number of groups of organisms can be studied collectively, differentiated by the shared intra-group functional characteristics for processing energy in the stream ecosystem. Individuals are categorized based on their mechanisms for obtaining food and the particle size of the food. This approach links aquatic food resource categories (coarse particulate organic matter [CPOM, particles >1mm], fine particulate organic matter [FPOM, particles <1 mm and >0.45 µm], periphyton, and prey), to invertebrate adaptations for their exploitation.

Functional Feeding Group analyses support the notion that linkages exist in riparian-dominated headwater streams between CPOM and shredders, and FPOM and collectors, and between primary production (e.g., periphyton in midsized rivers) and scrapers. The feeding of shredders on riparian litter affects detrital processing in aquatic systems. About 30% of the conversion of CPOM leaf litter to FPOM has been attributed to shredder feeding (Petersen and Cummins, 1974). In addition, shredder feeding enhances the release of dissolved organic matter (DOM; Meyer and O'Hop 1983). Thus we can use the assemblage of species and the associated functional feeding groups to link food resource categories and the predictable response of aquatic invertebrate assemblages in order to define to food resources prevalent under differing riparian conditions. The major Functional Feeding Groups, with their trophic and feeding characterizations, are described in Table 5-1.

Table 5-1. Functional Feeding Group categorization and food resources (from Merrit and Cummins 1996a). CPOM= Coarse Particulate Organic Matter; FPOM= Fine Particulate Organic Matter.

Functional groups	Abbreviation	Particle Size feeding mechanism	Dominant food resources	Particle size Range of food (mm)
Shredders	**SH**	Chew conditioned litter or live vascular plant Tissue, or gouge wood	CPOM-decomposing (or living) hydrophytes (vascular plants)	> 1.0
Filtering Collectors	**FC**	Suspension feeders-filter particles from the water column	FPOM-decomposing detrital particles; algae, bacteria, and faeces	0.01-1.0
Collector Gatherers	**CG**	Deposit feeders-ingest sediment or gather loose particles in depositional areas	FPOM-decomposing detrital particles; algae, bacteria, and faeces	0.05-1.0
Scrapers	**SC**	Graze rock and wood surfaces or stems of rooted aquatic plants	Periphyton-attached non-filamentous algae and associated detritus, microflora and fauna, and faeces	0.01-1.0
Predators	**P**	Capture and engulf prey or ingest body fluids	Prey-living animal tissue	>0.5

5.2.5 Using Indicator Species for Biomonitoring/discerning biodiversity

Biomonitoring is recognised as an essential tool for use in both routine monitoring of freshwater ecosystem biointegrity, and for assessment of impacts of pollution or other harmful pressures upon these systems. Because organisms and/or communities can display measurable reactions to the effects of habitat change or pollution, integrated over timescales ranging from days or less (e.g. microorganisms) to months or more (e.g. macrophytes), by using biomonitoring procedures based on differing groups of organisms, it is possible to detect changes occurring in a freshwater system over differing timescales of impacts. Currently, proposed measures for implementation of biomonitoring are primarily based upon assemblage-change measures, assessed against pristine or low-impact reference conditions.

Invertebrate assemblage-change river biomonitoring techniques are already well-established in the UK, and similar methodologies using macrophytes, diatoms, phytoplankton and fish are proposed or already in use here and elsewhere in Europe (e.g. the Swedish Environment Protection Agency River and Lake Biomonitoring Protocols; RIVPACS (River InVertebrate Prediction and Classification System: Wright 1995)).

These systems were developed in an attempt to classify aquatic systems using functional and taxonomic groupings of invertebrates, specifically benthic-invertebrates in river systems. This type of predictive model also looks to quantify "biointegrity", i.e. the degree to which a site supports its reference biota (the expected biota which would occur in the absence of human interference) and the overall diversity, evenness and abundance of populations and overall community composition. These studies and techniques have had mixed results. For example, reference sites have not always been representative, indicator species have often been hard to identify, or their tolerance/preference levels to different environmental factors were wrongly estimated (Norris and Hawkins 2000). However, these approaches (e.g. RIVPACS in the UK) are used by many of the current water protection agencies (including SEPA: Scottish Environmental Protection Agency) and so here, overall diversity (using diversity index scores), abundance of certain indicator species and overall macro-invertebrate assemblage structure will be used as an indication of river biointegrity.

5.3 Methods

Aquatic invertebrate samples were taken at the sites described in the introductory chapter (Chapter 1). Sampling was confined to the Cree catchment within the sampling year autumn 2003 to autumn 2004, and expanded to include Bladnoch catchment sites within the 2005 sampling season.

Benthic invertebrates were sampled using a three-minute kick sample with a 0.5m depth net with a mouth diameter of 0.25 m following methods used by SEPA. The hand net was standard (250× 250 mm frame, mesh size 500 µm). Both a transverse and diagonal transect of the stream were kick sampled taking care to cover all habitats types within the 10m-site stretch. An additional one minute was spent

collecting invertebrates from both sides of the lateral areas of the streams as these species are not as susceptible to the kick sampling technique.

The following physical/chemical parameters of the streams were measured at representative sample site locations within the 10m site stream-stretch (Table 5-2).

Table 5-2. Variables measured as part of invertebrate sampling methodology and the sampling technique summery.

Measure	Measurement Technique
Wet width (m)	3 replicate measures per visit
Depth (cm)	3 replicate measures of stream centre per visit
% Boulders/Cobbles (>64mm)	visual estimation
% Pebbles/Gravel (16-64mm)	visual estimation
% Sand (2 – 16mm)	visual estimation
% Silt/Clay (<2mm)	visual estimation
% Algal cover	visual estimation
% Sewage/fungi cover	visual estimation
% Bryophyte cover	visual estimation
Tree species richness	visual identification
% Overhanging vegetation	visual estimation
Corridor width (m)	3 replicate measures per visit
Tree height (m)	visual estimation
Altitude (m)	GPS reading
Light (% PAR)	2 x SKYE PAR light meter (simultaneous reading with open ref site)
Conductivity (mS/m)	Schott handylab pH/LF 12 probe conductivity electrode
pH	Schott handylab pH/LF 12 probe pH electrode

5.3.1 Benthic invertebrate identification

On completion of the three minute kick sample, 25ml of formaldehyde was added to each 150ml sample to stop biological activity. The treated samples were then stored in a cool box with ice packs until return to the lab. Samples were then frozen until identification, at which point, the sample was slowly defrosted to minimise damage to the tissue. Individuals were sorted from the debris also caught in the net. This was achieved by washing samples through consecutive stacked sieves of 2.0, 1.0 and 0.5 mm diameter to remove debris. The cleaned samples were then placed in a large white, water-filled tray. Kick sample material was hand sorted for invertebrate individuals (this was not for a set time period, but rather until there was confidence that nothing remained in the sample). The organisms from each sample were then stored in a 70% ethanol solution until identification. Invertebrates were identified to

family level, following Quigley (1977, Savage (1989), Macan (1973), Edington and Hildrew (1981), Elliott et al. (1988) and Holland (1972).

Invertebrate taxa were assigned to Functional Feeding Groups following Merrit and Cummins (1996a). Additionally, to determine diversity (H) at each site, the Shannon-Weiner Index was calculated (refer to Chapter 2, equation 1).

5.4 Results

In total 42 aquatic macro-invertebrate taxa were recorded and identified (Table 5-3). The dominant taxa were Pleocoptera larvae: *Leuctridae* and *Nemouridae* as well as Dipteran larvae; *Chironomidae* and *Simulidae.*

Table 5-3 Taxa sampled indicating mean and total individuals found at sites. Highlighted are the four dominant taxa.

Species	Mean	Total	Species	Mean	Total
Ancylidae	0.113208	12	*Isopoda*	0.292453	31
Baetidae	8.924528	946	*Leptoceridae*	0.028302	3
Chironomidae	**19.12264**	**2027**	*Leptophlebiidae*	0.462264	49
Chloroperlidae	2.264151	240	***Leuctridae***	**24.01887**	**2546**
Cordulegasteridae	0.254717	27	*Limniphilidae*	1.990566	211
Corixidae	0.04717	5	***Nemouridae***	**31.63208**	**3353**
Dytiscidae adult	1.160377	123	*Neuroptera*	0.962264	102
Dytiscidae larvea	0.311321	33	*Noteridae*	0.028302	3
Elmidae adult	4.754717	504	*Odontoceridae*	0.018868	2
Elmidae larvae narrow	1.433962	152	*Oligochaeta*	2.783019	295
Elmidae Larvae wide	0.433962	46	*Perlodidae*	4.698113	498
Ephemerellidae	0.490566	52	*Polycentropodae*	3.584906	380
Gammarus	2.566038	272	*Psidium*	0.556604	59
Gerridae	0.179245	19	*Rhyacophilidae*	1.537736	163
Glossiphorudae	0.056604	6	***Simulidae***	**11.30189**	**1198**
Goeridae	0.056604	6	*Siphlonuridae*	0.018868	2
Hebridae	0.066038	7	*Succinea*	0.018868	2
Helodidae	0.896226	95	*Tipulidae*	2.764151	293
Heptegeniidae	2.216981	235	*Valvatidae*	0.056604	6
Hydropsychidae	2.584906	274	*Veliidae*	0.584906	62
Hygrobatidae	0.075472	8			

5.4.1 The role of inter-catchment variability

Differences between the two catchments of the study (e.g. subtle geological changes or overall catchment land-use variability) may exert a greater influence on invertebrate assemblage structure than other stream-specific environmental parameters (corridor characteristics and specific energy source availability), of interest in this study.

Analysis of catchments individually was undertaken to assess whether the invertebrate population response from each catchment could be pooled in respect to the environmental measures taken at each site. This approach was also used to explore both inter, and intra-catchment variation and reduce the impact to the analyses of potential confounding variables associated with large-scales variation

between catchments. This approach was taken to help highlight site specific variables, potentially overshadowed by the dual catchment analysis, yet still of importance to site-specific ecological relationships.

Using the statistical package CANOCO (Ter Braak, 1990), multivariate analysis (Canonical Correspondence Analysis: CCA) produced two catchment-specific ordinations. TWINSPAN classification (Hill, 1979) was used to define statistically significant groups of samples and/ or species, and to explore correlations in species assemblage groups. As there was significant variation in abundances of specific species, TWINSPAN analysis was modified from the default settings so that pseudo-species cut points were defined as densities of 0-1.9, 2.0-9.9, 10.0 – 39.9 and ≥40.0 to account for the variation in abundances. These cut points were used for all TWINSPAN analyses done within this chapter.

5.5 Cree Catchment

The outcomes of ordination analyses of the data for sites located within the Cree catchment are shown as CCA1 (Fig 5.1). Assemblage groups are defined using TWINSPAN. TWINSPAN groups are illustrated by sample dot colouration and identification of group membership can be found in Table 5-4 (for the Cree).

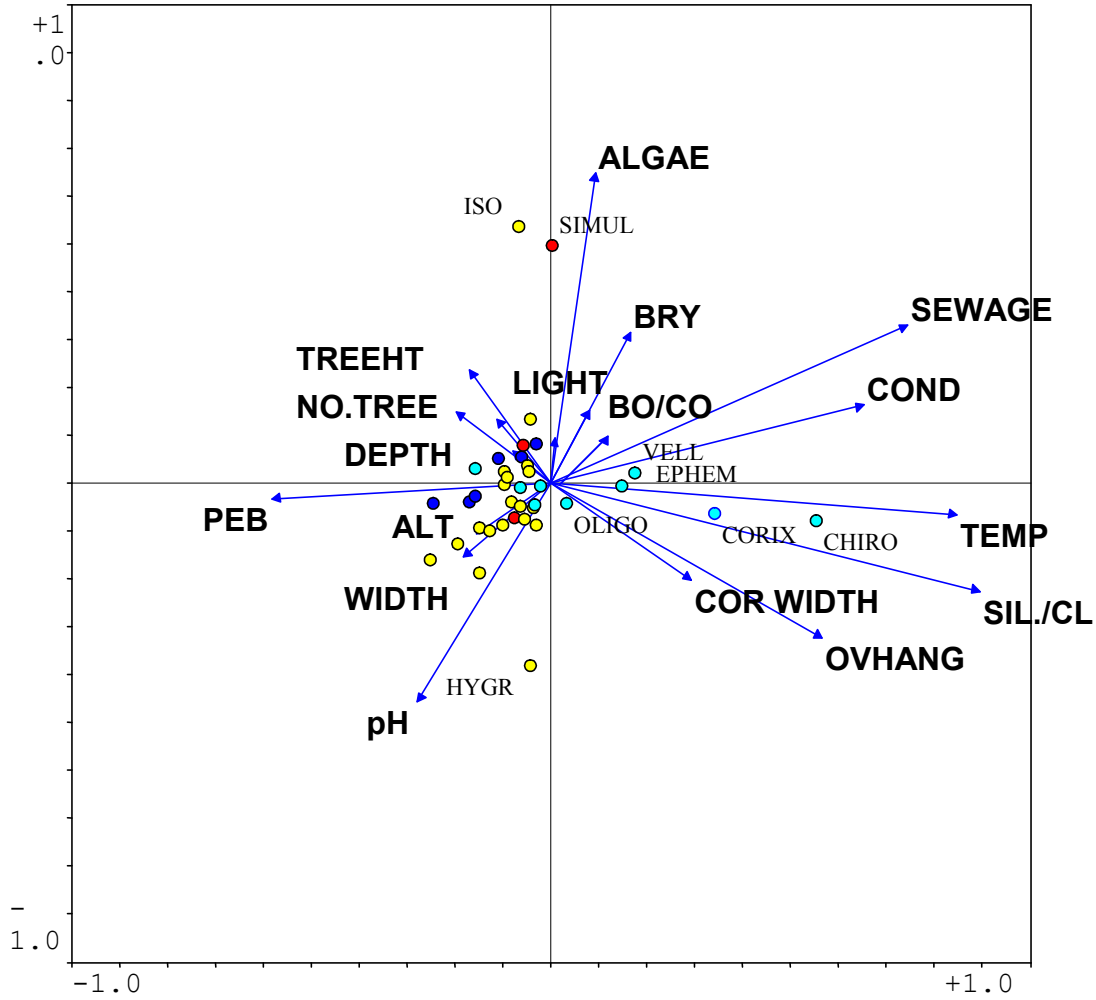

Fig 5.1. CCA1: species-environment ordination for samples located in the Cree catchment only. Colouration of dots indicates groupings as defined by TWINSPAN analysis. The contents of TWINSPAN groups are assigned in Table 5-4. A Monte Carlo test revealed the significance of the ordination (P = 0.005) with the majority of the variation explained within axis 1 (eigenvalue 0.648) and axis two (eigenvalue of 0.314).

Table 5-4. Species assemblage groupings as defined with TWINSPAN analysis associated with Cree-only CCA (Fig 5.1). Designation of groups 1 and 2 produced an eigenvalue of 0.361, and from groups 3 and 4, an eigenvalue of 0.423 was assigned.

Group 1 (yellow)	Group 2 (blue)	Group 3 (red)	Group 4 (light blue)
Ancylidae	*Baetidae*	*Hebridae*	*Chironomidae*
Glossiphorudae	*Leuctridae*	*Simulidae*	*Odontoceridae*
Perlodidae	*Elmidae* (larvae, narrow)	*Tipulidae*	*Corixidae*
Chloroperlidae	*Helodidae*		*Dytiscidae* (larvea)
Cordulegasteridae	*Heptegeniidae*		*Ephemerellidae*
Dytiscidae (adult)	*Hydropsychidae*		*Gerridae*
Elmidae adult	*Leptophlebiidae*		*Neuroptera*
Elminthidae (larvae, wide)	*Limnephilidae*		*Oligochaeta*
Goeridae	*Polycentropodae*		*Veliidae*
Hygrobatidae			
Isopoda			
Leptoceridae			
Nemouridae			
Psidium			
Rhyacophilidae			
Valvatidae			

Few species appear to be highly influenced by a single environmental variable. Instead, the majority of these samples appear to be associated with a wide suite of variables causing taxa points to be orientated towards the centre, rather than set out in the extremities of the ordination.

This analysis indicates that both Chironomidae and Corixidae occur positively correlated with axis one, and are associated with high values for attributes such as sewage fungi cover, silt/clay cover, conductivity, temperature, corridor width and percent riparian vegetation overhang. Isopoda and Simulidae are similarly positioned positively on axis two, which are associated with variables such as light, algae and bryophyte cover. Additionally, Hygrobatidae is negatively positioned away from these same variables and orientated more closely with increasing pH.

Overall, however, the majority of taxa are situated close to the centre, with the majority of taxa also negatively positioned against axis one. Table 5-5 illustrates the correlations of environmental variables with ordination axes. From the table, it is possible to suggest that the majority of taxa have a negative association with axis one (therefore associated with increasing stream width, depth, pebble cover, sand cover, pH, corridor tree diversity, tree height and altitude).

Table 5-5. Inter-set correlations of environmental variables of the Cree catchment with the ordination axes of CCA 1 (Fig 5.1). Significance of correlations indicated by eigenvalues. Only axes one (horizontal) and two (vertical) are illustrated within the CCA ordination.

NAME	AX1	AX2	AX3	AX4
LIGHT	0.0074	0.0774	-0.0992	0.1121
TEMP	0.824	-0.0558	0.1487	-0.2305
COND	0.6341	0.1336	0.1086	-0.112
WIDTH	-0.1769	-0.1262	-0.1099	0.0759
DEPTH	-0.0761	0.0539	0.315	0.2689
BO.CO	0.078	0.1256	-0.2023	-0.3712
PEB	-0.5654	-0.0284	0.1771	0.2045
SAND	-0.1925	0.1234	0.328	0.3804
SIL.CL	0.8707	-0.1882	-0.148	0.0397
pH	-0.2699	-0.3765	0.1945	-0.0096
ALGAE	0.0914	0.5327	-0.245	0.223
SEWAGE	0.7233	0.2725	-0.2879	-0.0765
BRYO	0.1626	0.2594	-0.0902	-0.1296
NO.TREE	-0.1091	0.1091	0.1407	-0.3139
OVHANG	0.5512	-0.2662	-0.1433	-0.1264
CORW	0.2859	-0.1679	-0.1829	0.2024
TREEHT	-0.164	0.1949	0.1708	-0.1341
ALT	-0.1506	-0.0865	-0.6002	-0.2298

5.6 Bladnoch Catchment

A species ordination plot for the Bladnoch catchment is shown in Fig 5.2. This analysis was of particular interest as it allowed species/community variation to be explored without the confounding variable of altitude, as sites from the Cree catchment were generally of higher altitude then the Bladnoch.

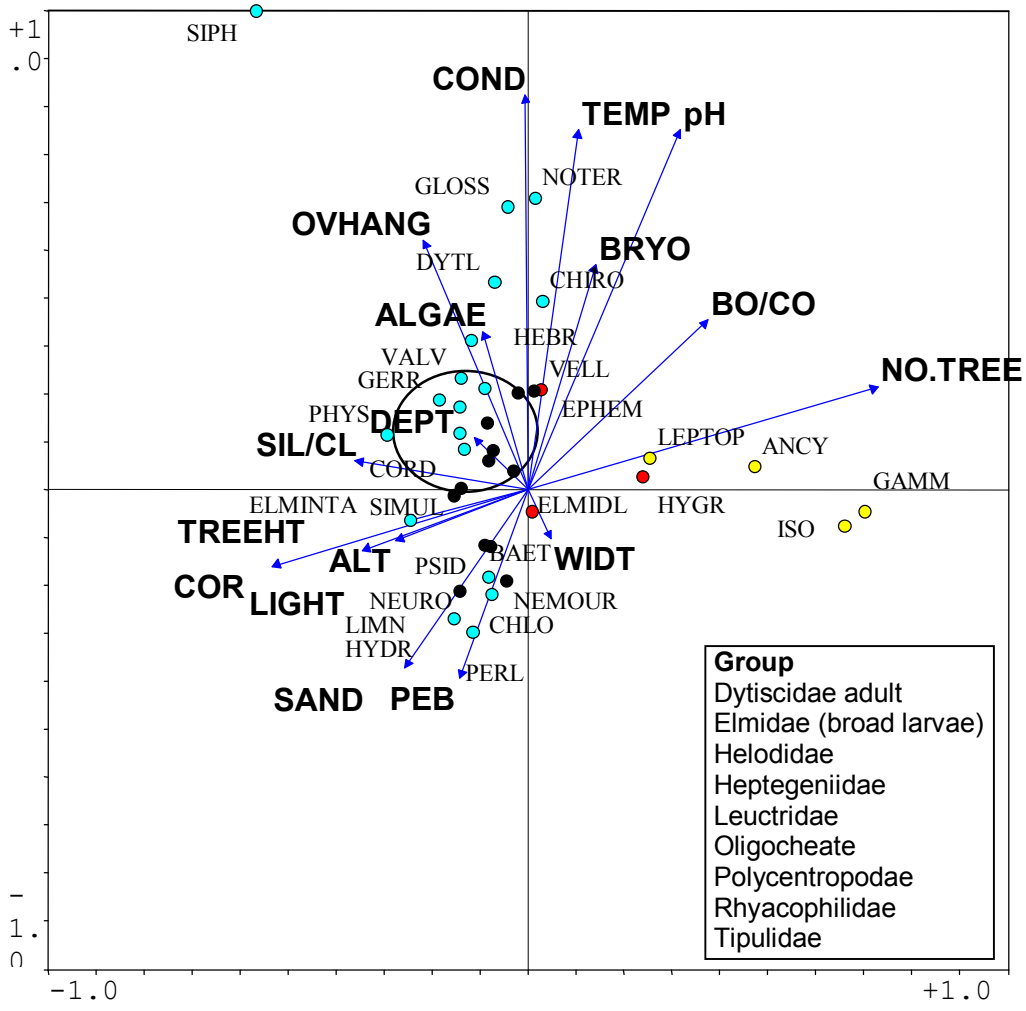

Fig 5.2. CCA2 indicating the relationship of Bladnoch only species with associated environmental variables. Colouration signifies TWINSPAN groupings as classified in Table 5-6 Taxa positioned within circled area have been identified at the corner of the ordination for clarity.

Table 5-6 Species assemblage groups as defined by TWINSPAN. Significance of group 1 and 2 delineation created an eigenvalue of 0.420. Delineation of groups 3 and 4 produced the eigenvalue of 0.595.

Group 1 (blue)	Group 2 (black)	Group 3 (red)	Group 4 (yellow)
Chloroperlidae	*Baetidae*	*Elmidae* (larvae narrow)	*Ancylidae*
Glossiphorudae	*Nemouridae*	*Ephemerellidae*	*Gammarus*
Polycentropodae	*Chironomidae*	*Hygrobatidae*	*Isopoda*
Cordulegasteridae	*Dytiscidae* (adult)		*Leptoceridae*
Dytiscidae (larvea)	*Helodidae*		
Elmidae adult	*Heptegeniidae*		
Elminthidae (Larvae wide)	*Leuctridae*		
Gerridae	*Neuroptera*		
Hebridae	*Oligochaeta*		
Hydropsychidae	*Psidium*		
Limnephilidae	*Tipulidae*		
Noteridae	*Velidae*		
Perlodidae			
Rhyacophilidae			
Simulidae			
Siphlonuridae			
Physidae			
Valvatidae			

A Monte Carlo test revealed that the significance of the Bladnoch ordination was lower than for the Cree ordination (which yielded P = 0.005) with a P value of 0.01. The majority of the variation within the ordination was explained along axis one (eigenvalue 0.530) and axis two (eigenvalue 0.341).

Consideration of the inter-set correlations (Table 5-7) indicates at the Bladnoch has a large number of environmental variables which influence the invertebrate community assemblage. For the purposes of this analysis, variables with an eigenvalue over 0.3 (or – 0.3) were deemed significant.

Table 5-7 Inter-set correlations of environmental variables of the Bladnoch catchment with the ordination axes of CCA 2 (Error! Reference source not found.**). Significance of correlations indicated by eigenvalues. Only axes one (horizontal) and two (vertical) are illustrated within the CCA ordination.**

NAME	AX1	AX2	AX3	AX4
LIGHT	-0.3337	-0.1255	-0.0088	-0.091
TEMP	0.1042	0.7415	0.0592	-0.3756
COND	-0.0068	0.8119	0.3815	0.1024
WIDTH	0.048	-0.1002	-0.1323	0.3708
DEPTH	-0.1093	0.1083	-0.0151	0.5015
BO.CO	0.3639	0.349	-0.1432	0.0287
PEB	-0.1378	-0.3879	-0.3049	0.2171
SAND	-0.2502	-0.3685	0.4144	0.302
SIL.CL	-0.3496	0.061	0.4443	-0.4256
pH	0.3092	0.7416	0.2503	-0.1585
ALGAE	-0.0904	0.3248	-0.0005	0.2591
BRYO	0.1377	0.4637	-0.2946	-0.248
NO.TREE	0.707	0.2116	0.2362	0.123
OVHANG	-0.212	0.5146	0.2267	-0.2226
CORW	-0.5179	-0.1567	0.1195	-0.4862
TREEHT	-0.2455	-0.0653	-0.1599	-0.0648
ALT	-0.2688	-0.1046	0.0517	0.2411

Using these analyses, it is possible to suggest that each of the groups defined through TWINSPAN appear to follow specific gradients of environmental variables. For example, group one, the largest of the groups, appears to follow a chemical gradient of conductivity, pH and water temperature. However, in addition, the majority of Group 1 taxa also seem situated close to increasing allochthonous and autochthonous energy sources (in the form of % algae and % overhanging vegetation). Group 2 is centrally located, and as such, much more likely to contain an assemblage with a generalist approach to both feeding and habitat requirements. However as a whole, group 2 is negatively associated with axis one, and thus more likely to be found in areas of increased tree height, increased cover of silt/clay and wider corridors with increased light. Groups three and four are much smaller in comparison to the previous two TWINSPAN groups and both are similarly positioned. These taxa follow a gradient dominated by increasing over-storey tree diversity.

5.7 Dual catchment analysis

5.7.1 Species-environmental variables ordination

Error! Reference source not found. shows the species-environment ordination plot (CCA3) resulting from the ordination analysis of both catchments combined. This permits examination of the wider relationships between individual taxa against the

gradients of environmental variables over a wider spatial scale compared with those recorded at each site. Related taxa assemblages have been defined using TWINSPAN classification (Table 5-8) and this analysis has been combined with information on functional feeding groups for each taxon, in order to examine the question of whether environmental factors might be related to feeding behaviour of the species assemblages present in the target streams.

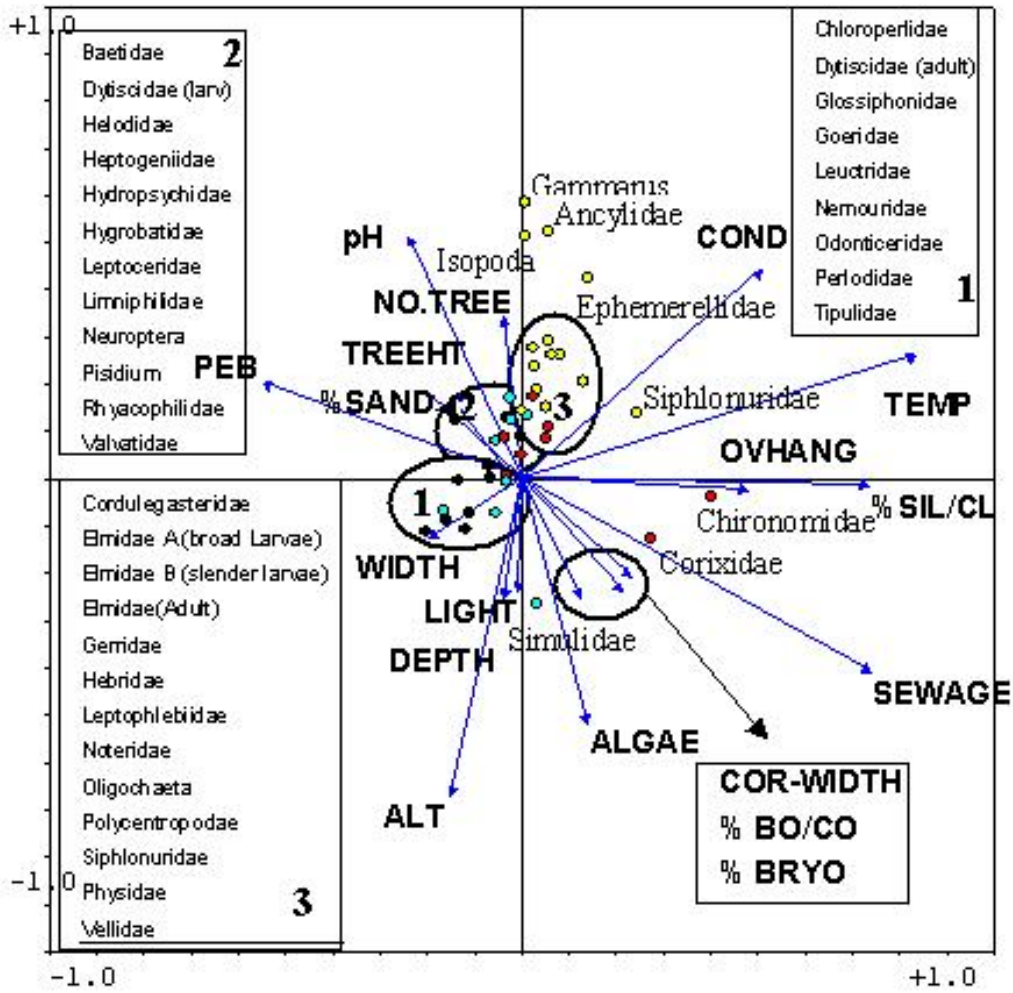

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Fig 5.3: CCA3 - Ordination of Species/Environmental Variables. TWINSPAN analysis of ordination assemblage has defined four groups. Coloration of dots indicates group classification. The defined contents of each group are detailed within Table 5-8. The majority of the variation in the ordination is explained in axes one and two. Axis one (horizontal) explained the greatest proportion of the variation in the ordination with an eigenvalue of 0.559. Axis two (vertical) has an eigenvalue of 0.303. Using the Monte Carlo permutation test, the ordination was found to be statistically significant (P= 0.005). Areas with closely positioned dots have been circled into groups (1, 2 and 3) and their contents labelled at the sides of the ordination to ease with identification taxa positioned within the centre of the ordination.

Table 5-8 TWINSPAN species classifications. The creation of groups 1 and 2 produced and eigenvalue of 0.354. Creation of groups 3 and 4 produced an eigenvalue of 0.330.

Group 1 (black)		Group 2 (light blue)		Group 3 (red)		Group 4 (yellow)	
Chloroperlidae	P	Baetidae	CG	Chironomidae	CG	Ancylidae	SC
Nemouridae	SH	Polycentropidae	FC	Oligochaeta	CG	Gerridae	P
Elmidae (adult)	SC	Dytiscidae (adult)	P	Corixidae	P	Siphlonuridae	CG
Goeridae	SC	Glossiphonidae	P	Dytiscidae (larvae)	P	Cordulegasteridae	P
Helodidae	CG	Heptegeniidae	SC	Hygrobatidae	P	Elmidae (larvae wide)	SC
Hydropsychidae	FC	Leuctridae	SH	Neuroptera	P	Elmidae (larvae narrow)	SC
Leptoceridae	CG	Limnephilidae	SH	Noteridae	P	Ephemerellidae	CG
Perlodidae	P	Simulidae	FC	Valvatidae	SC	Gammarus	SH
Rhyacophilidae	P	Tipulidae	P	Velidae	P	Hebridae	P
						Isopoda	SH
						Leptophlebiidae	CG
						Odontoceridae	SC
						Pisidium	FC
						Physidae	SC

The abiotic variables such as altitude, pH, conductivity, water temperature and proportion of small substrate size (specifically % silt/clay, % pebbles) have a greater importance in influencing assemblage structure as indicated by the increased length of the arrow. In addition, biotic factors such as percentage sewage cover and % algal cover show strong significance to invertebrate taxa variation.

Combining the TWINSPAN groups with Functional Feeding Groups (FFGs) defines the type of feeding mechanisms dominant with each of the four TWINSPAN defined assemblage groups. By combining this information with the environmental data (through CCA), the specific community structure and associated morpho-behavioural feeding adaptations can be assessed against gradients of changing environmental variables.

Table 5-9 shows all the taxa (mainly at family level, but with some genera included separately), and with life-cycle stages separately assigned, recorded during the project sampling period and their assigned FFGs.

Table 5-9. Taxa sampled and associated FFGs following Merrit and Cummins (1996). SH = Shredders, FC = Filtering Collectors, CG = Collector Gatherers, SC = Scrapers and P = Predators. Classes assigned using codes A = Arachnids, B= Bivalves, C = Crustaceans, G = Gastropods, H = Hirudinea, I = Insecta and O = Oligochaeta.

Taxa	FFG	Class Code	Taxa	FFG	Class Code
Ancylidae	SC	G	Isopoda	SH	C
Baetidae	CG	I	Leptoceridae	CG	I
Chironomidae	CG	I	Leptophlebiidae	CG	I
Chloroperlidae	P	I	Leuctridae	SH	I
Cordulegasteridae	P	I	Limnephilidae	SH	I
Corixidae	P	I	Nemouridae	SH	I
Dytiscidae adult	P	I	Neuroptera	P	I
Dytiscidae larvae	P	I	Noteridae	P	I
Elmidae larvae narrow	SC	I	Odontoceridae	SC	I
Elmidae adult	SC	I	Oligochaeta	CG	O
Elmidae larvae wide	SC	I	Perlodidae	P	I
Ephemerellidae	CG	I	Physidae	SC	G
Gammarus	SH	C	Polycentropidae	FC	I
Gerridae	P	I	Pisidium	FC	B
Glossiphonidae	P	H	Rhyacophilidae	P	I
Goeridae	SC	I	Simulidae	FC	I
Hebridae	P	I	Siphlonuridae	CG	I
Helodidae	CG	I	Tipulidae	P	I
Heptegeniidae	SC	I	Valvatidae	SC	G
Hydropsychidae	FC	I	Velidae	P	I
Hygrobatidae	P	A			

Interestingly, the most obvious observation which can be made from this data is that despite some gradients in potential food sources (e.g. % algal cover and % sewage cover) being important (as defined though the relatively long arrow length), all TWINSPAN groups (Table 5-8) appear to have a wide range in FFGs represented. The lack of distinctly dominant feeding groups within any one TWINSPAN group suggests that a wide range of energy resources is available along the environmental gradients on which the groups are positioned. This is of particular interest and relevance when considering the measured corridor conditions associated with variation in corridor management strategies. Increased light, corridor width or tree height does not appear to influence the community structure to the extent that any FFG becomes dominant.

Addressing taxa FFGs; with the exception of Ancylidae (scraper) and Corixidae (Predator), all the outlying taxa of the CCA ordination are either shredders or collectors. This would suggest detrital energy sources (primarily associated with

these guilds) are also associated with these environmental gradients with which each of these taxa is correlated. *Gammarus* and Isopoda are most closely positively correlated with riparian tree species diversity. As both these taxa are shredders, not unexpectedly, it appears that CPOM is the key source of carbon available within diverse broadleaf dominated streams. However negatively correlated with that axis is the Simulidae; a filtering collector resulting in confusion as to why this taxon appears to have a close association with the increasing gradient of algal abundance.

The lack of any dominant scraper feeding guilds present in the ordination diagram suggests that the algal cover is not the primary food resource assimilated. Instead, the remaining outlying taxa, positively correlated with axis one, are detritus based feeders, related to a set of variables which suggest more eutrophic conditions (as indicated by the higher levels of algal cover (ALGAE), sewage fungus cover (SEWAGE), temperature (TEMP) and conductivity (COND) as well as an association with sedimented substrates (SIL/CL) and a greater abundance of overhanging vegetation), yet none of the taxa appear from their FFG to utilise the autotrophic production often associated with eutrophic conditions.

5.7.2 Site-environmental variables ordination

Pooled catchment data was used to produce a CCA considering distribution of assemblages (as defined as the sites of which they were sampled from). Fig 5.4 combined CCA ordination with TWINSPAN (Table 5-10) in order to define comparable sites according to the assemblage of species within them. CCA4 (Fig 5.4) illustrates evidence of separation (and low level of group mixing) of TWINSPAN groups along environmental gradients.

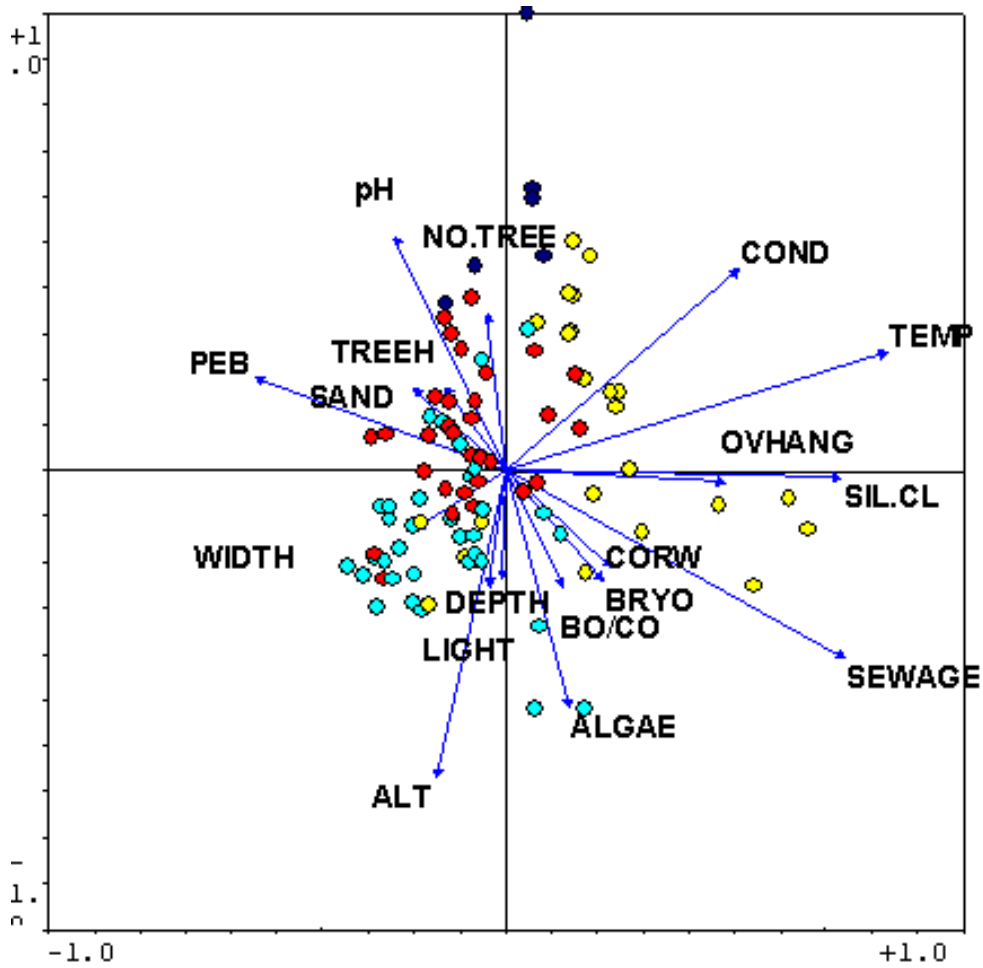


Fig 5.4. CCA 4 – Ordination of sites (as a function of the specific assemblages of species found at the site) with environmental variables. Colouration of site markers refers to TWINSPAN classification (Table 5-10) of invertebrate sample groups. Dark blue = Group1, yellow = Group 2, red = Group 3 and light blue = Group 4. For both the ordinations (CCA1 and CCA2), most of the variation in the ordination is explained in axes one and two. Axis one (horizontal) explained the greatest proportion of the variation in the ordination with an eigenvalue of 0.559. Axis two (vertical) has an eigenvalue of 0.303. Using the Monte Carlo permutation test, the ordination was found to be statistically significant (P= 0.005).

Table 5-10 Groups 1 – 4 (CCA1) defined by TWINSPAN analysis through differentiation of invertebrate species assemblages. Seasonal sampling point defined though lettering of months (J = July, M= March, N = November, and S = September) and year indicated by numbering (3, 4 or 5 for 2003, 2004 and 2005 respectively) Bladnoch catchment sites are highlighted in grey.

Group 1	Group 2	Group 3		Group 4	
SPPBRJ5	BBBS5	BUTBRN3	PCF2S4	AIRS5	BUTBRM4
SPPBRM5	BBMCFJ5	BUTOPN3	PCORN3	BBMCFM5	BUTOPM4
SPPSHJ5	BBMCFS5	GT1BRN3	PCORS5	BBMCOS4	GT1BRM4
SPPSHM5	BBMOPJ5	GT1BRS4	WOCS5	BBMOPM5	GT1COM4
SPPBRS5	BBMOPS5	GT1COJ5	AIRM5	BBMOPS4	GT1COM5
SPPSHS5	BBMSHJ5	GT1CON3	BBBM5	BBMSHM5	GT1SHM4
T33CF1S5	BBMSHS5	GT1COS4	FILIM5	BBMSHS4	GT1SHM5
T33SHS5	FILIS5	GT1SHN3	PCF2S5	BUTBRS4	LAGOPM4
	GT1SHJ5	GT1SHS4	T33CF1M5	BUTOPS4	LAGSHN3
	GT3J5	GT1SHS5	T33CF2M5	GT1COS5	PCF1M5
	PCF1J5	GT2N3	T33CF2S5	LAGOPS4	PCF1S4
	PCF2J5	GT2S4	T33SHM5	LAGSHM4	PCF2M4
	PCORJ5	GT3M5	WOCM5	LAGSHS4	PCF2M5
	PCORS4	GT3S5	PCF1N3	RBRJ5	PCORM5
	RBRS5	HMBN3	PCF1S5	ROPJ5	RBRM5
	ROPS5	HMBS4	PCF2N3	RBRS4	RBRN3
	ROPS4	LAGOPN3		RSHS4	ROPM5
	WOCJ5			RSHJ5	ROPN3
	AIRJ5			RSHS5	RBRM4
	BBBJ5			RSHN3	ROPM4
	FILIJ5				
	T33CF1J5				
	T33CF2J5				
	T33SHJ5				

The CCA ordination of sites (Fig 5.4) suggests that the sites are grouped not by site type characteristic (i.e., open, corridor, shaded, clear-felled, or broadleaf) but primarily by river site location. ANOVA testing confirmed that there was no significant difference in overall taxa diversity in any of the site types (n = 106, P >0.05). However, there is some evidence of seasonal changes in assemblage structure. Sites sampled in mid-summer are found more commonly towards the top of the CCA ordination and included mainly sample dates/sites from TWINSPAN groups 1, 2 and 3. Additionally, these assemblages also appear to be associated with low altitude, high over-storey tree diversity, higher pH, and higher conductivity.

Spring and autumn samples are found more commonly in TWINSPAN group 4 (situated towards the bottom left of CCA1). Group 4 is the largest of the groups and contains many of the sites within the upper Cree catchment. They therefore tend to be associated with greater altitude, and, interestingly, increased light. In addition the

occurrence of species associated with lower pH values is evidence for the presence of an indicator community characterised by acid tolerant taxa. TWINSPAN group 2 is interesting as it contains both Cree and Bladnoch sites. Group 2 sites are situated towards the right of the CCA ordination, and dominated by July and September samples. These species assemblages are therefore associated with increasing temperature, conductivity, and autochthonous/allochthonous production (% algae, % sewage and % overhanging riparian vegetation). Thus it can be inferred that these sites (and associated communities) have the highest level resource availability. The clear seasonal trends associated with the species composition ordinations suggest that seasonality is a confounding variable which needs to be considered when exploring relationships between invertebrate community composition and gradients of environmental change.

It is noteworthy that light is shown on the ordination as a relatively short arrow, indicative of an environmental gradient which plays only a minor role in constraining the ordination analysis, and hence likely to be of little importance in predicting the positions of site-groups within the plot. Therefore, it seems inappropriate to relate light intensity directly to autochthonous resource availability despite many studies suggesting direct and measurable influences by light intensity variability on in-stream algal abundance (Hill et al., 1995), and the highly influential role associated in defining macro-invertebrate community composition.

Overall analysis of the Cree and Bladnoch separately revealed that the Cree sites are of significantly greater altitude ($P < 0.001$) and have significantly lower mean pH ($P < 0.001$). In addition, overall diversity in terms of both species richness ($P < 0.001$) and Shannon-weiner index (H) ($P < 0.001$) was greater in the Bladnoch sites. Therefore, it appears that much of the variation in diversity across the two catchments was in response to chemical (pH) and physical (altitude) variables, on which corridor design modification cannot have a significant impact. The main drive of this study was to improve knowledge on forces driving diversity within corridor habitats and determination of optimal corridor design and management practices. Therefore, despite the need to increase sample size (and as a consequence, the applicability of the results to alternative geographical locations), analysis of each catchment in isolation, reduces the overall variance of any relationships and the effect of confounding variables (e.g. pH and altitude). As such, clearer information on

the role of more localised (corridor) physical variables is provided as the sites analysed collectively have similar chemical characteristics.

5.7.3 Functional Feeding Groups

Using invertebrate Functional Feeding Groups (FFGs) as indicators provides some information on the potential food sources available and the types of food sources being assimilated within each site. Overall abundances of individuals within the five main functional feeding groups (Groups and associated taxa are outlined in Table 5-9) for both catchments is shown here in Fig 5.5.

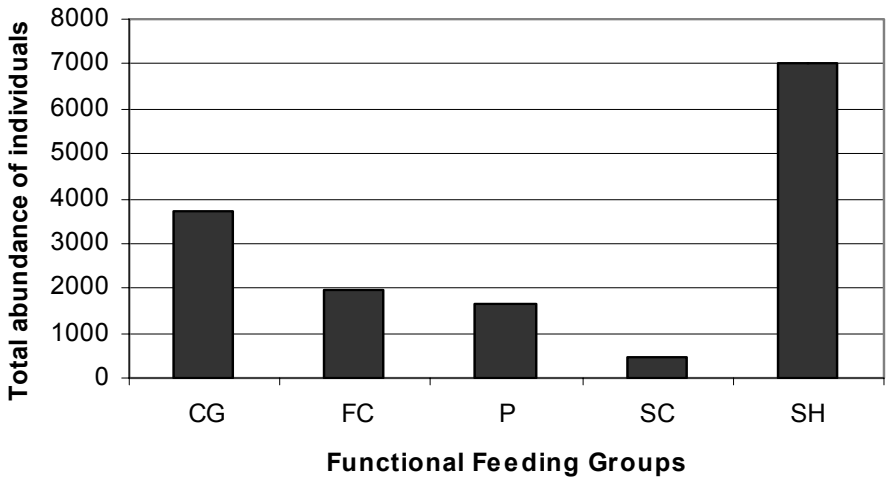


Fig 5.5. Total abundance of individuals within the Functional Feeding Groups. CG = Collector Gatherer, FC = Filtering Collectors, P = Predators, SC = scrapers and SH = Shredders

From Fig 5.5, shredders are significantly more abundant numerically than any other group (ANOVA, $P < 0.001$) with 7017 individuals caught in total and a mean per sample of 66.19 (± 0.6 S.E). The scraper guild shows the lowest mean relative representation (ANOVA, $P < 0.001$) compared with other guilds within the samples with a total abundance over both catchments and all sampling periods of only 473 individuals and a mean of only 15.5 (± 0.02 S.E) individuals per sample.

Collector Gatherers (CG)

Collector-gathers display a wide range of morphological and behavioural adaptations in order to collect and intake FPOM, predominantly from the terrestrial organic pool. The most numerically abundant taxa within this feeding group were the Baetidae and Chironomidae with 1072 and 2135 individuals respectively. The total abundance of these two taxa combined accounted for 86.1% of the individuals in the feeding guild.

Filtering Collectors (FC)

There were only four taxa present within this group, however, the group did include one of the most abundant of the taxa within the study; the Simulidae. The larvae of the black fly accounted for 62.7% of individuals within the group, with a total of 1234 larvae sampled.

Predators (P)

The predator group were, as expected for secondary consumers, higher up the food chain, numerically less abundant than primary consumer species. The most prevalent taxon was the Perlodidae with 534 individuals. Yet taxonomically, this group was the most diverse with a total of 15 taxa within the guild, as opposed to 8 taxa for CGs, 4 for FCs, 8 for SCs and 6 for SHs.

Scrapers (SC)

Scrapers are predominantly herbivorous species, often feeding on epilithic and epiphytic algal growths and biofilm material. Their utilisation of these food resources means that the suite of environmental variables which may influence their diversity and relative success, is likely to be very different to many of the other feeding guilds present.

In total 8 taxa were identified within the scraper guild, however, this was the least numerically abundant of the entire feeding guild with only 473 individuals found in total. The most abundant taxon represented within the guild was Heptegeniidae with a total of 244 individuals.

Shredders (SH)

Abundance of individuals within the shredder guild was the highest with a total of 7017. The two dominant taxa within this group were the Leuctridae and Nemouridae (Plecoptera) totalling 91.7 % of all of the individuals in this guild.

5.7.4 Regression Analysis

Multivariate ordination, as used above, allows us to establish testable hypotheses on the relationships between macro-invertebrate assemblage occurrences across environmental gradients. To explore these relationships in more detail, and to develop predictive models regarding the observed empirical relationships, regression analysis is an appropriate approach.

In order to increase sample size and widen the scope for application of any relationships, data from both catchments were analysed through regression analysis. Yet the varying responses in community assemblage meant that in order to maximise predictive power, only the environmental variables significant and common to both catchments were considered. Thus by using variables identified in the CCA ordinations (from inter-set correlation eigenvalues) (

Table 5-11), the role played in influencing the diversity of taxa (measured by the Shannon-Weiner index, H) within all sites was explored using regression analysis. Best-fit trend-lines were applied to data. Both linear and polynomial trend-lines were explored; the specific one used below is determined by which yielded the greatest r^2 value.

Table 5-11. Environmental variable significances defined through individual catchment CCA ordination correlations of greater than 0.3/-0.3. Common significant variables are used in linear regression analysis below.

NAME	CREE		BLADNOCH		BOTH
	AX1	AX2	AX1	AX2	
LIGHT	0.0074	0.0774	-0.3337	-0.1255	
TEMP	0.824	-0.0558	0.1042	0.7415	x
COND	0.6341	0.1336	-0.0068	0.8119	x
WIDTH	-0.1769	-0.1262	0.048	-0.1002	
DEPTH	-0.0761	0.0539	-0.1093	0.1083	
BO.CO	0.078	0.1256	0.3639	0.349	
PEB	-0.5654	-0.0284	-0.1378	-0.3879	x
SAND	-0.1925	0.1234	-0.2502	-0.3685	
SIL.CL	0.8707	-0.1882	-0.3496	0.061	x
pH	-0.2699	-0.3765	0.3092	0.7416	x
ALGAE	0.0914	0.5327	-0.0904	0.3248	x
SEWAGE	0.7233	0.2725			
BRYO	0.1626	0.2594	0.1377	0.4637	
NO.TREE	-0.1091	0.1091	0.707	0.2116	
OVHANG	0.5512	-0.2662	-0.212	0.5146	x
CORW	0.2859	-0.1679	-0.5179	-0.1567	
TREEHT	-0.164	0.1949	-0.2455	-0.0653	
ALT	-0.1506	-0.0865	-0.2688	-0.1046	

Interestingly, none of the riparian zone environmental variables, easily influenced by modification of corridor design, were found to be significant for both catchments in determining invertebrate assemblage structure. However, algal cover as estimated visually during sample visits arguably provides the best biotic link to the corridor characteristics; specifically light availability (as influenced by corridor design), as required for maximum algal photosynthesis and production (e.g. Allan at al., 1995).

However as seen in Fig 5.6, there is a negative polynomial relationship between increasing algal cover and invertebrate diversity (H). This is consistent with the results from Fig 5.3, where the majority of taxa are positioned away from a gradient of increasing algal abundance. This would suggest that the increase in autochthonous autotrophic production does not increase invertebrate diversity. As such, management strategies which aim to open up corridors and maximise light availability, may have the opposite effect on invertebrate diversity. The greatest diversity of invertebrates across both catchments appears to be found between the range of 15 and 40 % PAR. However the low r^2 value in the relationship indicates a large amount of variance in the relationship and relatively little power of predictability. This result may be due to the dual-catchment analysis approach. The low r^2 value lessens the applicability of this result for management purposes. However, despite the variance, this data indicates that high diversity can be achieved with very low levels of visually apparent algal cover. The result could either be related to discrete algal cover (thus, inaccuracy of estimation method), or an uncoupling of invertebrates and autotrophic biomass.

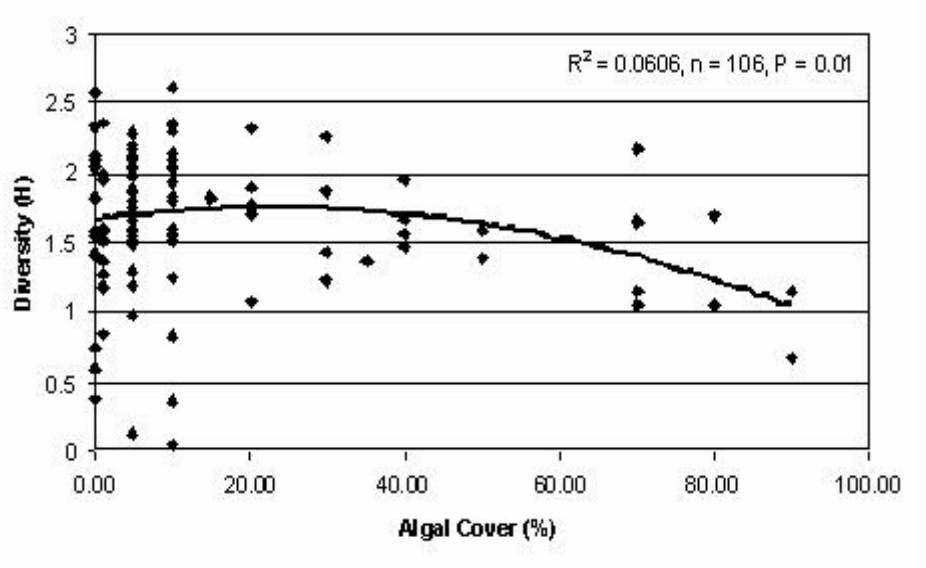


Fig 5.6. Polynomial curve describing relationship of estimated % algal cover and aquatic invertebrate taxa diversity (H).

Considering this data in conjunction with the designation of functional feeding groups to taxa; specifically the consideration of the relatively significant low number of taxa from within the scraper feeding guild (which are primarily herbivorous), strengthens

the suggestion that autotrophic production is utilised minimally. Thus, despite significant variability of autotrophic resource availability, and the comparatively high nutritional value of algal biomass, compared with allochthonous detritus (Taylor and Roff, 1984), algal cover does not appear directly to influence diversity or distribution of herbivorous benthic invertebrate taxa.

Therefore, it appears important to consider variables which will contribute to the quantity and quality of allochthonous resources (which appear to be the dominant resource utilised). As such, the functioning rate and capacity of many feeding groups will be dictated by the rate and efficiency of biological processing of the allochthonous material. Many breakdown processes are influenced by the abiotic conditions of the stream. The temperature of the system is important to processing rates (e.g. Hynes and Kaushik, 1969; Carpenter and Adams, 1979). Therefore, considering the role of stream temperature has obvious relevance to potential processing rate of all food groups and thus, potentially nutritional quality, and overall utilisation of allochthonous material (Rounick and Winterbourn, 1982; Bärlocher, 1985). From Fig 5.7, there is a highly significant ($P<0.001$, $n = 106$) polynomial relationship between taxa diversity and stream temperature. However the applicability of this data to reflect the corridor characterises and the role of shading on mean stream temperatures is questionable: such point samples are likely to be highly variable and potentially influenced by very short term temporal variability, not withstanding any season effects. However, despite this, many invertebrate taxa appear to be more prevalent in the more intermediate conditions (approximately $15^{O}C$) rather than either extremity.

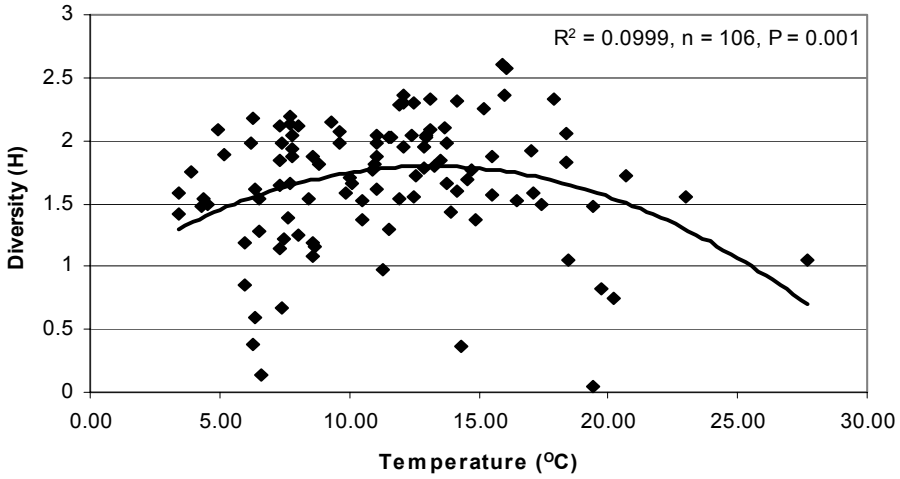


Fig 5.7. Polynomial relationship of invertebrate diversity (H) and water temperature (^{O}C) as measured on site during seasonal sampling trips.

From Fig 5.8, it is possible to see the positive linear relationship between conductivity and macro-invertebrate diversity (H). Although significant, the low r^2 value suggests that relatively little of the variation in this relationship is explained by the linear regression. Therefore, the predictive ability of any model produced using this relationship would be low. Further, it is likely that a number of alternative variables are influencing the diversity of invertebrate species across both catchments. The high variance of the relationship may be related to seasonal effects, as samples were taken throughout the year. However, the majority of invertebrate taxa have a life-cycle of at least one year (e.g. Lamouroux et al., 2004). Therefore, one would expect the community assemblage (and associated diversity) would reflect the mean habitat conditions present during the specific individual's life cycle, and not just the conditions of the sample period. This characteristic is one of the key benefits of using invertebrate sampling for water quality assessment as an alternative to spot measures of physical and chemical variables.

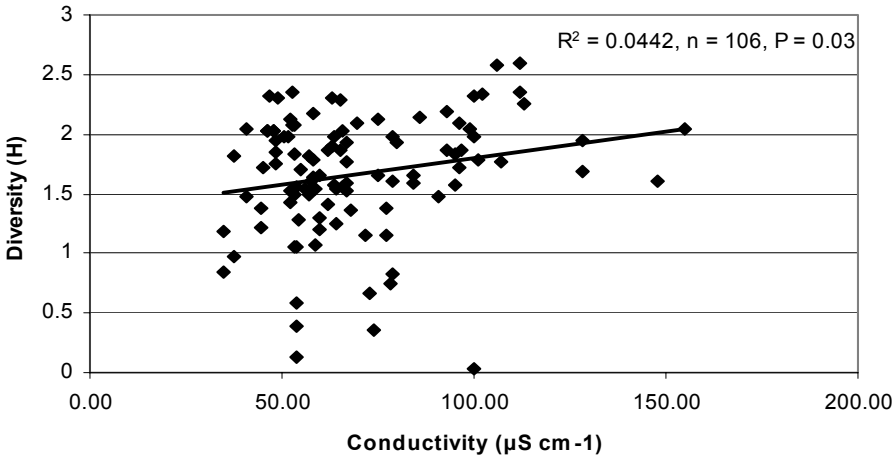


Fig 5.8. Positive linear relationship of macro-invertebrate diversity (H) and conductivity (µS cm^{-1})

Similarly, increasing pH appears to play a role in determining invertebrate diversity (Fig 5.9). The r^2 again indicates that much of the variation is not explained by this linear relationship, but, the relationship is still significant, with most diverse communities being associated with streams with a mean pH greater than 6.5.

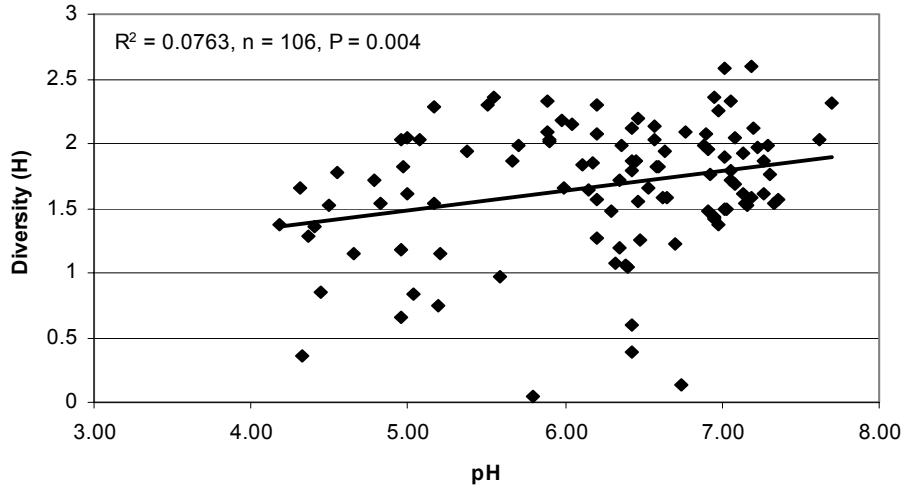


Fig 5.9 Influence of pH on invertebrate diversity (H)

Here Diversity of the invertebrate population was also found to have links with prevailing substrate size prevalence (Fig 5.10). A linear relationship derived for pebble cover ($P < 0.001$) suggests that there is a tendency for invertebrate diversity to increase with increasing prevalence of pebbles (16-64mm) in the substrate.

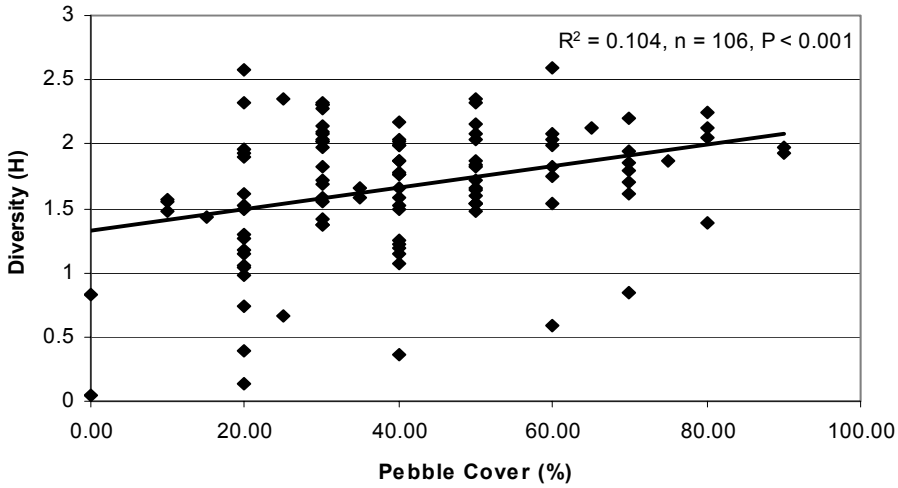


Fig 5.10 Positive linear relationship between estimated pebble substrate cover and benthic macro-invertebrate diversity.

The other variables which were deemed to be significant for both catchments (% silt/clay and % overhanging vegetation) were not found to be significant in any regression analysis performed ($P = 0.7357$ and $P = 0.82$ respectively) and therefore, appear not significant on a wide-scale, but rather on individual catchment-scale effects.

5.8 Discussion

5.8.1 The Cree Catchment

Analysis of the Cree catchment individually, suggested that the most significant variables to benthic invertebrates were temperature, conductivity, small sediment size (pebble/silt/clay cover), pH, sewage fungus, overhanging riparian vegetation, corridor width and to a lesser extent altitude. However, there is relatively little comparable significance in the role of the basic corridor features in explaining variation in benthic macro-invertebrate species assemblages. The most significant of the physical variables which one could describe as easily manageable, as far a forest corridor design features are concerned, appears to be mean corridor width. However even this still produced a shorter CCA arrow than many other variables, with a relatively low first axis correlation of less than 0.3. However, consideration of the increased significance of overhanging vegetation combined with increasing corridor width and increasing algal cover suggests that there is an influence gained from increased bank-side and in-stream light levels (to allow for this high primary productivity). However, the axis correlation for light individually is very low (Table 5-4). Therefore, the role of light in directly influencing invertebrate populations directly is questionable in the Cree catchment. This result is comparable to findings presented in Chapter 4; for autochthonous production: where corridor light levels could not always be used to predict autochthonous production. The results here suggest that on a catchment scale, the autochthonous production appears to be either light independent or not light limited.

However, as discussed previously, the autotrophic component of periphyton biomass is hard to assess visually and thus, without wide-scale stoichiometric and chlorophyll measures across the catchment sites, it would be difficult to assess accurately the food resource availability and thus predict invertebrate community response. Environmental agency habitat assessment often includes visual inspection of the algal and sewage content. The results gained here could be used to argue that although physical/chemical measurements of the corridor habitat alone provide limited information about resource availability, they are also not subject to the same visual inspection inaccuracies as visual inspection of periphyton/algal cover.

Many studies have shown that resource limitation within afforested catchments is the overwhelming factor involved in limiting diversity within similar habitats (e.g. Dobson and Cariss, 1999). However, within ordinations carried out in this chapter, the majority of taxa tended to be positioned in the opposing direction to increasing autochthonous production (algae and sewage fungus cover). However, in the case of the Cree and dual catchment ordinations, reduced correlation between diversity and autochthonous material may have been strongly influenced by the inclusion of the BB sites and the confounding effect of the felling event within the Black Burn sites in winter spring 2004/2005. Specifically, despite an increase of periphyton biomass (see Chapters 3 and 4) the potentially detrimental influence of habitat disturbance on community composition may have reduced coupling of autochthonous cover and invertebrate diversity, and skewed the ordination.

The felling event resulted in an abundance of organic matter availability within the benthos. Further, biodiversity and evenness dropped within BB sites. This sharp shift in community composition, habitat characteristics and associated reduction in diversity may have resulted in a negative correlation between most taxa and the highest in-stream periphyton cover estimations. Consequently, it is likely that the less important variables within the ordination may have been overshadowed by this event and were, in fact, likely be playing a greater role in influencing community composition, than was suggested by the ordination.

Although the Cree ordination may be of applicability at catchment-scale, invertebrate taxa diversity appears to correlate with increasing corridor width, tree diversity, increasing cover of intermediate sized substrates (pebbles), increasing pH, increasing stream width and increasing stream depth. However, as the lengths of these arrows within the ordination are low, it is unlikely that any of these variables strongly influence the community composition. Further, the complexity of the ordination, the short length of these positive associated gradient arrows, and the lack of any single dominating environmental variable, suggests high variance in any positive relationship between any single habitat parameter and invertebrate community composition. This result means that the predictive power of any one of the environmental variables mentioned above is low. Further, utilisation of these variables in modelling the response of corridor design on in-stream diversity is likely to have low powers of prediction and high variance.

As the majority of Cree taxa were positioned on the opposing side of the ordination to the variables of greatest influence (as illustrated by the longer arrow lengths) there was the suggestion that the majority of invertebrate diversity was negatively influenced by gradients of either autochthonous biofilm production or ground-flora vegetation. However, as the cluster of taxa is positioned away from the increasing gradients of these variables, there does not appear to be a specific limitation of resources driving the position of the invertebrate taxa. Regression analysis of algal resource cover and taxa diversity, (Fig 5.6) although significant, shows a polynomial relationship which did not suggest a specific limitation of autotrophic resources. Additionally although overhanging vegetation produced significant correlations within both catchments, the regression analysis proved insignificant. Thus, the direct release of allochthonous material from overhanging ground flora vegetation does not appear to influence invertebrate diversity either. However from analyses described in Chapter 2, this result was contradicted as invertebrate diversity was positively correlated ($P < 0.001$) with vegetation biomass from the bankside and 3 meter riparian zone sampling. This discrepancy between the two analyses strengthens the argument to suggest that the influence of the BB felling on the Cree ordination has confounded the relationships observed for the entire catchment. Therefore, consideration of the Bladnoch catchment in isolation may prove more useful in isolating the variables important to invertebrate community composition and diversity.

5.8.2 The Bladnoch Catchment

The isolated analysis of the Bladnoch catchment (CCA2 - Fig 5.2) indicated that the importance and influence of corridor features appears greater than was indicated in the Cree ordination. Focusing on the Bladnoch alone has revealed the importance of both corridor width and over-storey tree diversity in determining the community composition of benthic macro-invertebrates. In addition, the comparable spread of taxa across the ordination suggests that there is a greater diversity in the specific preferences of taxa to environmental gradients. Many variables appear to be closely related, for example, the chemical parameters all follow a similar gradient along axis one of the ordination. Further, many of the taxa appeared to follow this gradient of increasing pH, conductivity and temperature.

Temperature regulates the assimilation, respiration and overall populations of microorganisms processing detrital food resources, as well as modifying photosynthetic rates by primary producers and exerting direct control over metabolism of macroinvertebrates (Cummins and Klug, 1979). Due to this, it is difficult to separate temperature and food quality as regulators of invertebrate growth, population abundance and diversity. Thus within this project, variables which have the potential to change water temperature have additional importance in respect to the delivery and quality of food resources available to the in-stream community. Possible variables influencing temperature which were considered within this study include; season, shading by over-storey canopies and altitude. However, as the water temperature readings were point source measures, they are potentially subject to extreme temporal variation independent of spatial differences dictated by corridor cover and shading. The widespread applicability of the measure in this case is questionable. Analysis of season-specific sample points indicates clear seasonal separation of temperatures (Fig 5.11). This result adds to the evidence to suggest that pooling seasonal data causes an increase to variance. Further, the ability of invertebrates to integrate the influence of physical variability over their life span (often months, even years) may not be applicable to this study, as there is clear seasonal variation in diversity (even within a single year).

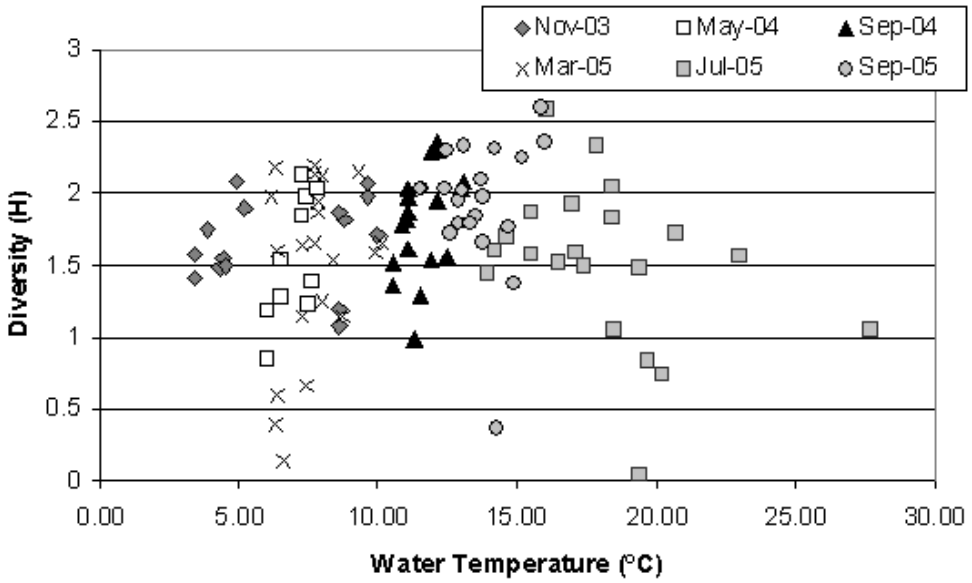


Fig 5.11. Polynomial relationship of invertebrate diversity (H) and water temperature (ºC) as measured on site during seasonal sampling trips. Seasonal effect indicated through isolation of sampling visits (as indicated on legend).

By pooling the seasonal sample data, a polynomial curve of temperature describes approximately 10% of the variation in the diversity (H) of invertebrate assemblage structure. The data suggest that the greatest diversity of invertebrates is found in the temperature range of 10-15^{o}C. Above this maximum, diversity drops, and therefore, despite the increased metabolism and food quality which would result from these higher temperatures, the optimal temperatures for supporting the highest invertebrate diversity were in fact, substantially lower than the maximum temperature of 27 $^{\circ}$C (from summer 2005). It is therefore suggested that the intermediate temperatures tend to support more communities based on both algal and detrital resources.

The disassociation between light and algal production and summer month invertebrate samples may be a reflection of the role of deciduous tree canopies in shading stream during mid summer months. As a consequence, production and the reliance of algal feeding groups may be greater during leaf senescence, in autumn to spring (and at lower temperatures/conductivity levels).

From the Bladnoch CCA (Fig 5.2), the importance of corridor features becomes apparent. Focusing on the Bladnoch reveals the importance of both corridor width and over-storey tree diversity in determining the community composition of benthic macro-invertebrates. These two variables are situated on opposing sides of the ordination, suggesting that the impact of forest diversity is increased with reduced corridor widths. There appears to be two main assemblages associated with increased tree diversity (namely TWINSPAN groups three and four and in particular the Crustacea taxa; *Gammaridae* and *Isopoda*).

Unexpectedly, both allochthonous and autochthonous production appear to be directly related as indicated by the closely positioned arrows of both algal cover and % overhanging ground-flora vegetation, suggesting that the abundance of overhanging vegetation is not in exclusion of autochthonous algal production. Unlike the Cree catchment where invertebrate abundance does not appear to be positively associated with autotrophic or allochthonous resources (or their delivery routes; i.e. overhanging vegetation), the greatest diversity of taxa (TWINSPAN groups one and

two) appears to be clustered in close proximity to both the main energy resource arrows.

Unlike the ordination produced with the Cree sites, most taxa appear to be positively correlated with increasing light and corridor width, thus, I can conclude that here there is evidence that algal resources are being utilised where available yet allochthonous resources are likely to be dominant overall as both algae and light have relatively short arrows and the majority of FFGs are not known for algal preferences.

Therefore, there is the suggestion of two separate community types, those with a preference for narrow corridors and high diversity of over-storey tree species (characterised by shredder species such as *Gammarus* and *Isopoda*), and those areas with wider corridors and well-established riparian ground-flora communities. The former group benefit from allochthonous inputs from overhanging vegetation and light intensities, supporting high algal cover and production levels and as such, support the most complex and diverse invertebrate assemblage structure with all feeding guilds represented. However, as overall there is a substantial amount of variation in the specific preferences of each taxon (as taxa are not positioned closely clustered to the centre of the ordination), it is difficult to define an exact optimal habitat for producing the most diverse invertebrate community. Thus, to maintain and maximise stream invertebrate diversity within a forested catchment, a diversity of habitat types which incorporate both open (with productive bankside ground-flora) and species-rich forest shade is required.

Substrate size and type has an influence on the type of invertebrate fauna supported and as a consequence, the relative diversity of substrate types (and associated microhabitats) available within a given area have substantial influence in determining the relative diversity and specific community composition of invertebrate fauna. Past studies have found a general correlation between increasing substrate size and higher diversity of invertebrate fauna (e.g. Allen, 1975). However, within this study, a significant linear relationship was found between increasing pebble cover within the stream and diversity (Fig 5.10). Additionally, the percentage cover of sand, silt/clay and pebbles all appeared to be positively associated with a large proportion of the taxa collected. Unlike past studies, % cover of boulders/cobbles cover was

orientated away from the majority of taxa of the Cree catchment. However, sampling error occurred here with the inclusion of bedrock in the boulders and cobbles category. Bedrock provides little shelter and is only important as a potential substrate for algal growth (Pennak and Van Gerpen 1947), thus confounding the results.

5.8.3 Pooled catchment data

Pooling the catchments revealed that the significant abiotic differences between the catchments appear to confound many of the small-scale corridor design variables which were highlighted in the Bladnoch ordination alone. The relative importance of these variables and specifically pH and altitude in dictating taxa diversity are highlighted in Fig 5.3.

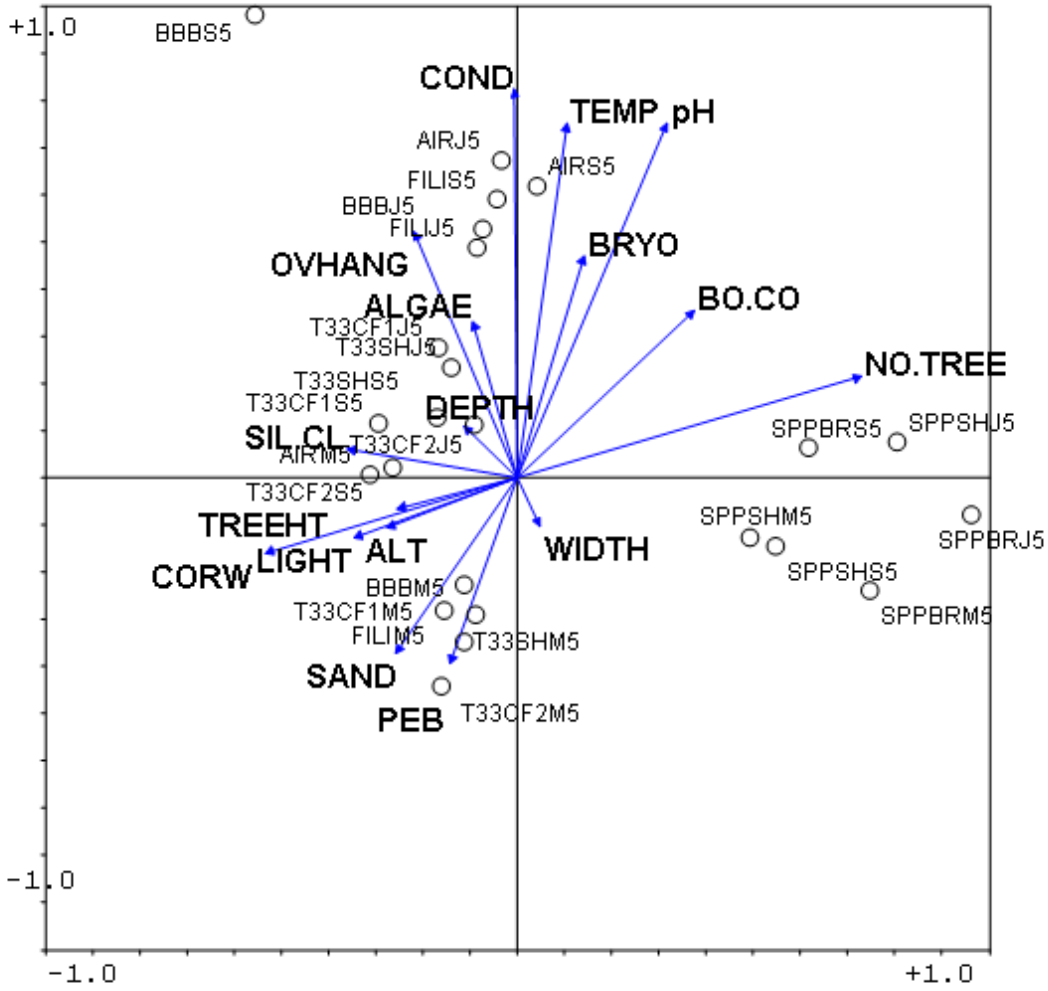
When catchments were pooled, the majority of species were situated towards the centre of the ordination and diagonally clustered along a gradient of both axis one and two. The orientation of the cluster suggests that species follow a gradient of stream width (although this variable has a shorter arrow), conductivity, pH, temperature and altitude. There are several distinct outlier taxa; to the right of the ordination, the dipteran taxa; *Simulidae* and *Chironomidae*, and the water boatmen (*Corixidae*) are all positioned such as to indicate associations with increasing levels of algae, sewage fungus, silt/clay cover and altitude. Referring to the site CCA (Fig 5.4), these species can be defined as primarily those of the Black Burn. All these taxa experienced a population explosion at BB, in 2005. The close association of these taxa with algal and sewage fungus abundance was likely to reflect a response to the felling event of the upper site in the chain BBCF (winter 2004/05) (described in greater detain in Chapter 7, clear-felling).

TWINSPAN analysis split the pooled assemblages into four distinct groups which separated themselves along the same diagonal gradient. However, there was no such specific separation of the FFGs associated with TWINSPAN groups. Thus, it appears that the distribution and specific assemblage structures present in the catchments, as a whole, was not directly influenced by either the biotic or corridor parameter, which control food type and availability. Rather, at this larger scale, the overwhelming control factors appeared to be the physiochemical components of the ordination.

Fig 5.4 relates the specific assemblages of species at a site with associated environmental variables measured at all sites within the two catchments. Similar site groups within the CCA were assigned using TWINSPAN (according to the similarity of the assemblage present). This dual analysis allowed for the relative similarities between catchment sites to be explored and to indicate whether site type, stream, catchment or season was most responsible for variation in the invertebrate community composition. Four main groups were identified and indicated evidence for both catchment separation and also temporal variation. For example, group one was exclusively made up of Bladnoch sites, and was orientated towards high pH and over-storey tree diversity. Group 2 was dominated by Cree sites and also summer sampling visits and, the associated increased in water temperature, % overhanging vegetation, sewage cover and conductivity. Co-correlation of these variables indicates increased levels of productivity within summer sample sites (associated with TWINSPAN group 2). However, as the algal cover arrow is in an opposing direction to temperature and the majority of summer samples, it is unlikely that autotrophic biomass is significantly increased in summer, or significantly limited in winter. The fact that summer samples and increased temperature cannot be directly related to increased algal production suggests that despite the potential for greater algal production during summer months, these assemblages are more influenced by the greater detrital quality and conditioning.

Group three was not separated by catchment location as sites of both catchments were grouped together. Instead, seasonal variation (only spring and autumn samples found) appeared to be more important in distinguishing the group from the rest of the ordination. Group 4 was similarly made up of primarily spring/autumn samples. Yet, there was also catchment specific variation as samples were Cree only. The seasonal separation of the sites was unsurprising given the fact that both these latter groups were associated with low conductivity and low stream water temperature. Within all the TWINSPAN groups there is a general mix of all corridor site types. As site types are not obviously grouped within the ordination, it is suggested the nature of the short scale changes in corridor design and the nature of riparian vegetation, has substantially less importance in determining community assemblage structure than the wider scale differences between the physio-chemical characteristics of different streams, and more especially different catchments. Consideration of

Bladnoch alone (Fig 5.12), removed the confounding effects of altitude, pH and the felling event, associated with the Cree. This analysis indicated that although there is separation of sites towards inter-river groupings, the majority of sites were still not distinguished by their specific riparian characteristics/site type. Therefore, it was not possible to suggest that the larger catchment-scale variables were overshadowing site-specific variation.



Error! Reference source not found.**Fig 5.12 Isolated analysis of Bladnoch site assemblages (Monte Carlo test (95% confidence) reveals significance of ordination: – P = 0.04).**

The role of coniferous forestry in causing or intensifying acidification in freshwaters due to their ability to intercept atmospheric acidifying pollutants is well documented (e.g. Harriman and Morrison, 1982). However, none of the CCA ordinations clearly illustrate this effect. Specifically, there was no correlation between increasing acidity and proximity of forest (narrower corridors). Therefore, the role of conifers here, in

accentuating acidity appears to be a general trend and not one which can be related directly to the specific design of the coniferous corridor. Thus, it is concluded that the acidifying effect is wide ranging and may be more a consequence of upstream and/or upper catchment acid deposition. Further, there is no evidence to suggest that modification of corridor design could influence, control or improve levels of acidification, despite numerous studies indicating a beneficial effect from riparian buffer zones.

The results obtained during this investigation are consistent with those of many other studies showing negative correlations between acidified streams and benthic macro-invertebrate biodiversity. Noteworthy, however, was that relatively high diversity was still found at remarkably low pH ranges. These results suggest an abundance of acid tolerant species are able to survive at pH levels down to 4.18. Sites where these low pH values were measured were all in the upper most parts of the Cree catchment. The *Simulidae*, *Corixidae* and *Chironomidae* were generally the taxa associated with the lowest pH ranges, suggesting a greater tolerance to acidity. Past studies have shown similar effects invertebrate communities. For example, Sutcliffe and Carrick (1973) and Townsend et al. (1983) both found that in studies of acidified streams, samples were often dominated by Diptera and Plecoptera species but rarely characterised by abundant populations of grazing invertebrates such as Ephemeroptera. Here, the results suggest that although Plecoptera taxa were not found to be more associated with acidified streams than non-acidified streams, these taxa remained numerically abundant in almost all streams. This was particularly apparent with Leuctridae and Nemouridae which were numerically very important within the study and showed no relationship with either altitude or acidity, suggesting a tolerance for changing gradients in both variables. Further, the densities of the algal grazers and scraper invertebrate species (particularly Ephemeroptera), are often found to be lower in areas of heavy shading by riparian trees when compared to open and treeless areas: Newbold et al. (1980); Gurtz and Wallace (1984); Behmer and Hawkins (1986); Dudgeon (1988). This phenomenon is commonly thought to be due to the lower algal abundance found in shaded streams. However, in upland areas of the UK with forestry plantations, this change in the assemblage structure is more commonly associated with low pH or increasing concentrations of dissolved aluminium (Harriman and Morrison, 1982).

5.9 Conclusions

Determining how corridor characteristics affect the biodiversity of in-stream biota is one of the key questions addressed by this study. ANOVAs of site types (i.e. corridor, open, clear-felled and broadleaf) against diversity proved insignificant. In addition, CCA ordinations separated sites in terms of catchment, season or a specific stream but not by site types within anyone stream system. In addition to this, the majority of variables related to corridor design (i.e. corridor width, tree height, tree diversity and % light) all had relatively shorter arrows than many of the other variables, with limited scope for large-scale manipulation. However secondary factors, often dependent on corridor design and associated with food availability and processing, were found to be of greater influence (e.g. % sewage fungus, % algae, % overhanging vegetation and temperature). Specifically, the results suggested that the variables which influence allochthonous production were the primary positive drivers of invertebrate community composition and distribution. Further, the variables which could be related to autotrophic production were negative drivers of diversity. Therefore, with reference to the functional feeding groups discussed previously, I suggest that species within these habitats have become adapted to energy resources dominated by detrital inputs. This shift in the community composition as a result of afforestation (either on site or upstream) has resulted in a relatively low dependence on autochthonous primary production. In addition to this, this survey and the results of the in-stream primary productivity studies (Chapters 3 and 4) have suggested that the availability of autotrophic resources is not significantly influenced by the immediate corridor characterises of the site. Therefore, one can conclude that even with modification to corridor design, the impact upon autotrophic resources is not predictable. In addition, even with a large range in algal availability within the sites measured, the utilisation of this resource appears low as the representation of grazing species within the community composition was minimal. Thus, to maximise biodiversity, the results mainly suggest that more benefit would be gained by maximising high quality allochthonous energy inputs, specifically with the increase of riparian over-story tree diversity.

6 Role of corridor characteristics in determining growth and survival of stocked Atlantic salmon (*Salmo salar* L.) and resident brown trout (*Salmo trutta* L.) populations within forested streams

6.1 Abstract

This chapter aims to investigate how aspects of conifer forestry riparian zone characteristics influence the survival of both stocked Atlantic salmon fry (*Salmo salar* L.) and resident trout populations (*Salmo trutta* L.).

Salmonid populations are of key importance and a target species due to their local economic and conservational value. The River Bladnoch catchment has been designated as a Special Area of Conservation (SAC) due to the importance of the Atlantic salmon population present therein. As such, Atlantic salmon were recently designated a priority species in the Dumfries and Galloway Local Biodiversity Action Plan (LBAP)

Within the spring and summer seasons of 2004 and 2005, a total of approximately 13,500 Atlantic salmon fry were planted out into the selected streams of both the Rivers Cree and Bladnoch catchments. In addition, natural fish populations were surveyed at all project sites during 2005. In this chapter, I seek to infer relationships between the growth and population density of juvenile salmonids, with variation of the physical habitat and corridor variables associated with conifer afforested stream systems. During this survey, all caught fish were identified and their fork length (mm) and wet weight (g) was measured. This allowed for the determination of growth and individual 'fitness'.

Low recapture success meant that statistical analysis of results was difficult. Light levels (PAR) were found to be the only factor which had a significant impact on the growth and fitness of both salmon and trout populations. Populations of both salmonid species appeared to fall into two population category scenarios: either comparatively more small fish within darker corridor habitats or fewer, but larger fish within lighter habitats. Significantly, although many chemical variables of forestry

streams have been found in the past to be the primary controls for buffer-poor catchments (for example pH and alkalinity; Dahl, 1927), these variables were not directly related to either population density of trout or the size class distribution of stocked salmon. Light-specific size-class separation is discussed in relation to individual and population fitness and the resulting potential consequences for the management and design of riparian zones.

6.2 Introduction

Most aquatic systems within the British Isles have at least some degree of influence from anthropogenic sources. The degree of this influence varies, yet some are degraded to such an extent as to cause deterioration of native fish populations (Hendry et al., 2003). This project concentrates on the impact of extensive land management in the form of widespread conifer forestry plantations on native Scottish fish populations.

Extensive investigation into the role of coniferous plantation has concentrated primarily on the role of increasing acidification within base-poor upland afforested catchments (Harriman et al., 1987). However, as management of forestry and forest streams is modified to reduce the impact of acid deposition (Forest and Water guidelines, 2003), this project considers how aspects of riparian design characteristics influence the survival of both stocked Atlantic salmon fry (*Salmo salar* L.) and resident trout populations (*Salmo trutta* L.).

Within this project, the fish species are the largest consumers studied. However, in addition to their importance ecologically as a top-down predator, both of the native salmonid species, Atlantic salmon and Brown trout, are very commercially important for both national fishing stocks and also on a more local basis for tourism and conservation (personal communication). Additionally, wild Atlantic salmon is an example of one of our native fish species in population decline (DAFS, 1983-84). Within this study, Salmon constitutes a target species due to its local economic and conservational value (e.g. priority Local Biodiversity Action Plan species), and represents a protected species within the Bladnoch catchment (key species within the Bladnoch SAC (Special Area of Conservation)).

6.2.1 Background

Catches of Atlantic salmon in countries which border the North Atlantic Ocean have declined rapidly in recent years (DAFS 1983-84). Significantly, Egglishaw et al. (1986) suggested a relationship between catch decline (Fig 6.1) and forestry plantations (Fig 6.2); suggesting that a number of effects of forestry activities, such as sedimentation, deposition of pesticides, increased evapo-transpiration from the

trees (this leads to reduced flows, making waterfalls harder to pass), increased woody debris (causing obstructions which can impede fish migration), acidification, shading and the loss of associated fauna and flora, were responsible for the decline in fish populations.

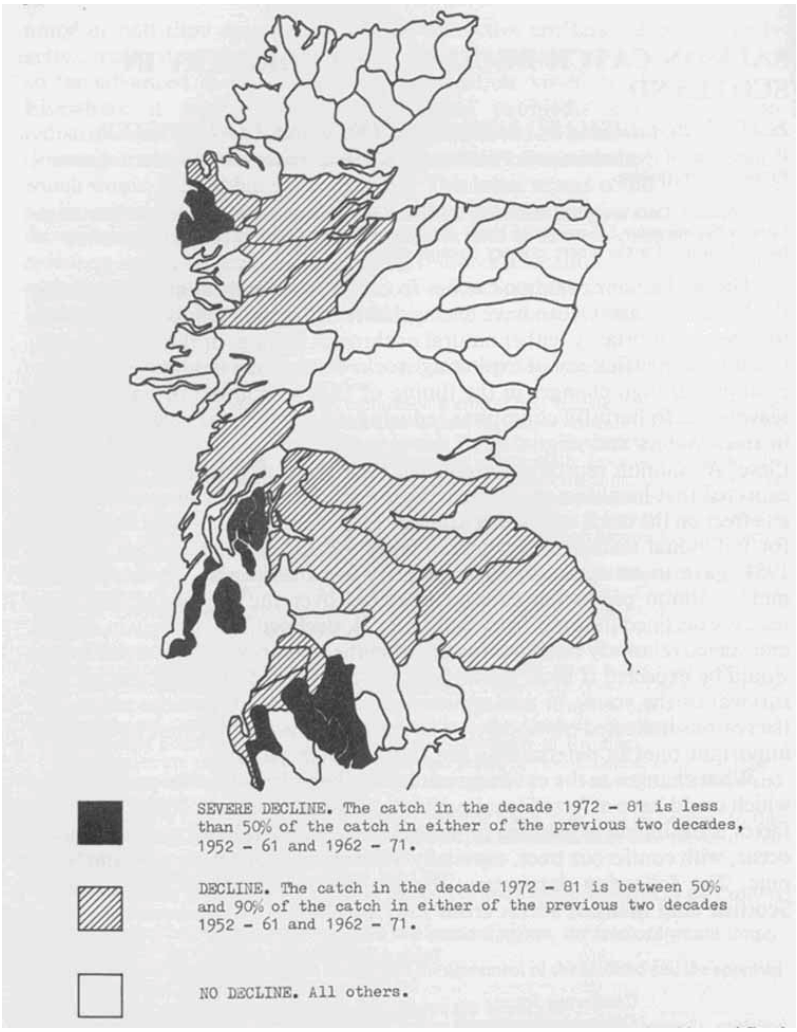


Fig 6.1. Trends in the catch by number by all methods (rod-and-line, net-and-coble and fixed engine) of Salmon of all sea ages (i.e. grilse and salmon combined), for the period 1952-81 for the 54 statistical districts on the Scottish mainland. (DAFS, Edinburgh 1983-84). Note that much of the Cree catchment in particular is in 'severe decline'.

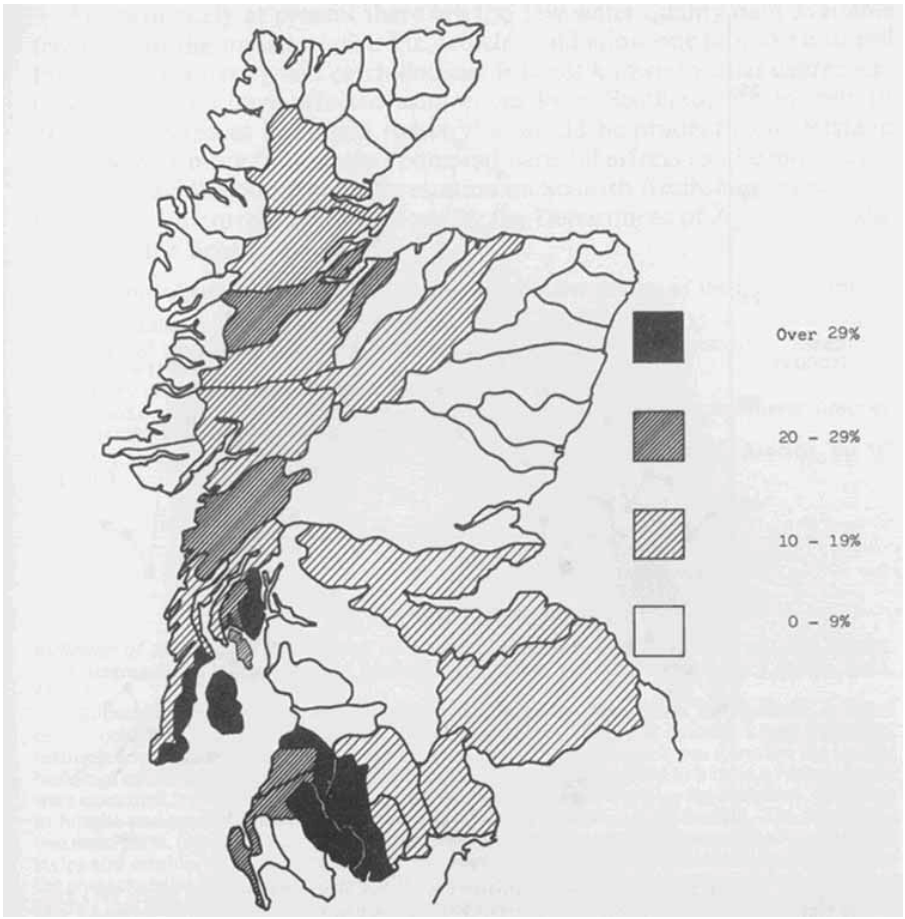


Fig 6.2. The proportion of forest (over 80% coniferous) found in the upland areas (altitude at least 183m) within salmon producing catchments, for the 54 statistical districts on the Scottish mainland in 1976.

Although chemical variation has been shown to be the major factor determining salmonid densities between rivers (e.g. Hesthagen et al., 1998), this project seeks to infer relationships dependent on variation of the physical habitat and corridor variables which surround river corridor design. However, the underlying chemical variation and specifically, the issue of acidity, cannot be ignored while interpreting data in a catchment known for its base-poor, low buffering capacity (Edmunds and Kinniburgh, 1986).

6.2.2 Acidification

The harmful effects of acid water on fish have been known for many years (Dahl, 1927). A pH range of 4.5-5.0 is likely to be harmful to the eggs and fry of salmonids (Alabaster and Lloyd, 1980). The decreased hatching success observed for Atlantic salmon eggs exposed to pH 4.5 and 5.0 in the present study has been reported previously (Runn et al., 1977; Peterson et al., 1980; Cleveland et al., 1986, Buckler

et al., 1995) and is related to reduced activity of the hatching enzyme chorionase (Haya and Waiwood, 1981). Additionally, in surface waters, a range of 4.0 - 4.5 is likely to be harmful to adult salmonids which have not been acclimatised to low pH values. However, resistance to low pH ranges increases with size and age (Harrison and Morrison, 1982), but the survival of the different stages of the life cycle can be influenced by the concentration of other ions, in particular aluminum ions, which cause toxic effects on the gill membranes and disrupt ion exchange mechanisms.

Freshwater fish, in unstressed environments, successfully regulate body fluid ion concentrations (Black, 1957; Evans, 1975), but as water acidity increases towards the lethal limit, the uptake of Na+ ions is strongly inhibited (Packer and Dunson, 1970). The transepithelial potential across the gill membrane changes from positive to negative thus allowing preferential absorption of hydrogen ions (McWilliams and Potts, 1978). A reduction in the permeability of the gill membrane to Na+ and H+ ions can be effected by increasing the calcium content of the water (Cuthbert and Maetz, 1972; Eddy, 1975). In natural waters where the pH is consistently below pH 4.5, it is likely that increasing mortality among eggs and fry will result in the reduction and eventual elimination of salmonid populations (Jensen, 1971; Carrick, 1979).

Consequently, studies exploring the effects of increased acidity of salmonid populations, suggest that population decline in acidified streams has been found to be primarily a consequence of recruitment failure of young (Hesthagen and Johnsson, 1998). There is thus the specific need to keep the low-order nursery and spawning streams in pristine condition, to maintain overall populations and reproductive success.

6.2.3 Riparian management

The specific characteristics of the riparian zone and in particular, the aquatic-terrestrial transitional zone (ATTZ), have been shown to have substantial influences on both the biodiversity and functioning of the aquatic and terrestrial habitats which it supports. Bankside vegetation influences not only the abundance and diversity of fish species, but also the success of differential life stages (Bilby and Bisson, 1992; O'Grady, 1993). Exploring work charting the relationship between riparian vegetation

and the aquatic zone highlights several important control factors including; food delivery, source and availability, cover for different fish species and different life stages, as well as temperature control.

However there still remains significant debate on the specific relationship between the type and quantity of streamside vegetation and fish production. Early work by Mundie (1969) demonstrated the importance of riparian vegetation in providing a food source in the form of terrestrial insects for salmonids. Leaf litter has also been shown to be an important habitat type for aquatic invertebrate production. However, Bilby and Bisson (1992), indicate that the fish populations in U.S. headwater streams are primarily supported by autochthonous production and so increasing incident light would increase fish stocks in affected streams. Therefore here, I address a number of differential habitat types within study streams in order to determine whether intra-site variation in over-storey tree type and canopy cover influences fish survival and growth.

O'Grady (1993) studied the effects of deciduous bank-side vegetation on salmonid stocks in Irish rivers. He found that the mean juvenile salmon density in shaded areas was only 19.4% of that found in areas of comparable habitat, which contained more open zones with dappled shade. Further, this reduction in mean density of salmon was attributed to the loss of aquatic plant cover such as epiphytic algae, mosses and aquatic macrophytes. However, O'Grady (1993) recommended only selective clearance of overgrown scrub, leaving partial shading to prevent over-proliferation of aquatic macrophytes, which may choke rivers, particularly in lowland and chalk streams with elevated nutrient levels. This debate over the relative merit of different levels of cover, combined with alternative dominance of either allochthonous or autochthonous baseline resources, means that in respect to conifer forest corridor design, it is important to determine which sets of conditions are most likely to promote fish growth and survival. The present investigation also seeks to integrate data collected in the previous chapter to determine any direct relationships between fish and benthic macro-invertebrates.

Hendry and Cragg-Hine (2003) suggest that riparian overhead cover is important in 'providing food and cover for juvenile salmon and other species'. They also emphasise the significance of riparian vegetation in maintaining bank integrity and as

a potential source of woody debris, which 'contributes to overall stream diversity'. However, studies concentrated within conifer afforested streams (e.g. Mills, 1969) note that afforested areas lacked bankside stability, leading to erosion and sedimentation.

Bjorn and Reiser (1991) warn against over-zealous removal of riparian vegetation cover, as this may result in excessive warming in summer by increased exposure to the sun, particularly in small streams. Indeed there have been many studies illustrating the use of bank-side riparian vegetation cover to reduce summer temperatures in streams reaching lethal levels for salmonids (e.g. Platts and Nelson, 1989) although the majority of these studies are concentrated in generally warmer climates.

Temperature is one of the main factors governing growth of juvenile salmon; it determines the date of emergence by fry as well as the length of the growing season, and as a consequence, determines the potential weight a salmon can achieve in any one year (Egglishaw and Shackley, 1985).

Elliott (1991) constructed a thermal tolerance polygon for juvenile Atlantic salmon, in which the ultimate lethal level for salmon parr was found to be between 30 and 33°C. This was found to be about 3°C higher than those of 26-30°C for brown trout (Elliott, 1981). In fact, the separation of the two salmonid species continues as brown trout are less affected by low temperatures because they cease feeding in the range 0-4°C whereas salmon cease feeding in the range 0-7°C depending upon the acclimation temperature (e.g. Gardiner and Geddes, 1980). In contrast, higher temperatures favour Atlantic salmon because they do not cease feeding until 21.6-22.5°C, whereas brown trout cease feeding at about 19°C (Elliott, 1981).

6.3 Aims

The primary aim of this chapter is to determine the factors influencing the survival of Atlantic salmon (*Salmo salar*) fry in selected afforested stream sites, in relation to habitat and corridor characteristics.

In addition, this study will consider differential energy resources likely to be most influential under a variety of environmental and riparian conditions, and determine

whether allochthonous or autochthonous resources are more influential in both the survival and growth of the stocked salmon.

Electrofishing surveys were used to examine native brown trout (*Salmo trutta*) populations in order to determine similar links with habitat and carbon source preferences as well as exploring any differences in these findings between the two key salmonid species.

It is hypothesized that variations in the availability of incident light as well as the specific characteristics of the riparian bankside habitat may have impacts such as:

- An increase in the volume of autochthonous production, which in turn might provide resources for invertebrates and therefore lead to an increase in the potential food available to salmonid predators

- Changes to temperature levels, causing either detrimental effects to feeding and survival if too high, or may reduce growth rates if not high enough. Further, there may also be variability in the results between salmon fry and brown trout fry.

- Variation in the population of terrestrial invertebrates contributing to the abundance of drift invertebrate resources available to fish populations.

- Reduced feeding success with shading, as fry are visually orientated predators.

6.4 Methods

Co-operation with the Galloway Fisheries Trust (GFT) permitted salmon population survival to be assessed by means of stocking-out and recapture experiments. The experimental release of salmon fry in Year One (2004) was confined to specific sites within the River Cree catchment. However, in Year Two (2005), the study was expanded to include some sites within the River Bladnoch Catchment (Special Area of Conservation - SAC).

6.4.1 Stocking of Salmon Fry

Approximately 13,500 Atlantic salmon fry were planted out over the two-year period into the selected streams of both catchments within the study area (Table 6-1). These sites were chosen on the basis that the study lengths were cut off from natural populations downstream by impassable obstacles in the river (waterfalls), thus excluding natural salmon populations from interfering or skewing the results of this stocking out experiment (Fig 6.3). Stocking was done in May 2004 and May 2005 at a density of approximately five salmon fry per m^2 at the locations indicated in

Table 6-2. Salmon were evenly distributed at each of the sites and also an additional further 50m upstream and 20m downstream, to account for the affect of migration in and out of the site, as following methods described by GFT.

Table 6-1. Stocking details for sites in years 1 (2004) and years 2 (2005) at a total of five streams and nine sites.

Stocking Site	Site Type	Total length of stocked site	Mean width	Stocking number	number stocked into 20m site	Salmon Fry retrieval	Salmon Parr retrieval
YEAR ONE				13/05/2004		20/10/2004	
Rowantree Upstream (SH)	Shaded	90	2.5	1125	250	1	N/A
Rowantree Middle (OP)	Open	85	2.1	892	210	4	N/A
Rowantree Downstream (BR)	Broadleaf	50	2.2	550	220	6	N/A
Pulnagashel Upstream (CF)	Clear Felled 1 (CF1)	90	2.9	1305	290	14	N/A
Pulnagashel Middle (CF)	Clear Felled 2 (CF2)	90	2.7	1215	270	10	N/A
Pulnagashel Downstream (CO)	Corridor (COR)	90	2.8	1260	280	33	N/A
YEAR TWO				02/05/2005		05/09/2005	
Rowantree Upstream (SH)	Shaded	90	2.5	1125	250	2	0
Rowantree Middle (OP)	Open	85	2.1	892	210	5	0
Rowantree Downstream (BR)	Broadleaf	50	2.2	not stocked	not stocked	1	0
Pulnagashel Upstream (CF)	Clear Felled 1 (CF1)	90	2.9	1305	290	20	1
Pulnagashel Middle (CF)	Clear Felled 2 (CF2)	90	2.7	not stocked	not stocked	6	2
Pulnagashel Downstream (CO)	Corridor (COR)	90	2.8	1260	280	34	5
Black Burn (Bladnoch) (CO)	Corridor	90	4.5	1575	450	1	0
Airies (Bladnoch) (SH)	Shaded	90	1.5	525	150	1	0
Fili (bladnoch) (CO)	Corridor (with BR. Trees within)	90	1.5	525	150	3	0

Table 6-2 . Locations of all the sites stocked in the Cree and Bladnoch in 2004 and 2005.

Cree	**Grid Reference**
Rowantree Broad	NX 35248 90688
Rowantree Open	NX 35662 90678
Rowantree Shaded	NX 34882 90848
Pulnagashel CF1	NX 37602 79738
Pulnagashel CF2	NX 37522 79748
Pulnagashel Cor	NX 37352 79078
Bladnoch	
Black Burn (B)	NX 28252 67292
Airies shade	NX 27527 67177
Fili	NX 28417 66347

Electrofishing surveys were carried out in October 2004 and September 2005, in order to measure the survival rates of stocked Atlantic salmon and native brown trout populations. The surveys were carried out following standard Scottish Fisheries Co-ordination Centre (SFCC) methodology. Stop nets (with a mesh size of approximately 20 mm) were placed at the upstream and downstream points of a 20 m stretch. This prevented any fish from escaping from the survey stretch. Three consecutive runs of the site were fished using a single anode and a DC driven generator (at the accessible sites) or a DC battery operated backpack system (at the more remote locations). The strength of electric current applied to the water varied depending on the conductivity of each individual tributary (which was measured on the day of the survey).

Fish caught during the survey were quickly transferred to a fresh bucket in order to recover. At the end of the survey, all fish were lightly anaesthetized, using a 0.1g L^{-1} solution of Benzocaine. Fish were then identified to species and their fork length (to nearest mm) and weight (0.1 g precision) was measured. Parr (1+) were

differentiated from fry (0+) using differences in markings and size category separation. These distinctions were made by a fisheries biologist who had undertaken a SFCC electrofishing training course. Once fish had recovered from anesthesia, they were returned to the site at the approximate area they were originally found.

Fig 6.3. Planting out Atlantic salmon into the Forested site on the Rowantree burn (May 2004)

6.5 Results and Interpretation

6.5.1 Environmental variables

Environmental characteristics of each stocking site are outlined in Table 6-3. The minimum pH measurements gained are illustrated in Table 6-4. Values were obtained during fish and invertebrate sampling visits at each site within 2004 and 2005. This information is included to determine if fish survival is possible with water chemistry at site.

Table 6-3. Environmental measurements of the salmon stocked sites (mean ± S.E). Cree sites measured throughout 2004 and 2005 (thus, n = 6). Bladnoch sites (FILI, BBB and AIR) only measured in 2005 (thus, n = 3).

	RBR	ROP	RSH	PCF1	PCF2	PCOR	FILI	BBB	AIR
Light (% PAR)	29.06 +/- 8.12	72.34 +/- 8.82	14.30 +/- 4.26	88.34 +/- 2.03	86.93 +/- 3.34	38.35 +/- 8.64	67.26 +/- 7.09	92.38 +/- 4.86	13.68 +/- 2.32
Conductivity (mS/m)	44.50 +/- 2.99	50.53 +/- 3.56	53.12 +/- 2.00	55.18 +/- 3.36	55.34 +/- 3.11	56.43 +/- 2.89	116.33 +/- 11.67	96.33 +/- 6.06	132.00 +/- 19.60
Wet width (m)	2.44 +/- 0.27	1.95 +/- 0.15	2.59 +/- 0.22	2.18 +/- 0.17	2.37 +/- 0.14	2.73 +/- 0.34	1.24 +/- 0.03	3.28 +/- 0.22	1.36 +/- 0.06
Depth (cm)	22.00 +/- 3.45	24.78 +/- 3.37	20.67 +/- 2.74	21.94 +/- 1.58	15.80 +/- 1.61	24.27 +/- 2.47	16.33 +/- 0.33	35.22 +/- 5.72	12.67 +/- 1.50
Bedrock (%)	30.57 +/- 3.21	50.08 +/- 6.63	15.93 +/- 7.85	0.00 +/- 0.00	7.35 +/- 4.26	15.00 +/- 3.58	0.00 +/- 0.00	15.67 +/- 7.25	0.00 +/- 0.00
Boulders/cobbles (%)	15.00 +/- 8.47	21.00 +/- 6.32	45.70 +/- 5.57	45.00 +/- 6.19	45.00 +/- 7.35	41.00 +/- 9.30	20.00 +/- 11.55	40.67 +/- 8.82	28.33 +/- 13.02
Pebbles (%)	50.83 +/- 7.79	30.00 +/- 6.83	38.00 +/- 6.63	40.00 +/- 7.30	32.00 +/- 4.90	34.00 +/- 7.48	40.00 +/- 15.28	33.33 +/- 3.33	55.00 +/- 10.41
Sand (%)	4.17 +/- 2.01	0.00 +/- 0.00	3.00 +/- 2.00	6.67 +/- 3.33	10.00 +/- 3.16	6.00 +/- 2.45	20.00 +/- 5.77	10.00 +/- 5.77	10.00 +/- 5.77
Silt/clay (%)	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	8.33 +/- 3.07	4.00 +/- 2.45	4.00 +/- 4.00	26.67 +/- 17.64	0.00 +/- 0.00	6.67 +/- 6.67
pH	5.75 +/- 0.46	5.92 +/- 0.44	6.19 +/- 0.48	6.18 +/- 0.12	6.14 +/- 0.11	6.21 +/- 0.07	6.82 +/- 0.18	6.98 +/- 0.21	7.06 +/- 0.35
Algal cover (%)	6.83 +/- 4.73	3.67 +/- 1.54	3.20 +/- 1.11	31.67 +/- 12.22	34.20 +/- 16.81	21.00 +/- 7.81	35.00 +/- 22.91	11.67 +/- 4.41	8.33 +/- 1.67
Sewage/fungi cover (%)	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	3.33 +/- 2.11	2.00 +/- 2.00	2.00 +/- 2.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00
Bryophyte cover (%)	12.50 +/- 4.23	15.83 +/- 3.75	13.00 +/- 3.00	12.67 +/- 3.48	7.20 +/- 3.29	20.00 +/- 5.48	3.33 +/- 1.67	30.67 +/- 14.62	5.00 +/- 2.89
Tree species richness	2.67 +/- 0.42	2.17 +/- 0.17	1.80 +/- 0.37	0.33 +/- 0.21	0.00 +/- 0.00	2.60 +/- 0.68	3.33 +/- 0.33	1.33 +/- 0.33	1.33 +/- 0.33
Overhanging veg (%)	1.00 +/- 0.82	7.67 +/- 2.73	3.40 +/- 1.03	8.50 +/- 1.50	20.20 +/- 5.30	0.60 +/- 0.40	40.33 +/- 19.67	10.33 +/- 5.49	5.00 +/- 2.89
Corridor width (m)	7.44 +/- 0.73	40.46 +/- 5.34	9.00 +/- 1.00	100.00 +/- 0.00	66.00 +/- 20.82	7.53 +/- 2.00	16.56 +/- 2.56	23.56 +/- 4.89	7.22 +/- 1.56
Tree height (m)	17.89 +/- 3.02	9.89 +/- 1.35	9.07 +/- 2.73	1.00 +/- 0.45	8.00 +/- 4.90	24.00 +/- 6.68	11.22 +/- 1.37	16.33 +/- 3.51	29.44 +/- 2.00
Altitude (m)	310.00 +/- 0.00	320.00 +/- 0.00	330.00 +/- 0.00	130.00 +/- 0.00	115.00 +/- 0.00	105.00 +/- 0.00	100.00 +/- 0.00	110.00 +/- 0.00	120.00 +/- 0.00

Table 6-4. Minimum pH measurements of the salmon stocked sites. Cree sites measured throughout 2004 and 2005. Bladnoch sites (FILI, BBB and AIR) only measured in 2005. Lowest value obtained at Rowantree Broadleaf, during March 2004. Alabaster and Lloyd, 1980 suggest that a range of 4.5 – 5 can be detrimental to salmonid fry if maintained for long periods. All Rowantree sites have suffered levels below 5, but this level is not reflected by mean values (Table 6-3).

AIR	BBB	FILI	PCF1	PCF2	PCOR	ROWBR	ROWOP	RSH
6.43	6.57	6.47	5.89	5.90	5.99	4.45	4.95	4.97

6.6 Basic population and growth analysis of 2004 fish

A total of 68 fish were caught in the electrofishing surveys carried out in October 2004 (Fig 6.4). The Atlantic salmon (*Salmo salar L.*) recovery totaled 54 fry, from the six stocked sites in 2004. In addition, 14 brown trout parr (*Salmo trutta.*) were also caught within the Pulnagashel sites. The highest single site abundances of both species were recorded in the Pulnagashel sites (Fig 6.4), with all trout and a total of 43 of the salmon recovered compared to only eleven salmon recovered at Rowantree Burn.

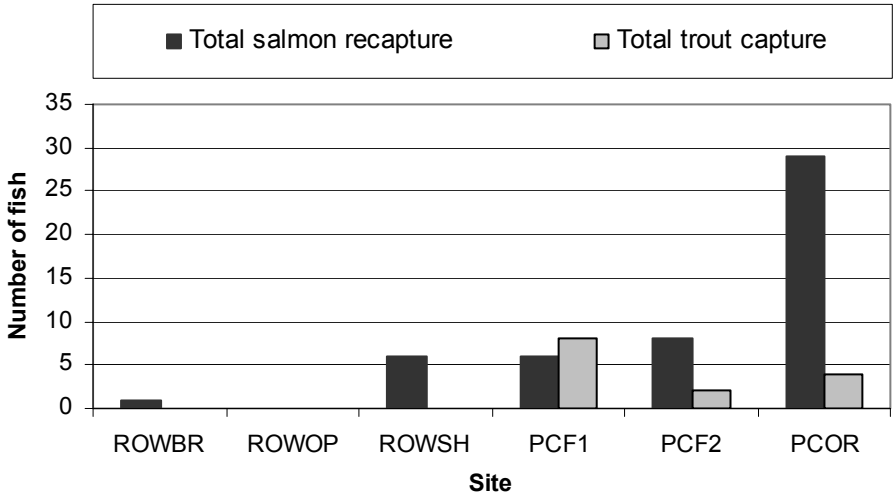


Fig 6.4. Total number of Fish caught (trout) or recaptured (salmon) in the two streams; Rowantree (ROW) and Pulnagashel (P) within the six sites (BR, OP, SH, CF1, CF2 and COR).

In order to provide some indication of the potential likelihood of salmon fry reaching a reproductive age (and thus potentially contributing to the overall health and biological functioning of the systems), it is important to consider factors other than simply the salmonid density. In order to further identify the fitness and health of the stocked salmon fry at each site, information on growth characteristics was collected, as the size of the fish often determines the likelihood of survival (e.g. Parker 1971; Juanes 1994; Elliott 1989*a*, 1989*b;* Thorpe 1977, 1989; Wright et al. 1990).

Salmon Fork Length

The results from the electrofishing surveys revealed that there was a difference in the growth rates between fish captured at different sites. It can clearly be seen (Fig 6.5) that stocked salmon recovered from the Pulnagashel Burn were larger than those recovered from the Rowantree Burn (ANOVA: $n = 54$, $P < 0.001$).

Additionally, there appeared to be inter-site differences. Results from the limited inter and intra-site specific variation in fork lengths achieved by fry (Fig 6.5), suggested that preferential growth conditions were present at all Pulnagashel sites, but particularly at PCF2 (ANOVA, $P < 0.001$). However with such low recapture rates, statistical analysis was limited to the sites with fish numbers = $n > 3$. Therefore, the results from RBR have been removed from statistical analysis.

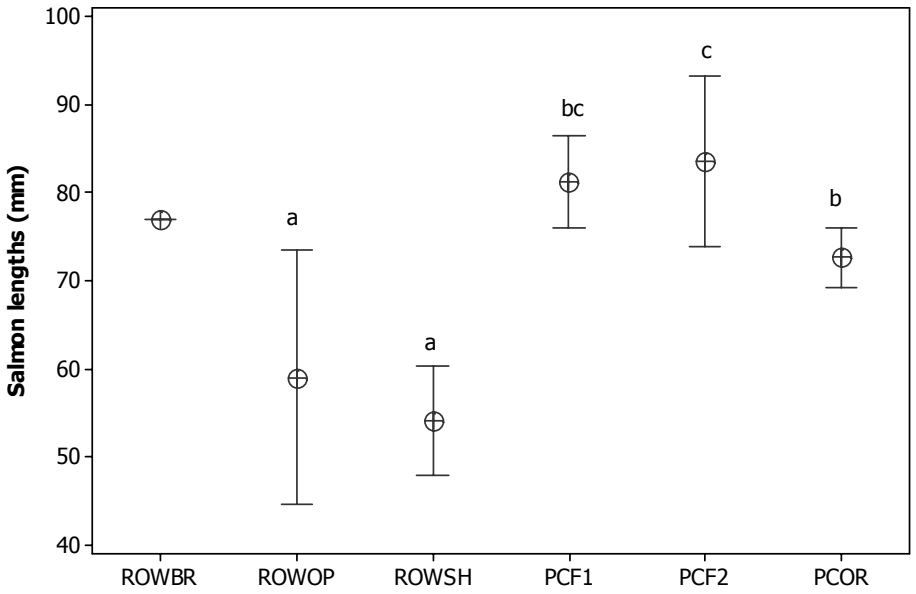


Fig 6.5. Mean fork lengths (mm) of salmon fry caught in October 2004 (± 95% confidence interval) at stocked sites. Differences in the letterings (a-c) indicate significant differences (Tukey test; P < 0.05) in salmon lengths of all sites minus ROWBR (where n = 1).

There is some suggestion of density dependence at Pulnagashal as although the greatest number of fish survived in the corridor site of the Pulnagashel (over the two clear-felled sites (Fig 6.4)), individuals from the Pulnagashel corridor site (PCOR), had significantly reduced body lengths (ANOVA, P = 0.007) than those fish recovered from within the two clear felled sites (CF1 andCF2) (Fig 6.5).

A total of only 11 fish (all Atlantic salmon) were recovered from all of the Rowantree sites (ROWSH, ROWOP and ROWBR). Fry appeared to survive best within the shaded site (which yielded a total of six fish) whereas only one fish was recovered at the broadleaf site. Interestingly this fish was the largest (in terms of fork length) of all recovered. This may have been due to high intra-site competition for resources, or the presence of a waterfall at the lower end of the site stretch (influencing movement out of the site). However due to the low survival rates of the salmon fry on this river, it is difficult to successfully apply any meaningful statistical analyses. Only the individual from the broadleaf site was large enough to be comparable with the lengths of the fry recovered from the Pulnagashel clearfelled sites (as it is within the 95% confidence interval of CF1 and CF2) (Fig 6.5). Individuals from both the

open site (ROWOP) and the shaded site (ROWSH) were significantly shorter than any of the Pulnagashel site Atlantic salmon. Significantly, as light levels at ROWOP and PCF1/PCF2 were all similar (Table 6-4), survival and growth (in terms of fork length) differences between site populations, can not be attributed to variations in light availability. Therefore, it seems likely that in-stream habitat quality as well as variation in water chemistry (specifically pH) is likely to be responsible for the variation between sites.

Salmon body weight

Salmon from the Pulnagashel sites were significantly heavier (P <0.001) than those recovered from Rowantree Burn (Fig 6.6).

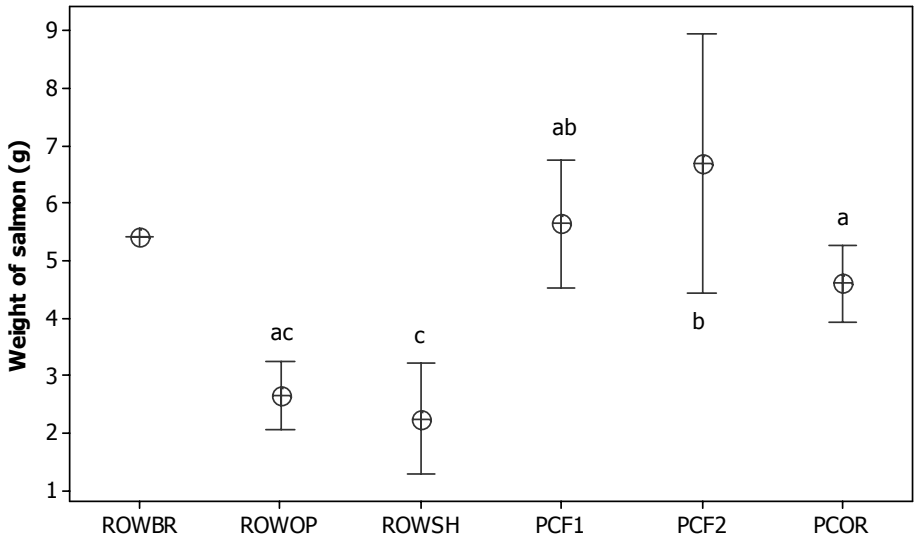


Fig 6.6. Mean wet weights (g) of salmon fry caught in October 2004 (± 95% confidence interval) at stocked sites. Differences in the letterings (a-c) indicate significant differences (Tukey test; P < 0.05) in salmon weights of all sites minus ROWBR (where n = 1).

Again, there was a trend occurring, with fry of greater weight occurring in the Pulnagashel sites (similar to the fork length data) (Pooled stream data – ANOVA, P <0.001). However, in this analysis, both the results from the first clearfelled site (PCF1) and the corridor site (PCOR), of the Pulnagashel are comparable for the data range of fry recovered from the Rowantree open site (ROWOP), indicating that weight ranges of these populations are comparable.

Weight gain is probably a better indication of overall salmon health, as it better demonstrates the feeding success of the fry (Elliott 1989a, 1989b). Further analysis

of the weight data from the individual sites indicates that within Rowantree Burn, fry found in the shaded Rowantree site were the lightest (Fig 6.6) and the shortest (Fig 6.5) of all the fry to survive. RSH fish had a mean weight of 2.25 g and mean length of 54.1 mm (however, it is also important to consider that the highest densities of stocked salmon fry in the Rowantree Burn were collected from this site).

2004 Brown trout data

A total of 14 brown trout were captured in the sampling period of October 2004. All the brown trout were confined to the Pulnagashel sites. The presence of a large impassable waterfall at the bottom of the lower Rowantree site, may account for the lack of resident trout population. The waterfall obstructing the Pulnagashel although impassable for salmon populations, may have allowed for a resident trout population to colonize during past high flow events.

The highest densities of brown trout were found in the uppermost clearfelled site (PCF1), with a total of eight caught. There were no trout fry (+0) caught; all individuals were identified as parr (1++). There was no significant difference between the fork lengths or weights of trout parr found at each of the Pulnagashel sites (ANOVA, n = 14, P = 0.998 and P = 0.919, respectively).

6.7 2004 Discussion

The presence of the brown trout combined with the significantly greater density, mean fork length and mean weight of the salmon reared in the Pulnagashel Burn, compared with Rowantree Burn (ANOVAS all, $P < 0.001$), all suggest that the conditions present at the Pulangashel were considerably more favourable than that of Rowantree Burn. The significant differences in both growth and overall survival of salmonids in the two stream systems suggest that there may be significant variation in either the in-stream or riparian zones influencing fish success. The reduced number of riffle habitat types as indicated by GFT staff, at Rowantree may have confounded any relationship between salmonids and riparian characteristics. The expansion of sites in 2005 and the removal of less suitable sites from 2004, was partly done to reduce this influence.

The unexpected presence of brown trout (although indicating the suitability of conditions for fish and providing a more long-term indication of favourable conditions), presented the potential for additional competition of resources at the Pulnagashel sites. In this respect, the results of Atlantic salmon density, fork length and weight are somewhat unexpected.

Furthermore, a substantial area of riparian zone upstream of PCF1 (the uppermost site) was felled in mid-summer of 2004. Following this event, there was some suggestion for increased nutrient loading of the sites downstream (evidence was provided by the notable increase in the observed algal growth - author's observations). This effect decreased noticeably at each downstream sampling station respectively (PCF2 and PCOR). However, during later sampling trips, there was evidence of large-scale and rapid decomposition (distinct, unpleasant smell – especially at the uppermost site (PCF1)). Similar incidents are described in a study by O'Connor (2002). Following a study in County Antrim, and review of the literature, O'Connor (2002) found that diatom frustules physically caused the thickening of the gill tissue and severe hyperplasia through the presence of diatoms within the gills of the young salmon. However, initial results do not suggest a hindrance to salmon survival or growth within this stream (or the site most proximate to the disturbance), as a result of forestry activities.

However, the original hypothesis of this project was that an increase in levels of autochthonous primary productivity would produce beneficial results to survival and densities of salmon fry. Although the autochthonous algal increase present at Pulnagashel was not directly attributed to a specific increased light regime (but rather to an upstream felling event), the nutrient increase was nevertheless transformed into autochthonous energy, as a result of adequate light availability in-stream at the two upstream clearfelled sites on the Pulnagashel Burn (PCF1 and PCF2). Consequently, despite the potentially detrimental influences of felling to the ratio of autochthonous to allochthonous carbon supply (chapter 4 and 7) and invertebrate consumer diversity (chapter 5), there was a clear increase in both the fork length (Fig 6.5) and bodyweight (Fig 6.6) of the salmon fry in the two clear-felled sites compared to all of the Rowantree sites (and to a lesser extent PCOR). However, despite the initial results from both Rowantree and Pulnagashel streams suggesting that growth (both length and weight data) of salmon may potentially be

limited by reduced autochthonous resource availability (specifically algal growth), the survival data alone, was not consistent with this pattern. A significantly greater number of salmon were recovered and thus survived in the reduced light level conditions of both the Pulnagashel corridor site (PCOR) and the Rowantree Shaded site (RSH) in 2004, over the more open alternative sites (Fig 6.4). This suggests that although the open sites may provide the conditions favourable for the production of larger and ecologically fitter individuals, it is also possible that the canopy cover provided within the corridor/shaded sites increases overall survival by either providing cover from visually orientated predators (both in stream and external) or through the contribution of increased allochthonous carbon to the base of the food web. Thus, there appears to be a trade-off between survival and growth of salmon fry within their first season.

There is debate over the comparative advantage to overall population survival with a scenario of either few larger fish or a greater number of small ones. It is generally thought that larger and faster growing juvenile fish are more likely to survive, with mortality being greater for smaller, slower growing fish. Smaller fish are more susceptible to starvation and predation due to their lower social status and predators of young fish are thought to preferentially seek out and consume smaller, more vulnerable prey (Parker 1971; Juanes 1994).

Additionally, larger, faster growing fish occupy and defend the most profitable territories in the stream, while slower growing fish are forced into less suitable habitats and may eventually die (Elliott 1989a, 1989b). This process may not only influence mortality rates, but also the life history patterns of surviving fish. Individuals that are superior competitors and thus grow fastest, have the potential to migrate to sea after only one year in freshwater, while slower growing fish are more likely to delay migration, remaining in freshwater for at least a second year (Thorpe 1977, 1989; Wright et al. 1990).

On the other hand, predators (e.g. herons) may preferentially choose larger juveniles, possibly due to their higher visibility and the larger net energy gains associated with consuming larger prey (Pepin and Shears 1995; Gleason and Bengtson 1996*a*).

The data collected in 2004 provides some initial indications and hypotheses of possible trends relating fish survival to environmental conditions, however, with such a limited data set, the statistical power and answerable questions, are limited. Attempting to designate specific variables responsible for the survival and growth of fish species within such a limited pilot-study dataset would likely be misleading. Therefore, in order to better explore some of the issues and questions relating fish (specifically focusing on the conservational key salmonid species) to on-site conditions and specifically light levels and corridor characteristic, expansion of the data set (both area and timescale), should provide this study's findings with greater confidence and applicability.

6.8 Fish data from 2005

In 2005, site locations were expanded and modified. From the sites stocked in 2004, two sites were removed (RBR and PCF2). This was due to the salmon stocking number restrictions combined with the requirement to include representative Bladnoch sites. RBR was excluded due to its proximity to a waterfall, whereas PCF2 was closely replicated by PCF1 in 2004 and as such, it was felt that it was the most suitable to be sacrificed. A further three sites within the Bladnoch catchment were added to the stocking experiment (BBB, FILI and AIRIES). Within sites surveyed in 2005 (Table 6-1), approximately 7,200 Atlantic salmon were planted out in May 2005. From this initial stocking, only 73 salmon fry were recovered in October of the 2005 season. In addition to the stocking results, all other 2005 sample sites (unstocked) were also surveyed in order to determine the diversity and abundance of naturally occurring fish populations.

6.8.1 Salmon data 2005

Fig 6.7 displays population density of salmon fry and parr recovery within the 2005 sampling season. Again, the Pulnagashel sites had the highest recapture success. No fry from 2004 survived within the Rowantree burn to become parr. However as opposed to the 2004 initial discussion, the density of fry within Rowantree Burn was highest at the open site. Again, PCOR yielded the greatest population density of fry and significantly, yielded the greatest survival of fry stocked from 2004.

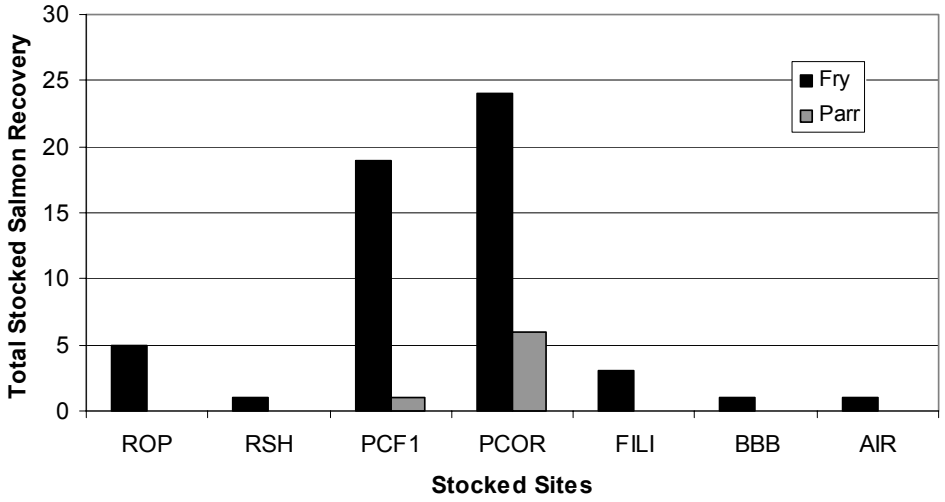


Fig 6.7. Recovery success of Salmon fry and parr at the stocked Cree and Minnoch sites from 2005.

The appropriateness of applying salmon density variation data for measurement of habitat preferences is debatable. Sites were chosen due to their biological isolation and therefore, using the presence of salmon as an indicator of optimal salmon conditions is questionable as their positioning is not through natural reproduction and survival processes. Survival should provide some information on comparable preference of stocked habitat conditions. However, I felt that by concentrating on size data (fork length and wet weight), more information could be gained on the biotic variables associated with variation of fry size, and such as, the long term potential for survival and breeding success. Food availability is likely to be an important control parameter for growth and survival. Table 6-5, indicates the mean invertebrate diversity and abundance data for the stocking sites.

Table 6-5. Mean ± SE diversity and abundance of invertebrate taxa within stocking sites for all sample visits (2003 – 2005).

Site	H	No. Spp	Total Abundance
AIRIES	1.83 ± 0.12	14.66 ± 1.33	159 ± 48.64
BBB	1.87 ± 0.13	13 ± 3.05	211 ± 103.17
FILI	1.94 ± 0.14	11.66 ± 1.45	126.66 ± 35.09
PCF1	1.86 ± 0.22	14 ± 1.01	115.2 ± 31.48
PCF2	1.79 ± 0.16	11.33 ± 0.61	124.33 ± 28.51
PCOR	1.83 ± 0.15	9.2 ± 1.31	37.6 ± 8.7
RBR	1.35 ± 0.22	8.16 ± 0.60	63.66 ± 20.18
ROP	1.37 ± 0.22	9 ± 0.77	130.33 ± 53.68
RSH	1.39 ± 0.33	7.8 ± 0.96	70.8 ± 28.86

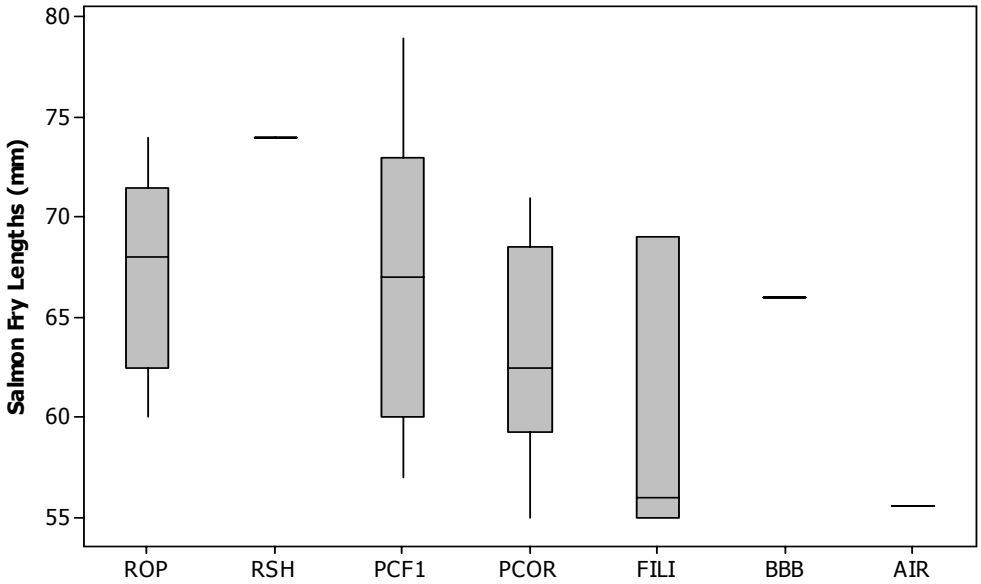


Fig 6.8. Boxplots of salmon fry fork lengths (mm) recovered from 2005 stocked fry. Plots illustrate median ± 1st and 3rd quartile and upper and lower limits. No significant differences between samples where n > 3 (ANOVA, P = 0.194).

However, Fig 6.8 indicates that there was no significant difference in the fork lengths attained by salmon fry at ROWOP, PCF1 and PCOR sites in 2005. The greatest lengths were found at PCF1 sites, adding to the hypothesis of a trade-off between survival and growth of salmon fry within their first season. However as recovery of individuals from some of the sites was very low (n = 1 at AIR, BBB and RSH and n = 3 at FILI), making reasonable conclusions about size-class distributions is difficult. This variation in growth between sites may be due to invertebrate food availability (Table 6-5). Low abundance of invertebrate individuals with PCOR and RSH may have contributed to the low fork lengths of low abundance found at these sites throughout 2004 and 2005.

When wet weight is considered, a similar pattern occurs (Fig 6.9). Again there is no significant difference in the mean wet weights achieved from fry at any of the stocked sites where n > 3, within 2005 (ANOVA, P = 0.325).

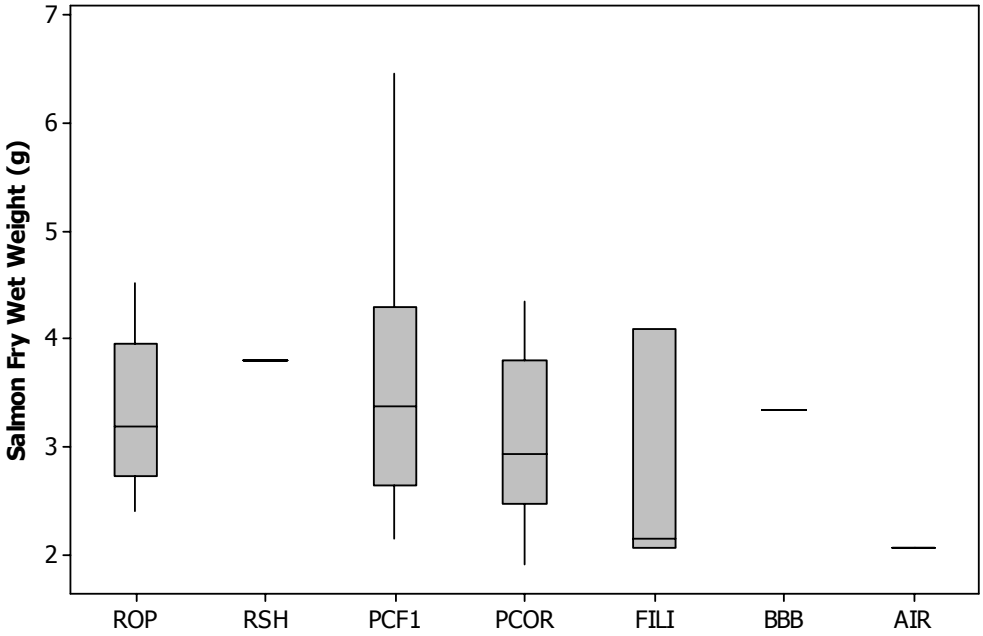


Fig 6.9. Boxplots of salmon fry fork lengths (mm) recovered from 2005 stocked fry. Plots illustrate median ± 1st and 3rd quartile and upper and lower limits. No significant differences between samples (ANOVA, P = 0.325).

Multivariate Analysis

Canonical Correspondence Analysis (CCA) was utilized for analysis of the 2004 and 2005 fish data. Salmon fry were separated into size classes in order to delineate the environmental variables responsible for different growth responses. Further, natural populations of trout (two size classes - above and below 100mm) and eel were included to determine any correlations with specific site variables or corridor characteristic preferences.

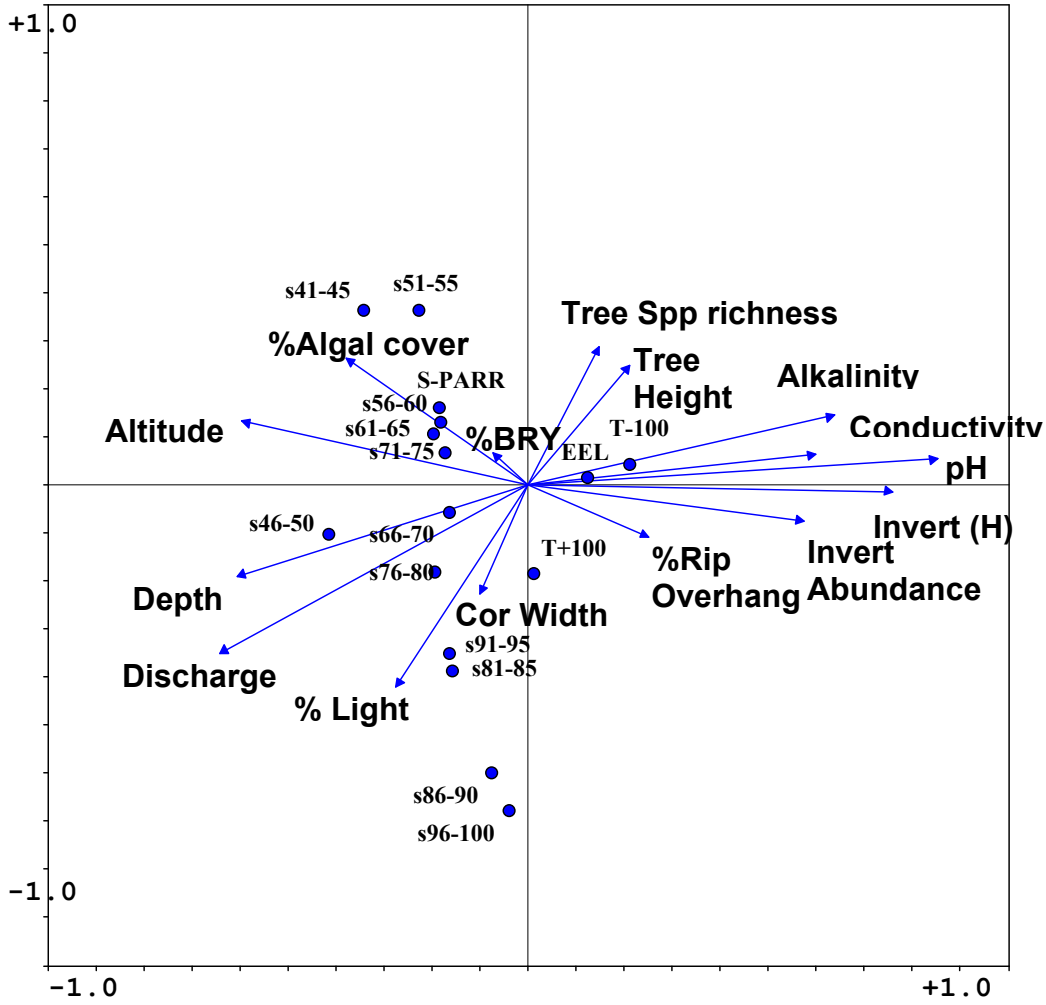


Fig 6.10. CCA ordination of data from both 2004 and 2005 stocking and recapture experiments combined with wide-scale sampling of natural populations. Salmon separated into size classes and parr category. Trout were more simply separated into +100mm and -100mm size categories. Eel abundance also included. Ordination significant (Monte-Carlo test, P < 0.05). Correlations of environmental variables with axis 1 – 4 defined in Table 6-6 with eigenvalues.

From arrow lengths produced within the CCA of Fig 6.10 and the inter-set correlations shown in Table 6-6, environmental variables plotted along the horizontal axis (axis one), appear to be the primary controls over fish size and population distribution.

Table 6-6. Inter-set correlations of environmental variables with axes 1 to 4. Particularly influential correlations (as defined by high positive or negative values) highlighted in bold.

NAME	AX1	AX2	AX3	AX4
%LIGHT	-0.2717	-0.4039	-0.0206	0.2637
CONDUCTIVITY	**0.5873**	0.0613	0.3246	0.2311
DEPTH	-0.5949	-0.1839	-0.0033	0.402
pH	**0.8372**	0.0517	0.2074	0.1992
ALKALINITY	**0.6282**	0.1389	0.269	0.2443
%ALGAE	-0.3721	0.2537	-0.2237	0.1184
%BRYOPHYTE	-0.0711	0.0635	-0.0318	0.7268
%RIP OVERH	0.2452	-0.1041	0.1272	-0.234
ALTITUDE	**-0.5861**	0.128	0.1568	-0.0822
COR WIDTH	-0.0975	-0.2175	-0.078	0.0388
TR HEIGHT	0.209	0.2387	0.0418	0.1057
TR SPP RICH	0.1448	0.276	-0.1675	0.0043
DISCHARGE	**-0.631**	-0.337	-0.1901	-0.1668
INV SPP RICH	**0.7468**	-0.0141	0.0964	0.157
INV ABUND	**0.5633**	**-0.0718**	0.1272	0.3539

From the species data, the main result is a gradient in the salmon fry size classes plotted vertically along axis 2. Increasing salmon fry fork length appears to most closely follow a gradient of increasing % light and also a widening of the corridor. This result confirms the initial hypothesis gained from the 2004 data set; increasing the availability of light within the stream appears to have a significant positive influence on the salmon fry lengths achieved within the first years growth. However, the abundance of salmon parr is more closely correlated with lower light conditions and a higher diversity of riparian tree species. Interestingly, there is no direct relationship between increasing light availability and algal cover (%). This could be an indication that the conditions within the shaded sites of the study were not light limited enough to reduce algal/bryophyte cover.

Although stream temperature at each site was measured, these measurements were only taken three times a year and so it was felt that such spot measurements would be misleading as seasonal and daily fluxes of temperature ranges would be greater than site to site mean variation. Therefore, temperature data was not included within the CCA ordination. However, one possible reason for the increased growth rate of the salmon fry in open areas is the increased temperatures associated with greater solar flux.

Temperature influences the date of emergence by fry as well as the length of the growing season; often it is these factors which are thought to increase the potential

weight that a salmon can achieve in any one year (Egglishaw and Shackley, 1985). However, as salmon stocking was done with approximately two-month old fed-fry, and the electrofishing survey of all sites was carried out within a one-week period, before stream temperatures dropped below feeding thresholds, the effective growing season for fry was the same. As a result, it is possible to discern that any changes in weight were as a consequence of the conditions, which the fry were under during stream-site location. Koskela et al. (1997) showed that growth rates of *Salmo salar* fry at 6°C were higher than those fry incubated at 2°C. Further, it was also shown that the lipid intake of fry at higher temperatures was significantly greater.

6.8.2 Trout Data 2005

Trout data used in this part of the study are from wild, unstocked populations. This may have implications on the comparable applicability of the trout results over salmon data. Trout have survived whole life cycles in the environment in question and have potentially been present at the site for longer than salmon fry of a single season, which were not spawned at site. Therefore, trout provide an amalgamated indication of condition suitability over a longer time period. Conversely, trout habitat requirements/preferences are often described as different from those of salmon, which makes the trout a poor proxy for indicating salmon survival potential.

Both brown trout size classes and the eel population are comparatively centralized within the CCA ordination (Fig 6.10), suggesting that there are no extreme condition preferences. However, as these populations illustrate natural distribution patterns and not specific stocking requirements, the positioning of these species within the ordination better describes actual condition preferences. The eel and trout populations appear to be correlated with higher pH, conductivity, alkalinity, benthic invertebrate diversity and abundance. As all these parameters have relatively long arrow length, it is likely that their influence over the distribution of species is strong (Table 6-6).

Linear regression reveals no significant relationship between trout population and pH ($P = 0.059$). No trout were found at mean pH levels at <6.1.There is no significant relationship between trout population and alkalinity ($P = 0.373$), conductivity ($P = 0.363$), benthic invertebrate diversity ($P = 0.095$) or invertebrate abundance ($P =$

0.383). However, there is a significant negative linear relationship between % light and trout abundance at site (P = 0.016), but also a significant positive linear relationship between trout length and light levels (P = 0.044). Therefore, a similar trend to the salmon data is occurring whereby population density of trout is greatest in the shaded sites; however growth rates are increased in light intensive habitats. Unfortunately attempting to relate a trout density to specific corridor characteristics did not yield any direct significant relationships; corridor widths (P = 0.087), tree height (P = 0.311) or diversity of tree species (P = 0.13).

Considering influential arrows on the left hand of the CCA ordination (Fig 6.10) and the inter-set correlations of the axes (Table 6-6), there is evidence to indicate that altitude, depth, discharge and algal cover are all important variables (negatively correlation with axis 1). Using linear regression, altitude was found to have a negative linear relationship with trout density increase, however, the r^2 reveals that this trend is not significant (P = 0.056, r = 0.236). Additionally, water depth, was not an important variable (P = 0.335). Spot measurements of discharge also proved a poor predictor of trout densities despite the appearance of a negative linear relationship (P = 0.062). Even algal cover was not significantly related to trout density (P = 0.192), despite the potential carbon contribution this material makes to the base of the food chain.

6.9 2005 discussion

The fact that both the salmon and trout populations appeared to fall into two specific scenarios; many small fish or few large fish, seemed to speculatively point towards density dependent populations. However, the demonstration that it was light levels, which dictated which scenario was found at a specific site, has significant implications for habitat management.

There is also some evidence that small fish are less affected by changes in cover than larger fish (Parkinson and Slaney, 1975). However, the mean reduction in fish size within shadier sites may be just a reflection of reduced growth rates in the cooler waters associated with reduced solar flux. However, Jenkins et al. (1999) found that larger, more competitive brown trout were less affected by increased density and generally these populations were less density dependent. Therefore,

having reduced mean population density within the open sites does not agree with the findings of Jenkins et al. (1999) as these trout/salmon had a greater mean weight and fork length. The increased predation risk accompanied with more open sites and larger prey (Pepin and Shears 1995; Gleason and Bengtson 1996a, 1996b) may have influenced fish survival at open sites. However as the overall survival likelihood is greater for larger fish (e.g. Parker 1971; Juanes 1994; Elliott 1989a, 1989b, Thorpe 1977, 1989; Wright et al. 1990), using the limited results gained though this study, it is possible to tentatively suggest that through promotion of open canopies, overall survival of salmonids, and population success may be increased. However, this suggestion is limited by water chemistry. Survival of trout and salmon was very low throughout the stocking experiment and, although mean pH at all sites was above, lethal limits suggested by a number of studies (see introduction), minimum levels (especially at Rowantree Burn) measured suggest that during precipitation events, pH levels may be damaging to young fry populations. These events may well have contributed to the low recovery success of salmon. Further, the greater success of trout was likely to reflect their greater overall tolerance to acidity. Therefore, any suggestions made for riparian modification come secondary to the confounding variables associated with water chemistry (especially pH).

7 Implications of clear-felling forestry activities on in-stream and riparian corridor integrity

7.1 Abstract

Unforeseen clear-felling occurred within the Cree catchment at the uppermost Black Burn site (BBCF) between the 11th of November 2004 and 8th of March 2005. The impact of clear-felling was examined through observation of the biological changes which occurred within one of the field sites. The effects of this event were assessed in terms of changes in the allochthonous contribution of carbon within stream biofilm. Energy source and availability were combined with community structure analysis of in-stream biota. Diversity of in-stream macro-invertebrate and algal taxa reduced. A bloom in *Sphaerotilus* spp. indicated a change to water nutrient status. Changes in the composition and biomass of the biofilm were considered in relation to downstream Total Organic Carbon (TOC) concentrations and related to possible changes in the functioning capacity of the stream biofilm.

The buffering capacity of the riparian zone is discussed in relation to vegetation type with the aim to suggest optimal conditions to reduce the ecological impact of felling on in-stream biota. By considering the vegetation structure, I explore the optimal conditions by which the riparian zone can buffer nutrient discharge through nitrate removal and retention.

7.2 Introduction

As a consequence of forest planting strategies during the 1960s and 70s, large areas of UK commercial forestry plantations are now approaching maturity or fully matured and ready for harvest. Harvesting is viewed as the most disruptive stage of the forestry cycle (Nisbet, 2001). Of foremost concern is exposure of soil through operations and an increase amount and velocity of run-off through plough furrows and drainage ditches (especially in the steep upland areas). Such effects increase the rate of sedimentation and nutrient input (Rounick and Winterbourn, 1982) to catchment streams.

Soil organic matter, particularly the litter layer, is important in regulating errosion level of forest soils (Bormann et al., 1969). Accumulated litter protects soil from the erosive energy of raindrops, promotes soil particle aggregation, and accelerates rainwater percolation. Disturbances that remove the litter layer or compact forest soils promote overland flow and erosion of mineral soil (sediment) into stream channels. Sediment yields decrease as vegetation re-grows. However, in-stream redistribution and transport of sediment may continue for many years (Brown and Krygier, 1971). These effects are exacerbated when soils are easily erodable and periods of wet weather follow (Nisbet, 2001). In addition, a combination of heavy machinery on soft ground, a planting and drainage infrastructure based on old designs, many of which have little or no buffer strips, and often a poorly designed drainage system creates the risk of significant erosion and loss of sediments and carbon to streams when old-style plantations are felled and harvested (Guo, and Gifford, 2002; Turner and Lambert, 2000).

The biological and ecological effect of soil/sediment losses to streams can be extensive, and represent the commonest concern from felling activities. Suspended sediments and increased turbidity (following a disturbance event), severely reduces in-stream diversity (Vuori and Joenssu, 1996) by smothering algae, invertebrates and fish eggs (Giller et al., 2002) and render traditional gravel spawning beds of salmonids unusable (Stretton, 1984). Siltation of spawning gravels is a particularly common risk from forestry activities (Herbert et al., 1961, Neill and Hey, 1992). Under natural conditions, most spawning rivers in the UK would have suspended concentrations of sand, fine silt and clay of less than 5 mg/l during low flows and

may be essentially clear-water rivers (Hendry and Cragg-Hine, 1997). High concentrations of suspended solids in the water may physically choke fish or disrupt feeding behaviour (Barrett et al., 1992). The fine particles released from forest drainage and surface runoff smother salmonid eggs by preventing intra-gravel currents (Moring 1982, Thibodeaux and Boyle, 1987), and reducing contact with dissolved oxygen in the flowing water. This prevents or disrupts alevin emergence (Phillips et al., 1975, Hausle and Coble, 1976) and reduces the fitness of the fry and parr, and hence their ability to cope with the natural pressures faced within the riverine environment (MacCrimmon and Gotts, 1986, Olsson and Persson, 1988). These effects have been found to persist for many years after the felling event (Yount and Niemi, 1990).

In addition to short term disturbance related changes affecting sediment release and nutrient runoff, the long term impacts on potential food source availability and acquisition are likely to have severe implications to ecological communities whose structures are based upon a specific balance of allochthonous and autochthonous production. For example, clear-felling can cause an increase in incident light within the stream environment due to canopy removal (Rounick and Winterbourn, 1982). This can cause an increase in photosynthesis and autochthonous production (e.g. algae and/or macrophytes). However, the potential for increased production is also dependent on nutrient availability.

Although short-term effects of clear-felling can increase the inputs of allochthonous energy sources (such as needles, twigs and branches) during the disturbance event, the long-term consequences may be either an overall reduction in allochthonous inputs with the removal or riparian over-storey trees, or increase in allochthonous material from increased ground vegetation biomass. Consequently, this uncertainty and potential variability in the production of allochthonous and autochthonous material has the potential to substantially alter the community dynamics dependent on these specific food sources.

Increased algae growth following canopy removal can subsequently increase the productivity of macro-invertebrates and fish in the medium term (e.g. Behmer and Hawkins, 1986). However, this increase may be offset by the reduced inputs of allochthonous food sources which were previously available (e.g. Wallace, 1988).

Potential overall energy balance will be dependent on the physical, chemical and nutrient status of the stream and thus the ability of the autochthonous production to compensate fully for the loss of allochthonous production (OECD, 1982; Johnson et al., 2000). However, excessive algal production can also cause detrimental effects upon the physical habitat of the stream, such as reduced dissolved oxygen during dark respiration in warm weather. O'Connor (2002) describes incidents in the literature, and within his own study in County Antrim, where diatom frustules have physically hindered the emergence of salmon alevins as they create a thick mat on the top of the gravel beds which have the potential to reduce the supply of oxygen to developing fish eggs within spawning gravels, where the eggs were laid. Also described were thickening of the gill tissue, severe hyperplasia and the presence of diatoms within the gills of the young salmon.

Other studies have also found shifts in functional feeding groups of macro-invertebrates. For example, an increase in herbivorous macro-invertebrate species may follow increased algal production after clear-felling events (Behmer and Hawkins, 1986; Wallace and Gurtz, 1986), together with up to a 30% decrease in the densities of detritus-feeding invertebrates (e.g. Wallace, 1988). Benefits to fish populations may occur with the increased abundance of grazing macro-invertebrate populations by enriching the supply of food, enhanced by feeding efficiency (of the visually orientated predator fish species) due to increased incident light (Valdimarsson and Metcalfe, 2001).

However, increases in light penetration following clear-felling are not always accompanied by increases of in-stream primary production (Johnson et al., 2000). This may be a result of limitations in nutrients (OECD, 1982) or a consequence of increased turbidity and the reduction in the associated light penetration (Johnson et al., 2000).

There are also many cases where nutrient release has occurred. When land adjacent to streams is afforested, forest vegetation regulates nutrient inputs to streams by two primary mechanisms: through uptake of nutrients from soil solution and storage in biomass, and by decreasing water movement through soils (Bormann et al., 1969; Vitousek and Reiners, 1975; Vitousek, 1977). Following disturbance, vegetative nutrient uptake is reduced/halted and soil conditioning activities and changes to

drainage patterns accelerate organic matter movement. As a result, post-felling concentrations of Ca, K, Na, Mg, and NO_3^{2-} are elevated in stream water (e.g. Swank, 1988). Nutrients that are relatively mobile in soil solution or cycle biologically appear to be most affected. The nitrogen cycle of forested catchments is extremely sensitive to disturbance (Vitousek and Reiners, 1975; Vitousek, 1977). Changes to concentrations of the nitrogen species in stream water, within afforested catchments, occur most commonly through soil leaching post-felling (e.g. Staaf and Olsson, 1994).

Total nitrogen output following felling has been considered in a number of studies. Weis et al. (2006) estimated organic nitrogen losses post felling, and found that the organic N component can account for up to 70% of total N lost. However, from a review of catchments studied in Britain (Neal et al., 1998), higher concentrations in NO_3- occurred only in the minority of felled sites, where aluminium leaching was also high. Concentrations declined several years after the felling events.

In addition to nitrogen release, DOC levels can exhibit marked increases following felling events (e.g. Cummins and Farrel, 2003). This trend can be amplified by seasonal weather patterns (DOC is naturally higher in mid/late summer) and has been found to be associated with increases in monomeric aluminium concentrations (Cummins and Farrel, 2003). However, the presence of increased DOC in itself, tends to reduce the toxicity of the aluminium (Howells et al., 1990), and thus does not necessarily pose a threat to fish survival.

Additionally, following clear-felling, soil temperatures and moisture levels usually increase due to removal of canopy cover and reduction in the water requirements from trees. From this, nutrient cycles within the soils of the riparian zone are modified leading to changes in biogeochemistry (Iseman et al., 1999). Changes to the water requirements and usage within the adjacent (now felled) area have significant implications to water availability. Evapotranspiration can account for 40-60% of the annual water loss from forested catchments (Kovner, 1956). Therefore, vegetation is an important factor in regulating stream flow. Removal of forest vegetation generally decreases evapo-transpiration and increases stream flow (e.g. Dunford and Fletcher, 1947). This effect has been found to be approximately

proportionate to the catchment area cleared (Hewlett and Hibbert, 1961; Hibbert, 1966).

In an attempt to minimize the impacts of such disturbance, the Forest and Water Guidelines were produced as a guide to best management practices (Forestry Commision, 1998, 1990, 1993, 2003). Following adherence to the guidelines, substantial modification to management of activities and planting design strategies has reduced the effects of forestry activities. Disturbance to soils was minimized though early modification of drainage systems (Thompson, 1979) and through the development of buffer strips (Mills, 1980). Emphasis on minimizing soil disturbance resulted in minimal forms of cultivation such as mounding or scarifying and the modification of drainage channel design in order to minimize soil disturbance and control water flows.

There has been much recognition that although the guidelines are based around sound principles, they lacked scientific verification. As a consequence, confirmation of guideline effectiveness is important, and widespread scientific testing of FWG application is required. In a recent study of the impact of felling processes which followed a former version of Forest and Water Guidelines (published in 1993), Nisbet et al., (2002) describe how shallow ploughing combined with furrow-end buffer strips on steeper slopes ($>5^{o}$) retained mobilized sediments, and the control of land drainage resulted in little disturbance of the freshwater environment. Samples of macro-invertebrates and trout suggested that the community compositions remained largely unaffected. Only one site appeared to show a decline in invertebrate diversity due to localized accumulation of silt and brash.

Effective buffer zone function depends on a number of different characteristics of the soil, for example, drainage flow paths, vegetation, soil moisture content and soil temperatures. Excess nutrients, pollutants and acidic waters can be retained, transformed and/or cleansed from the system through mechanisms such as assimilation and biostorage by plants, denitrification, microbial assimilation, and mineralization in soils (Correll, 1997).

Nitrate removal and retention is of key importance to the functioning requirements sought from a buffer zone. The specific mechanisms responsible for widely

documented cases of nitrate retention are often elusive (Correll, 1997). Many suggest a combination of denitrification, assimilation and retention by the vegetation as well as transformation to ammonium and organic nitrogen (and retention of this within soils) (e.g. Correll et al., 1997). However, what is clear from a number of studies is that nitrate does not simply transform into other more soluble forms of nitrogen and be discharged into the stream system (e.g. Lowrance et al., 1983; Correll et al., 1997).

Many of the characteristics of the riparian zone, such as the species composition of the vegetation and rates of processes such as denitrification, require that the soils be anaerobic or of low oxidation/reduction potential (redox) for at least part of the year. The species composition of riparian vegetation is fundamental to maintaining this redox potential, as the organic matter production (through high photosynthetic rates) and mechanisms of delivery of organic matter to the soil's activity drives and facilitates these biogeochemical reactions (e.g. Pinay et al., 1995). Thus in the long run, maintaining high rates of nitrogen processing and retention within soils requires a low redox potential to be maintained through high primary productivity. Thus, analysis of community composition, specifically plant functional types abundant within the community, allows predictions of the hydrology of soil, and ability of vegetation buffering capacity. Specifically, differences in community composition may vary rates of nutrient and pollutant (in this case, nitrate) to containment and transformation within the buffer zone.

The efficiency of riparian buffer zones in removing pollutants from surface and ground water is highly dependent upon hydrology. For the effective removal of particulates and dissolved nutrients as well as toxic materials, surface flows must occur as sheet flow rather than highly focused flows, and ground water must flow at a shallow enough depth to be within the rooting zone of riparian vegetation. Overall, it appears that studies have found uncertainty in which riparian vegetation best promotes nitrate removal or retention, but that grass or dense herbaceous vegetation is more effective at trapping particulates from overland storm flows (e.g. Osborne and Kovacic, 1993). However woody vegetation with its relatively deeper root systems (more likely to intercept groundwater flows) may be more effective at removing nitrates from ground water through more effective release of organic matter at depth (e.g. Parsons et al., 1994).

It was not anticipated that Black Burn would be felled during the course of this research and as such, this investigation into the impact of felling on biodiversity and baseline energy dynamics can only be considered as pilot data. However, the design of the Black Burn corridor provided insight into both the potential impacts of modern felling approaches to 'old design' sites (at one sub-site, BBSH) and in addition, to designs consistent with the current FWGs (2004) (at the felled site –BBCF, and middle site, BBOP). Felling occurred on site from 11th October 2004. As the coup felled covered an area greater then the Black Burn catchment alone, it is not certain when exactly the disturbance event stopped in the catchment. However, the area was cleared prior to my arrival on-site on the 8th of March 2005.

As a consequence, this study aims to discern the impact of felling within a single stream felled within the study period (Fig 7.1). Although chemical and nutrient variables were not analysed as part of this study, this focused, short-term study, infers changes to nutrient source and availability from the ecological status and community composition. Additionally, the riparian zone diversity is discussed with respect to the felling event and the possible limitations of the present buffer zones with respect to onsite and downstream ecological status.

Fig 7.1. Upstream corridor site after clear-felling in the winter of 2004-2005

Additionally, I focus on biofilm composition and biomass and assessed post felling changes to measured characteristics in order to estimate variation in stream trophic status and biofilm material. Further, the specific composition of the biofilm can affect the retention and processing capacity of organic enrichment and non-organic pollutants. From consideration of the literature, it appears that this approach has not previously been applied in estimating carbon sources and availability following forest clearance activities.

This chapter specifically addresses the influence of the felling, without the confounding influence of other variables associated with the remaining sites of both catchments. The chapter explores the results from each trophic level potentially influenced by the felling. The chapter integrates the response from multiple trophic levels within the Black Burn, in an attempt to indicate the ecological response to a disturbance event. Further, by combining all datasets within this chapter, the effect of the felling is explored as ecological conditions vary spatially and temporally. Further, there is the opportunity to determine how influential the event was to the overall patterns of abundance and diversity compared to that found in areas unaffected by disturbance and allow assessment of the impact to ecological status of modern felling techniques on both 'old style' and 'modern' riparian zone designs.

7.3 Methods

Routine measurements of primary productivity were on going throughout the project, as the felling event at Black Burn was unexpected; no additional measurements were taken to account for the full extent of physical, chemical and biological changes to the system. As a consequence this chapter addresses the changes to the three Black Burn sites within the constraints of the existing sampling protocol. The ecological sampling programme in place addressed diversity and productivity of in-stream biofilms, riparian vegetation, macro-invertebrates and fish, with ongoing measurements of basic water chemistry and corridor physical characteristics.

This chapter uses data from other chapters and the methods corresponding to each component can be found as follows: riparian vegetation: Chapter 2; autotrophic

biofilm content: Chapter 3; autochthonous and allochthonous carbon, Chapter 4; and Invertebrate diversity, Chapter 5.

7.4 Results

7.4.1 Buffer widths

The Forest and Water Guidelines (Forestry Commission, 2003) recommend a buffer width of 20 m on either side of the stream for watercourses with a channel width > 2 m (including lakes and reservoirs). For smaller streams (1-2 m width) the minimum distance from the river edge for planting is 10 m, and 5 m for channels <1 m width, unless highlighted as important for fish spawning, when 10 m is required. Within this study, both the upstream and middle (BBCF and BBOP) sites are within the minimum requirements of the Forest and Water Guidelines (FWG), and although, there are few native deciduous tree species growing within the corridor as recommended in the FWGs, and the majority of riparian vegetation being in the form of coniferous seedlings and riparian ground-flora, the two sites still represent minimum buffer zones requirements. However, only the forestry adjacent to the upper-most site was cleared (BBCF). The downstream site (BBSH) however, has very little buffer-zone, and below FWG minimum width requirements. Therefore, these sites provide an opportunity to consider in-stream and riparian response to felling at a site which falls within FWG requirements and to observe downstream influences of the event within a single system with different riparian zone characteristics (Table 7-1).

Table 7-1. Stream and corridor widths (± S.E) for sites of the Black Burn (prior to felling activities at the upstream site). Samples are mean of 3 width measurements per visit, within the 10m site stretch. Each site (minus CF, which represents measurements of a single pre-felling visit) was sampled four times, totalling 12 replicate measurements.

Black Burn

Relative Position	Site ID	Stream Width (m)	Corridor Width (m)
Upstream	BBCO (pre-felling)	1.13 +/- 0.28	12.6 +/- 1.45
Middle	BBOP	1.86 +/- 0/18	17.5 +/- 2/88
Downstream	BBSH	1.83 +/- 0/12	4.6 +/- 1.2

7.4.2 Biofilm characteristics and productivity

Following the felling event (

Fig 7.2.), several changes occurred to the visual characterisation of the biofilms collected in spring 2005 multiple visits, compared to samples taken in spring 2004. These changes to the biofilm material included a visible loss of green autotrophic algal, thickening and darkening of the material, and often a general peaty appearance. This was combined with a bloom of abundant grey gelatinous growths in spring, covering the entire benthos.

Fig 7.2. Black Burn Corridor site before felling in summer 2004 (left) and after, in spring 2005 (right).

Composition analysis indicated a significant increase ($P < 0.001$) in biomass settlement within the stream benthos following the felling event (Fig 7.3). The biomass increase was reflected in both the carbon and nitrogen contributions to benthic energy sources, which were generally significantly greater ($P < 0.001$) in 2005 sampling points (Fig 7.4 and Fig 7.5). However the contribution from carbon in mid-summer 2005 is substantially greater than that of nitrogen, reflected by the molar C:N (Fig 7.6), suggesting significant ($P < 0.001$) carbon loading of sites post felling. These results suggest substantial increase in nutrient availability within 2005 season and especially during the July collection.

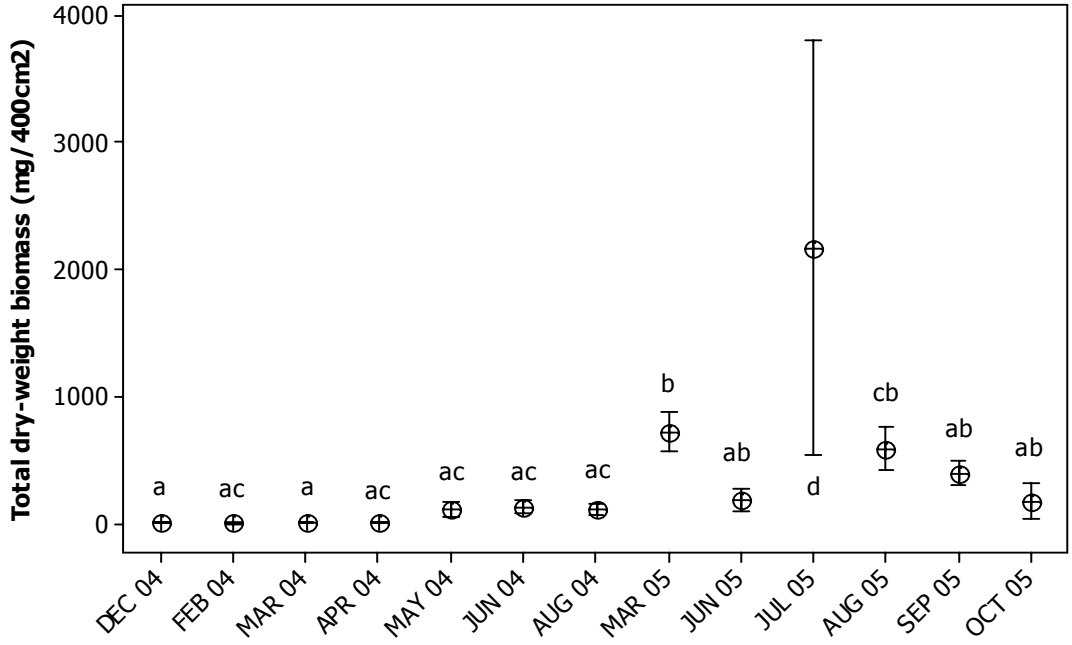


Fig 7.3. Significant differences (ANOVA, P < 0.001) of biofilm dry-weight biomass (mg). Differences in groups defined with Tukey Test (95% confidence) and signified with differential lettering (a, b, c and d).

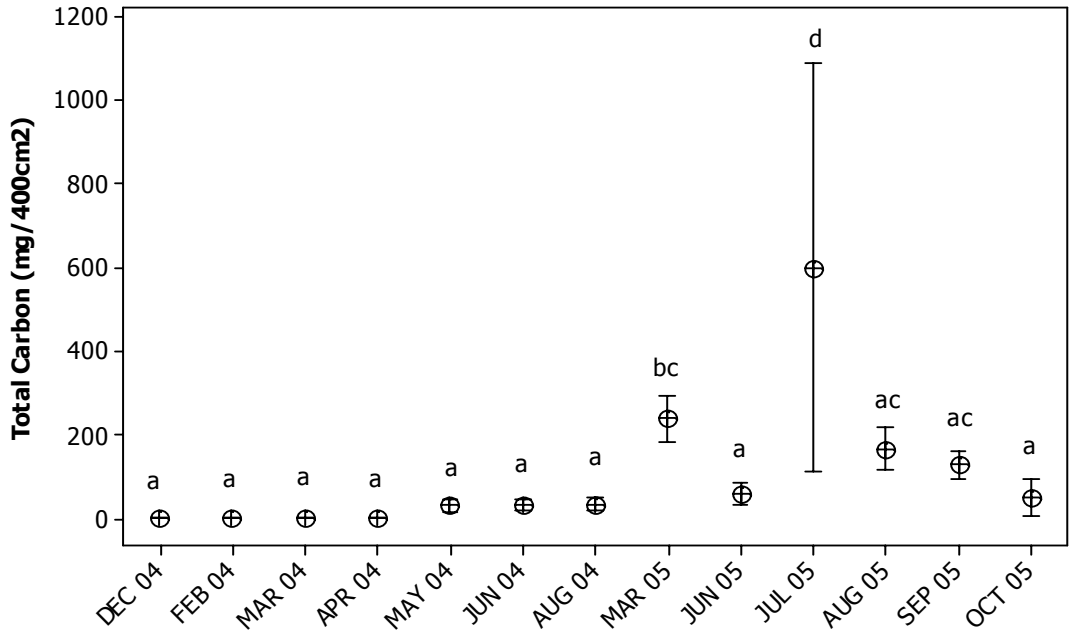


Fig 7.4. Significant differences (ANOVA, P < 0.001) of biofilm total carbon (mg). Differences in groups defined with Tukey Test (95% confidence) and signified with differential lettering (a, b, c and d).

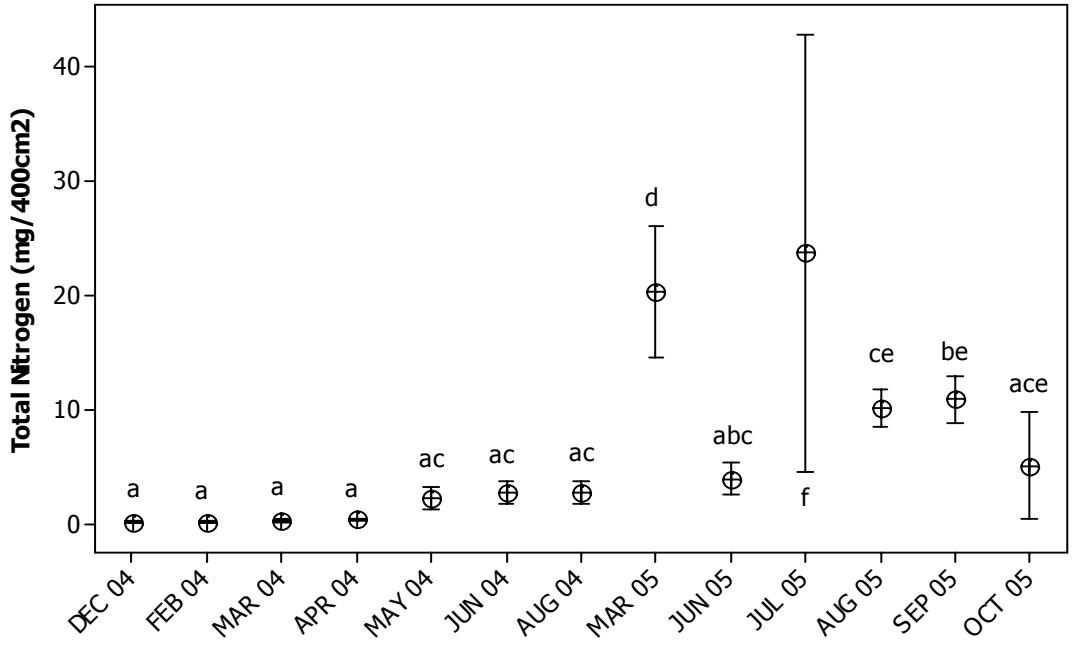


Fig 7.5. Significant differences (ANOVA, P < 0.001) of biofilm total nitrogen (mg). Differences in groups defined with Tukey Test (95% confidence) and signified with differential lettering (a, b, c, d, e and f).

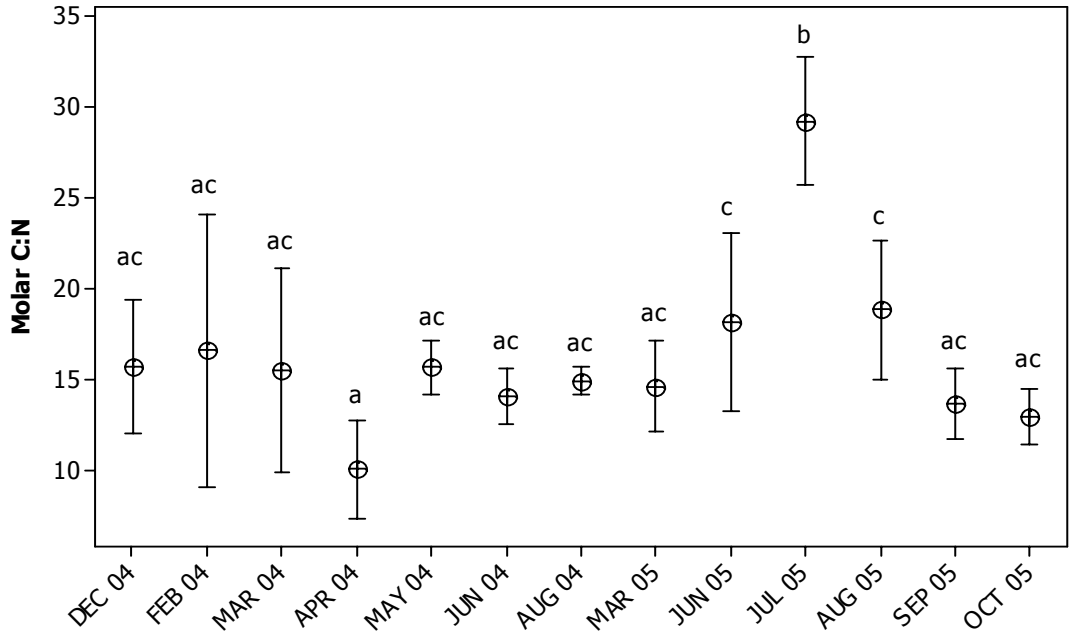


Fig 7.6. Significant differences (ANOVA, P < 0.001) of biofilm molar C:N. Differences in groups defined with Tukey Test (95% confidence) and signified with differential lettering (a, b, c and d).

Autotrophic biomass, as outlined in Chapter 3, is shown again here. The data illustrates that chlorophyll *a* production within the biofilm material reached levels comparable to 2004 data post felling by July 2005 (Fig 7.7). There was evidence for full autotrophic concentration recovery within 6 months of felling. However this autotrophic production was spatially variable. Chlorophyll standing stock was significantly ($P<0.001$) higher at the clear-felled site (BBCF) compared to the shaded site, with the narrowest corridor (BBSH). Therefore, it appears apparent that during disturbance and nutrient enrichment, corridor design parameters significantly influence autotrophic biomass within streams (Fig 7.8).

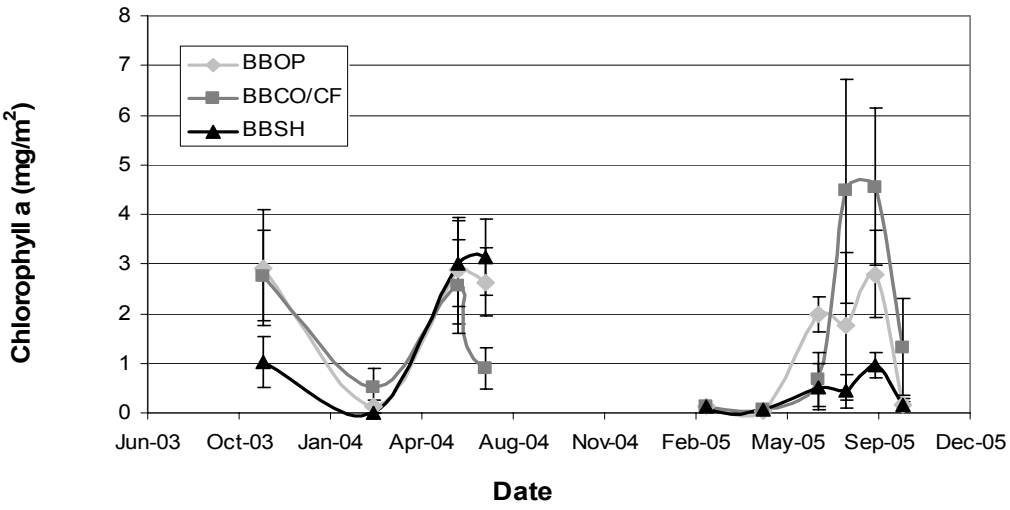


Fig 7.7. Chl *a* from Black Burn biofilms collected during 2004 and 2005 (mean ± SE). BBCO and BBCF constitute the same site, BBCO, represents pre-felling conditions. Gap in data is representative of unsampled, felling period at BBCO.

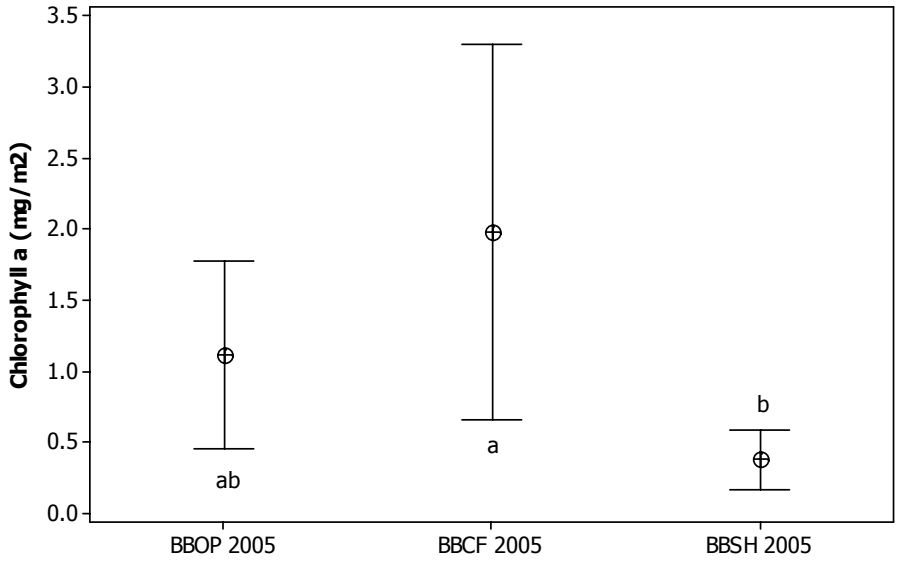


Fig 7.8. Mean Black Burn biofilm Chl *a* concentrations (± 95% confidence interval) from each sample site in 2005 (upstream, BBCF; middle, BBOP; downstream, BBSH). Tukey test reveals significant differences (indicated with differing letters: a or b) between BBCF and BBSH (P = 0.030); where chlorophyll production is reduced.

Even with the delay of measurable autotrophic biomass within 2005, the overall levels of chlorophyll measured between the two years appeared to be approximately comparable. However, when chlorophyll is analysed in comparison to overall biofilm carbon mass, the concentration of autotrophic material within the biofilm material was low. Specifically, using C:Chl *a* (C:Chl) measurements as an indicator of % autochthonous autotroph concentration of the biofilm, suggests significant (ANOVA, P = 0.049) variation amongst groups for C:Chl content (Fig 7.9). The increased C:Chl in 2005 biofilms indicates a relatively low algal prevalence (<100 is generally regarded as high algal cellular content - Geider, 1987) and increased concentration of allochthonous material.

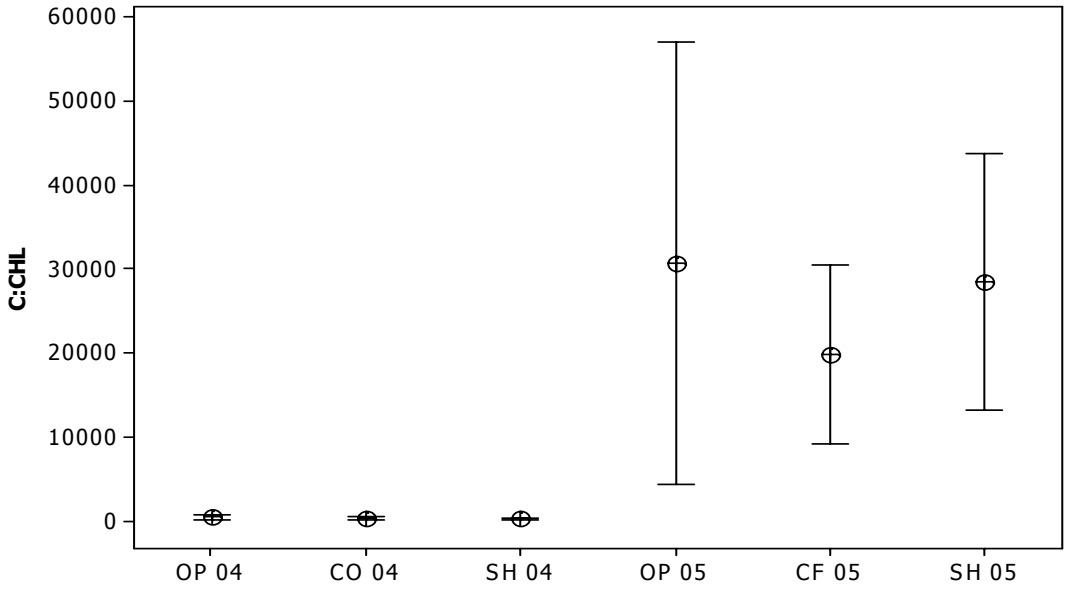


Fig 7.9. Comparison of site specific C:Chl (± 95% confidence interval). Kruskal-Wallis analysis indicates significant differences between groups (P < 0.001) Distribution of means suggests all BB 2005 sites have significantly greater C:Chl.

This result is confirmed through calculations of algal biomass (algal C mg/m^2), transformed from chlorophyll *a* density using the algal conversion factor of 60 following; Romani and Sabater, 2000 (Fig 7.10). From Chapters 3 and 4, it was shown that variation in the chlorophyll conversion factor (CF) has minimal implications to the algal content of the biofilm in comparison to the allochthonous and heterotrophic component. Therefore, the intermediate CF was used here for analysis. In general, the proportion of biofilm material which is autotrophic is much greater within 2004, before the felling event occurred. However biofilm chlorophyll content became comparable between 2004 and 2005, by July 2005 (Fig 7.7), suggesting that the autotrophic contribution here does not increase proportionally to the increase in overall biofilm biomass. Rather, the overall ratio of the autotrophic matter to heterotrophic and allochthonous organic matter is low within post-felling biofilms despite chlorophyll recovery comparable to pre-felling levels.

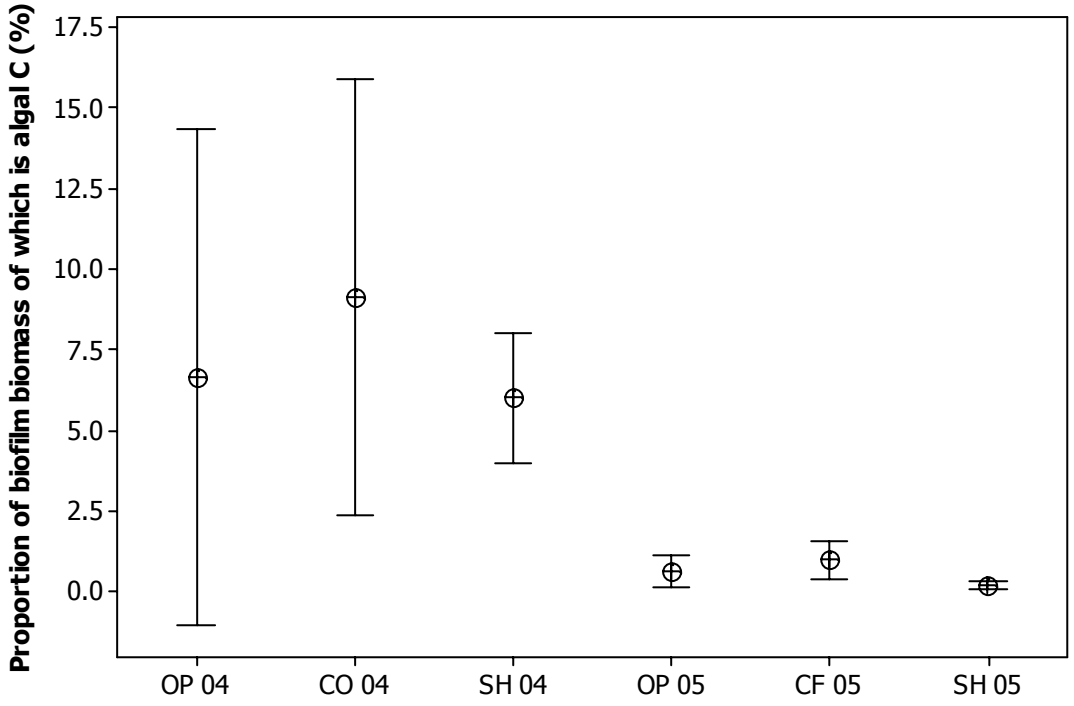


Fig 7.10. Proportion of biofilm biomass derived from autochthonous algae. Significant differences (Kruskal-Wallis, P <0.001) between BB sites in 2004 and BB 2005, indicated by distribution of means, suggests lower biofilm carbon derived from algae in BB 2005 biofilms. Also suggestion of reduced algal production in shaded BB 2004 site, indicating possible light limitation to PP.

The high proportion of non-autotrophic material following the felling event and the relative proportional contribution of allochthonous material is likely to have a significant influence on both biofilm internal diversity and functioning as well as influencing the community composition dependent on biofilm material. However, in comparison to past studies (e.g. Romani and Sabater, 2000); the contribution of autotrophic material is low during both years with autotrophic ratios of ~1:9 for 2004 and ~1:99 in 2005.

7.4.3 Algal species assemblage

Green algae (Chlorophyceae) dominate in the initial stages after felling (Fig 7.11), but only in the immediate area of the felling (BBCF), not downstream sites, despite the relatively close proximity to the felled area (~150 and 200m for open and shaded respectively). This suggests that community composition may respond to nutrient enrichment only on very small spatial scales or that nutrient availability may decline quickly over a short area. The latter suggestion supports the hypothesis that efficiency of biofilm processing and nutrient retention capacity at BB during 2005 was

high. Specifically, the processing and drawdown, and the resulting reduction in available nutrients limited the spatial advance of the taxon (Guasch et al., 1994) away from the enrichment source.

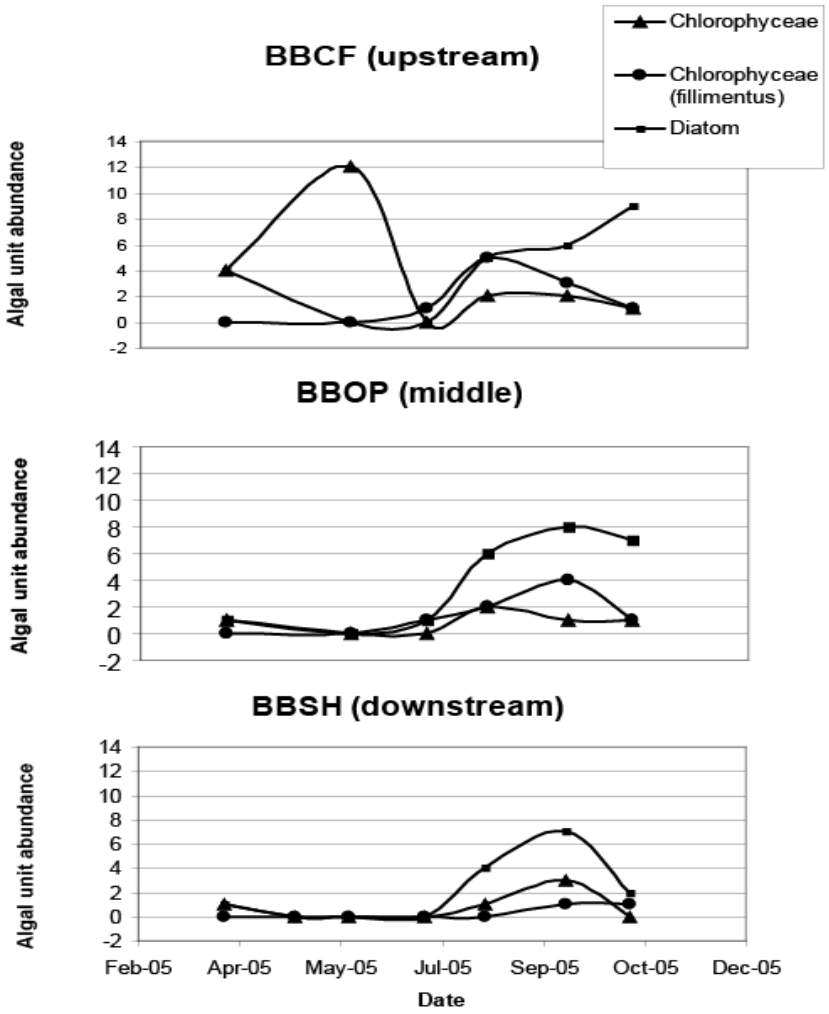


Fig 7.11. Temporal variation in the dominant taxonomic groups in 2005 Black Burn biofilm samples, with all three sites displayed, post-felling.

Additionally, diatoms show late summer increased abundance in all sites, Diatoms are a siliceous class of algae reputed for being very sensitive to chemical conditions. They usually account for the highest number of species (up to 80%) among the primary producers in aquatic systems (Pan et al., 1999). Yet in the samples immediately following felling, their prevalence is very low and not comparable with that of Chlorophyceae in BBCF. As diatoms have frequently been used as biological indicators of water quality (Kelly et al., 1998; Prygiel et al., 2002; Leira and Sabater, 2005; Sabater, 2000), the temporal variation of abundance here, suggests both unfavourable conditions post-felling, but also recovery of conditions in late

summer/autumn 2005. The reduction of diatom abundance at all sites at the end of the sampling season is not clear but may reflect seasonal variation.

Immediately following felling (March – May 2005) there was also notably abundant hetrotrophic growth of the colonial bacterium: *Sphaerotilus natans* (Fig 7.12). This population was most abundant at the two uppermost sites (BBCF and BBOP), but declined in all sites towards mid to late summer, 2005.

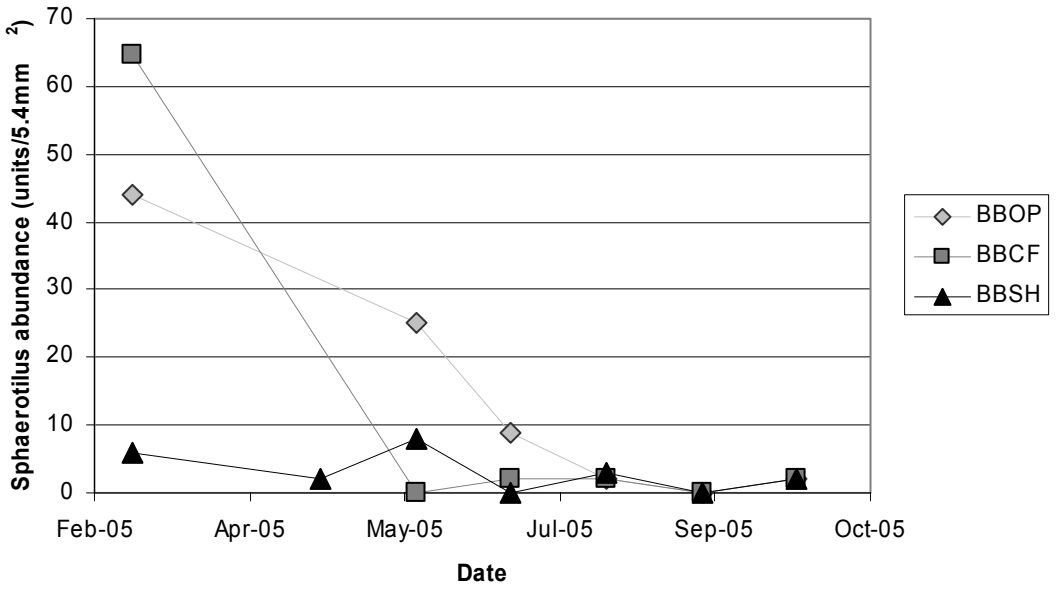


Fig 7.12. Abundance of *Sphaerotilus natans* at BB sites within 2005, following felling event. Abundances for 3 fields of view (x 10 magnifications).

7.4.4 Chemical and physical data measurements

Physical and chemical data for the Black Burn were limited to measurements taken on four sampling trips. The unexpected nature of the event, and limitations on project resources meant that only routine water chemistry samples were taken. These measures were only originally designed to aid in the general characterisation of differential sites and not to distinguish temporal variation within a site and as such, were not sufficient to infer changes to the chemical characteristics of the stream environment post felling. Limited data is included here as an illustration of the data ranges measured in order to provide background information on site characteristics. However this data is not repeated frequently enough to be able to infer impacts of felling activities.

pH data was only collected once prior to felling and three times over the following season. At each site, pH varied from a minimum of 4.18 to a maximum of 5.80. This maximum range of 1.62 pH units showed no significant spatial variation (ANOVA, n = 12, P = 0.979). Although temporal variation was significant (ANOVA, n =12, P = 0.003, with July 2005 having significantly higher pH than the pre-felling sample), it was felt that inferring chemical changes to the system from a data variability range which is smaller in comparison than that observed over 24 h variation arising from photosynthetic activity and/or precipitation (Tetzlaff et al., 2007), would not be meaningful.

A similar situation arises with conductivity data. Here, n still = 12 and there is no significant spatial variation (ANOVA, P = 0.470). Temporal variation (ANOVA, P = 0.049), over the four sampling points indicated a greater absolute conductivity in July 2005 compared to pre-felling conditions. However the range of data is still low (67 -100ms/cm), and as such, it was felt it was inappropriate to infer chemical changes to stream water from felling activities from these limited and low ranging data points which could be as much to do with natural variation in the system.

There was an increase in light availability at the stream water surface immediately following felling of the clear-felled site at that site from 54% PAR in September, 2004 to a mean of 81.1% for the 2005 sample season. However any accompanied variation in stream water temperatures are from spot point measurements which cannot be relied upon to reflect long term changes in corridor characteristics over those likely to occur due to seasonal or even hourly temperature variation. The addition of long term data-logging of the physical and chemical changes of the water may have contributed to the understanding of water chemical and nutrient level variation following felling. However, by using biological indicators such algal composition, bacterial colonisation and macro-invertebrate species composition, as studied here, it is hoped that some evidence for chemical change can be inferred and contribute to evidence suggesting a change in conditions.

7.4.5 Benthic macro-invertebrates

There were distinct changes in the invertebrate community composition following felling. However, as the sampling methodology comprised a single kick sample per fieldwork visit, the total sample size for all visits and all sites on the Black Burn was

relatively small (n = 12), resulting in limitations of the statistical power of the data set.

In total 20 taxa were found at the three sites of the Black Burn (for both before and after the felling event). The dominant taxa, present in almost all samples, were the Diptera (Chironomidae, Tipulidae and Simulidae), Plecoptera (Nemouridae and Leuctridae) and the Trichoptera, Polycentropidae (Table 7-2).

Table 7-2 Results from invertebrate kick samples at the Black Burn (Sep-04 to Sep-05).
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The ability to detect any effects of forestry clearance may depend on the choice of response measures. In respect to benthic macro-invertebrates different characterisations of benthic communities, such as the biomass, taxonomic community structure or total abundance can emphasise different aspects of ecological responses (e.g. Rodrıguez and Magnan, 1993). Therefore, evaluation of a wide range of biological metrics is useful in detecting and understanding the impact of forestry, as well as the response and subsequent recovery of macro-invertebrate communities (Resh et al., 1988). Thus, here both multivariate analysis and spatial and temporal variation analysis of the differential measurement parameters (diversity index, species richness and numerical abundance of individuals) are used to assess the community and ecological response of macro-invertebrate taxa.

The following ordination of Cree only sites (Fig 7.13) is a replicate of that of Chapter 5 and has been repeated here for ease of discussion. Due to the small sample size available, statistically, it was not possible to analyse BB alone. However, the ordination of the whole Cree catchment provides comparable influences of various environmental variables as well as evidence to suggest that the majority of taxa are negatively correlated with any environmental variable which could be associated with the felling event (i.e. increasing silt/clay, sewage fungi cover (as identified as *Sphaerotilus* spp.) and algal cover). The only taxa which were correlated with positive increases in these variables were *Corixidae*, *Chironomidae*, *Ephemerellidae* and *Vellidae*.

The ordination provides evidence of co-related variables associated with the felling event. From within the Cree catchment, sewage fungi were only detected within the Black Burn. Therefore, it is assumed that this variable can be directly associated with the felling event. As such it appears that associated with increased heterotrophic production (and as such the felling event) are the variables positively correlated with axis one: increased conductivity, water temperature, sedimentation (% silt/clay), overhanging vegetation and widening of corridors.

There was some evidence of separation of a group influenced by the felling event using TWINSPAN (Hill, 1979) (group 4, Table 7-3) into a distinct 'felling community', suggesting that the response by the invertebrate community is marked enough to influence the entire Cree catchment ordination, causing a marked separation of those species associated with the felling and the rest of the catchment assemblage. Further, the community response was fast enough to be comparable with the rate of change in conditions. Additionally, this survey period, although short, was sufficient to detect higher trophic group affects of a consumer assemblage structure dominated by taxa dependent on detrital resources (e.g. collector gathers (Chironomidae) and collecting filterers (Simulidae)).

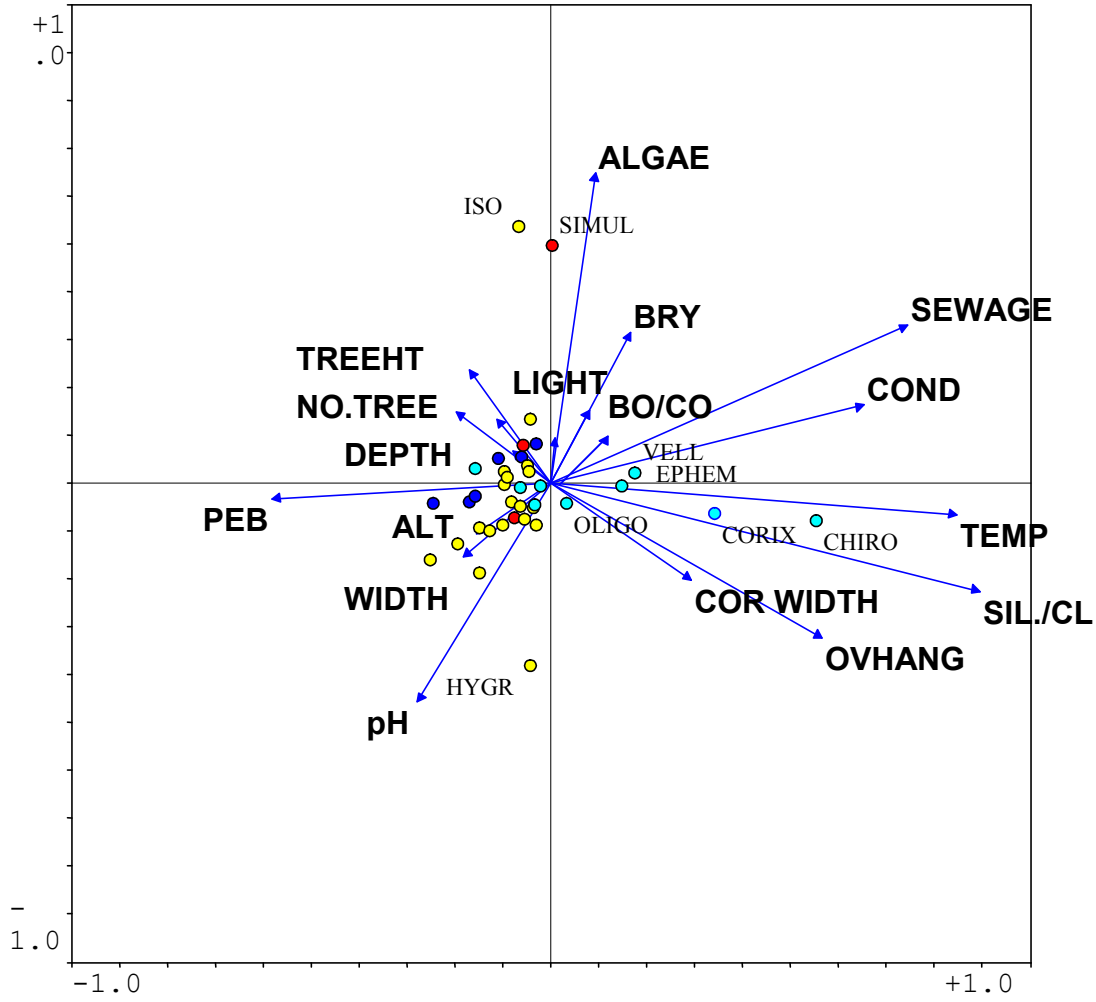

Fig 7.13. CCA1 from invertebrate chapter: species-environment ordination for samples located in the Cree catchment only. Colouration of dots indicates groupings as defined by TWINSPAN analysis. The contents of TWINSPAN groups are assigned in Table 7-3. A Monte Carlo test revealed the significance of the ordination (P = 0.005) with the majority of the variation within the ordination explained within axis 1 (eigenvalue 0.648) and axis two (eigenvalue of 0.314).

Table 7-3. Species assemblage groupings as defined with TWINSPAN analysis associated with Cree-only CCA (Fig 7.13). Designation of groups 1 and 2 produced an eigenvalue of 0.361, and from groups 3 and 4, an eigenvalue of 0.423 was assigned.

Group 1 (yellow)	Group 2 (blue)	Group 3 (red)	Group 4 (light blue)
Ancylidae	*Baetidae*	*Hebridae*	*Chironomidae*
Glossiphonidae	*Leuctridae*	*Simulidae*	*Odonticeridae*
Perlodidae	*Elmidae* (larvae, narrow)	*Tipulidae*	*Corixidae*
Chloroperlidae	*Helodidae*		*Dytiscidae* (larvae)
Cordulegasteridae	*Heptegeniidae*		*Ephemerellidae*
Dytiscidae (adult)	*Hydropsychidae*		*Gerridae*
Elmidae (adult)	*Leptophlebiidae*		*Neuroptera*
Elminthidae (larvae, wide)	*Limniphilidae*		*Oligochaeta*
Goeridae	*Polycentropodae*		*Velidae*
Hygrobatidae			
Isopoda			
Leptoceridae			
Nemouridae			
Psidium			
Rhyacophilidae			
Valvatidae			

There was a reduction in diversity (H) at all sites immediately following felling (Fig 7.14) in March 2005. The clear-felled site experienced the greatest loss in diversity following felling. That this reduction is spatially constrained supports the conclusion that the biological effects of felling were mainly limited to an area immediately adjacent of the felling activities (BBCF), and there were reduced impacts downstream (at BBOP and BBSH). However, there was a recovery in diversity at both the clear-felled (CF) and shaded site (SH) (top and bottom sites), suggesting recovery of environmental conditions at these sites by the end of the sampling period (Sep 2005). It is unclear why this response was not also apparent in the Open site (middle). It is possible that the proximity to the road and under-road tunnel downstream and deep, slow moving, depositional areas of waters upstream reduced facilitation of inter and intra site migration of individuals for repopulation. Further, using diversity indices to indicate temporal variation of diversity has potential limitations, as data is manipulated to the extent of possibly reducing the sensitivity of the overall diversity measure. However, species richness within sites, over a temporal scale is indicated in Table 7-2. Similar spatially-specific recovery rates were observed in macro-invertebrate communities in riffle and depositional habitats of mountain streams in North Carolina. Invertebrate assemblages of the above mentioned habitats took much longer to recover from logging disturbance than those

on moss-covered bedrock, a more stable habitat (Stone and Wallace, 1998). Clearly, environmental context should be considered when evaluating forestry impacts, which may otherwise be masked by natural variation among sites.

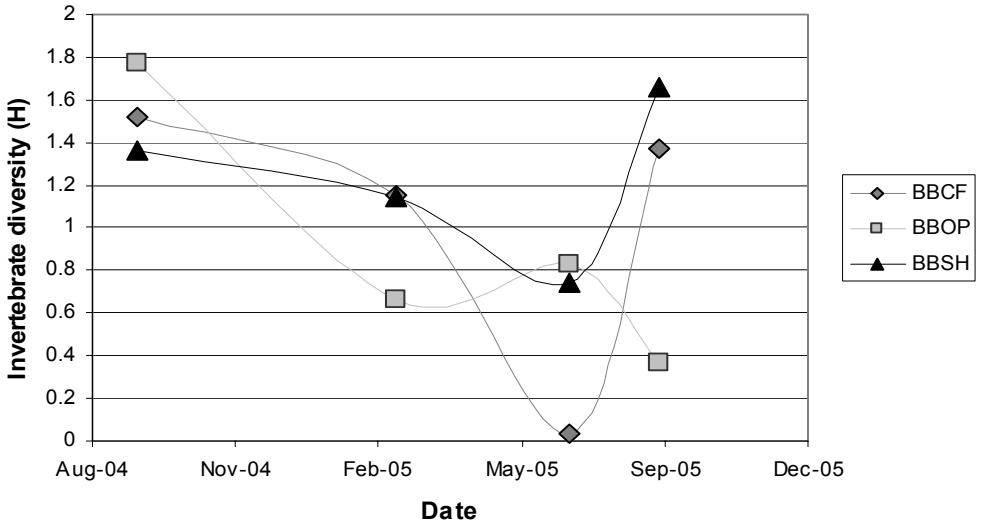

Fig 7.14 Changes in benthic macro-invertebrates Shannon-Wiener diversity indices post felling (2004-2005) at BB sites (BBCF – upstream, BBOP middle, and BBSH, downstream).

Consistent with a number of previous studies concerning recently felled systems, high abundances of certain species has been reported to cause unevenness of populations (e.g. Bisson and Sedell, 1984; Wilzbach, 1985; Kiffney et al., 2003). At Black Burn there was a sharp increase in the number of individuals from the Dipteran taxa (Table 7-2). However consideration of Shannon-Weiner diversity indices which account for unevenness of populations, indicated that when all sites where pooled there was no significant temporal variation in overall diversity scores (H) (ANOVA, n = 12, P = 0.114). This result may increase scepticism in the appropriateness of using Shannon-Wiener indices within this system, where temporal variation is the main consideration. However with consideration of sites individually (Fig 7.14), there was the suggestion of both temporal and spatial species-specific shifts. Initially, there was an increase in abundance of Simulidae at BBOP (middle) and BBCF (upstream) (in March), closely followed by a spatial shift to abundance of Chironomidae in BBSH (downstream) (during the July sampling trip) (Table 7-2). In addition, the presence of *Sphaerotilus natans* at sites following felling may have significantly damaged populations of invertebrate taxa. Sewage fungi have been noted to clog gills of invertebrates, as well as hindering movement and feeding success (Hynes, 1978).

Here, samples were discovered with severe infestation of bacterial colony growth (Fig 7.15).

Fig 7.15. Nemouridae (Pleocoptera) sample from BBCF 2005, showing colonisation of *Sphaerotilus natans.* Field of view shown here = 1cm width.

7.4.6 Allochthonous Vegetation

As mentioned earlier, two out of the three Black Burn sites fell within the minimum width requirements of the forest and water guidelines. Consequently, it seems appropriate to explore the riparian vegetation at these sites in detail to determine possible reasons for the impacts to both the basal resource characteristics and invertebrate diversity and assemblage structure described in the previous sections.

In a study by Nisbet et al., (2002), the use of the forest and water guidelines on forest clearance strategies was assessed. This study illustrated minimal impacts from clear-felling on macro-invertebrates and resident trout populations as well as water quality. Consequently, here, one must question why, when modern felling practices would have been used, such significant impacts occurred to invertebrate populations and biofilm characteristics/content within the short period of sampling following the event? Although the entire length of Black Burn does not strictly follow the 'New Style' planting regimes, in that the downstream site has a corridor width below the

minimal FWGs, the sites upstream, which fell within the FWGs, still showed significant disruption.

Despite the close proximity of the BB sites, all three sites have very different riparian vegetation classifications. Of particular interest is the riparian vegetation at the clear-felled site as it is important to define why this vegetation type did not contribute adequately to the buffering capacity of the clear-felling event.

A total of 19 vegetation species were found during sampling at the Black Burn clear-felled site (Table 7-4). From the species list, it is clear that the majority of species are grasses, reeds or *Carex* species and there are no large shrub species or deciduous trees.

Table 7-4. Black Burn Clearfelled site (BBCF) mean riparian community composition for March (M5), July (J5) and September (S5) sample trips for both bankside (B) and three-meter from bank (3) sites, using the DOMIN scale of plant abundance assessment in accordance with Rodwell et al. (1991).
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Consequently, it is proposed that this riparian species assemblage will be best adapted to removing surface runoff organic particles (Osborne and Kovacic, 1993) and the ability of the vegetation to remove nitrate from ground water will depend on the flow path of water and the relative proximity to the roots of the vegetation (Correll, 1997).

Anaerobic conditions are required to produce the redox potential required for denitrification, but as soil oxygen content was not measured, it is difficult to determine whether conditions are optimal for denitrification or not. However, as many studies have noted that the redox potential is not low enough in soils subject to dry periods or droughts (e.g. Weller et al., 1994); one can assume that the anaerobic conditions are produced within high soil moisture/waterlogged conditions. I did not measure soil moisture saturation, thus to estimate conditions, species assemblage at Black Burn CF site was used to assess the potential for optimal redox potential conditions through inference from the optimal soil moisture preferences of the specific plant community.

TABLEFIT was used to analyse the plant communities of each of the three Black Burn sites (Table 7-5). TABLEFIT automatically classifies vegetation groups according to the National Vegetation Classification (NVC) and identifies habitat types according to the EC CORINE system The program identifies vegetation types by means of an index of goodness-of-fit, which measures the degree of agreement between the sample under study and the association tables in British plant communities (Hill, 1989).

Table 7-5. TABLEFIT analysis of Black Burn sites for riparian vegetation types.

Site	Corine Type	Descriptor	Goodness-to-fit score
BB Shade	C41.21	Atlantic oak wood + Bluebell	42
BB Open	C31.86	Bracken fields	57
BB CF	C37.217	*Juncus effusus* meadow	47

Although the assigned goodness-to-fit score is poor for BBCF (47) the clear-felled site was categorised as *Juncus effusus* meadow (Corine – 37.217, NVC M23). *Juncus effusus* and many of the *Carex* species found in abundance at the clear-felled site represent a community structure adapted and associated with moist, even waterlogged soil conditions (Chittendon, 1956).

7.5 Discussion

7.5.1 Biofilms

Biofilm biomass increased significantly following felling activities. This mass increase was assumed to be proportional to thickness of biofilm material. When biofilms are sufficiently thick, steep redox gradients occur, creating anoxic zones. This gradient can facilitate the cycling of certain elements, such as nitrate, which can be denitrified (Nielson et al., 1990; Triska et al., 1993; Claret et al., 1998). Denitrification causes a net loss of nitrogen to the atmosphere. Thus, this activity in the biofilm would lead to a net loss of nitrogen from the river system, aiding recovery of waters enriched by diffuse pollution events and activities such as felling.

However, studies have illustrated a trade-off in functioning by biofilms with differential compositional characteristics. For example, nutrient uptake and consequently, the capacity of a biofilm to improve water quality decreases with increased thickness (Freeman and Lock, 1995). A thick biofilm becomes increasingly self-sufficient with increasing cycling of internally produced algal and bacterial

exudates and lysis products. However, increased thickness of biofilms also increases the ability to store organic nutrients from external sources (Romani and Sabater, 2001). Further, the redox potential achieved with increased biofilm thickness promotes both denitrification of excess nitrates and allows the biofilm to be more resilient to the effect of inorganic pollutants (e.g. heavy metals). In a purely biological process, biofilm organisms (algae, bacteria and fungi) are responsible for the uptake of organic carbon (Kaplan and Bott, 1983) and inorganic nutrients (Portielje and Lijklema, 1994; Tate et al., 1995). Nutrient decline downstream has been observed elsewhere (e.g. Sabater et al., 1991) and has been tentatively attributed to biofilm activity. This hypothesis has been tested in artificial channels, and unambiguously related to biofilm uptake (Mulholland et al., 1995 Kaplan et al., 1987; Mason and Jenkins, 1995). Autotrophs and heterotrophs in the biofilm use nitrogen and phosphorus from the river water, the former to build up their growing cells (Bothwell, 1988), and the latter during the degradative use of materials of high C:N and C:P (Mulholland, 1992).

Using the stoichiometric mixing model developed in chapter four, the proportion of allochthonous material within the biofilm sampled over temporal and spatial scales is illustrated (Fig 7.16). During pre-felling sampling, approximately 80% of the biofilm material was defined as autochthonous in origin, following felling, this ratio changes to a dominance of allochthonous inputs (up to 70 %). The simplest interpretation here is that following felling; there was a substantial contribution of allochthonous carbon from the riparian zone to the stream which was then incorporated into the matrix of the biofilm material.

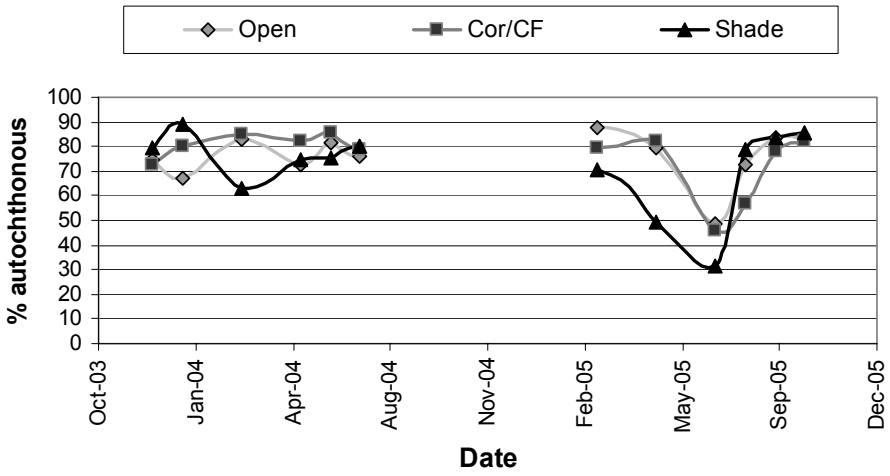

Fig 7.16. Assessment of proportional contribution of autochthonous carbon to the Black Burn biofilms using molar C:N based mixing model.

Within this study, the thickest biofilms were found during July sampling (Fig 7.3). During this same period, biofilms also incorporated the greatest proportion of allochthonous material within the matrix (Fig 7.16). This drawdown and retention of organic material into the biofilm removes it from the water column and thus should aid recovery of the stream water biochemical composition. However, the active processing of this material within the biofilm is primarily accomplished through bacterial processing and decomposition (Freeman and Lock, 1995). The presence of autotrophs enhances the degradative capacity of the biofilm (Romani and Sabater 2000), thus, increasing the capability of the biofilms to ameliorate water quality. Consequently, those biofilms present post-felling are likely to be negatively influenced by the low proportion of algal carbon associated with the biofilms present post felling (Fig 7.10). It is apparent that the conditions on-site which facilitate the maximum autotrophic production are likely positively to influence processing of excess allochthonous material.

Riparian conditions were found to significantly influence autotrophic production (Fig 7.8), resulting in the clear-felled and open sites having a greater production of chlorophyll *a*. Thus, it is suggested that a greater light availability in-stream at BBCF and downstream of the felling site BBOP facilitated the recovery of the stream environment from the nutrient enrichment effects of the felling event.

However, maximum biofilm biomass during July 2005 could also reflect maximum retention of material during that point. Consequently, despite the dominance of allochthonous material and the depletion of autotrophic material (Fig 7.9 and Fig 7.10), biofilm growth appears to retain much of the excess organic material released into the stream system. As such, biofilm activity removes organic nutrients from the water column and may reduce impacts to downstream areas even with low autotrophic content. This finding suggests that even heterotrophic allochthonous based biofilms are important in organic matter retention. However, this finding contradicts previous studies which note that although bacteria are important in the uptake and processing of nutrients (Caron, 1994), optimal retention and processing of organic pollutants requires biofilms to have a greater proportion of autotrophic material (Romani and Sabater, 2000) for optimal bacterial functioning. Within August

2005 sampling, chlorophyll content of the upper two sites (BBCF and BBOP) was comparable with maximum chlorophyll mass recorded during 2004. With primary production increase, it is unsurprising that the autochthonous proportional content of the biofilm (as calculated using molar C:N) showed signs of increase (Fig 7.16).

One of the most visually conspicuous aspects of the felling event was the appearance in the stream of a greyish mucilaginous material which appeared to contribute significantly to the early 2005 biofilm biomass. This material was identified as a colonial bacterium species; *Sphaerotilus natans.* Samples with this species in abundance had low molar C:N measures and as such, were rightfully classified with the stoichiometric based mixing model as autochthonous material. Abundance of this material immediately following felling (Fig 7.12) may explain why autochthonous content of the biofilm remained high until July 2005 (when abundance of *Sphaerotilus* dropped), despite felling occurring during the winter months. The increase in autochthonous material following July sampling appears to be related to the shift to autotrophic production (Fig 7.7).

Water nutrient concentrations were not measured as part of the original project design and with the unexpected nature of the felling event; the total data and associated conclusions possible are limited. Yet, the response of the baseline resources and higher consumer groups measured as part of the classification of stream and riparian zone ecological status have proved useful as indicators of the chemical and physical variations occurring in the sites. The biological impact provides evidence to support the theory that significant nutrient enrichment occurred post felling. For example, high concentration of organic matter (evidenced though biofilm biomass increase) within the stream system is likely to have resulted in an increased biological oxygen demand through increased heterotrophic respiration. These combined effects of felling were likely to have provided the conditions appropriate for high production of *Sphaerotilus natans*; a species which is commonly referred to as sewage fungus, and is often associated with organic pollution events (Curtis and Harrington, 1971). Therefore, much of the evidence for nutrient enrichment comes from the biological response. Yet without chemical analysis, this assumption is still somewhat speculative. However, further evidence of nutrient enrichment comes from data collected independently by the Scottish Environmental Protection Agency (SEPA). Measurements of total organic carbon (TOC) and dissolved organic carbon

(DOC) within the water are shown in Fig 7.17. Significant increase in carbon was identified within these analyses, during the period immediately following clear-felling at Black Burn. Although this increase may have been partly due to seasonal fluxes in nutrient release, such a sharp incline, corresponding with the period of BB disturbance, is suggestive of a direct correlation between BB and Minnoch TOC/DOC levels. This suggestion indicates that the release of organic material from the felling site caused a notable increase in carbon content at a point in the main Minnoch tributary approximately 7km downstream of Black Burn.

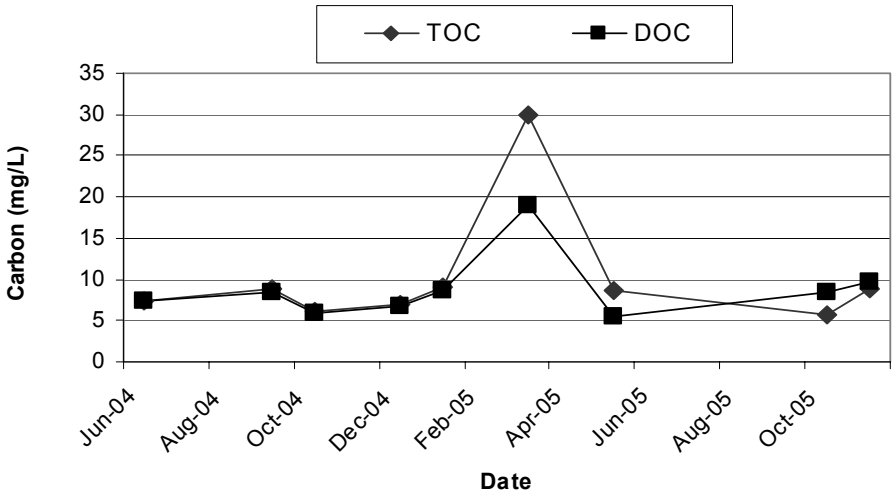

Fig 7.17. Total organic carbon (TOC) and dissolved organic carbon (DOC) (mg/L). Measurements taken by SEPA, at Minnoch Bridge location (NX 36220 74840).

The temporary nature of the flux, and specifically, the sharp reduction in carbon concentration downstream from April 2005 (Fig 7.17) coincided with the mid-summer period of maximum biofilm biomass (Fig 7.3), and initial chlorophyll recovery (Fig 7.7) This suggests that although the proportion of autotrophic (and autochthonous - Fig 7.16) material was low (Fig 7.10) within the thickened biofilms of summer 2005, the retention of organic material by biofilms on-site, may have contributed to the reduced downstream carbon loading. Thus allochthonous dominated biofilms may be playing a significant role in buffering organic enrichment release to downstream areas.

Algal community composition and biofilm efficiency (as photosynthesis per unit chlorophyll) are related (Guasch et al., 1995). At BBCF, the community of stream biofilm algae changed from a green algae dominated community structure (reflecting

the high nutrient availability required by this taxa: Guasch et al., 1995) to a community diatom-dominated (at all sites), although with a lower photosynthetic efficiency per unit cell (Guasch et al., 1995). Yet consideration of the relative abundance of diatom cell units and the comparative restricted area utilised by the chlorophyceae, overall photosynthetic activity increased in latter summer months reflecting the increased efficiency to the biofilms during this period.

However, there was evidence that diatoms could possibly be used as an indicator species for the forest clearance event. The reduced diatom population within the BB sites and the relative population recovery towards the end of 2005 suggests that this species was negatively affected by the felling event (Fig 7.11). This result also suggests that recovery from felling within the biofilm in terms of chlorophyll (Fig 7.7) production and algal species diversity assemblage only took one growing season (approximately 5 months) before results were comparable with pre-felling conditions.

7.5.2 Benthic Macro-invertebrates

Logging activities were linked to a decline in taxonomic richness, increase in numerical densities and shift in community structure of benthic macro-invertebrates. These changes primarily reflected marked increases in the abundance of chironomids and simulids

Although there was some evidence of a 'post-felling adapted' community composition from the Cree catchment CCA (Fig 7.13), the rate of change of environmental conditions following the event and shifts in potential energy sources (e.g. Fig 7.10 and Fig 7.16) was likely to be too rapid for all but those with the fastest reproductive rates (e.g. *Chironomidae* and *Simulidae*), to demonstrate a temporally adaptive response to the changing conditions.

The shift in dominance from Simulidae in March to Chironomids in July could reflect both changes in food availability/form and the differential feeding mechanisms and behaviours of the two taxa. The larvae of the Black Fly (*Simulidae*) concentrate dilute sestonic carbon through filter-feeding, into benthic biomass. Food quality and quantity are amongst the strongest predictors of Black Fly larvae distribution (Richardson and Mackay, 1991). Larvae feed non-selectively (Chance, 1970), and individuals ingest differential particles in proportion to abundance rather than quality,

selective only that the particle is within a size range capable to be captured efficiently on their cephalic fans (Wotton, 1984). Thus, abundant presence of a filtering collector species during the early stages following the felling event, suggests that the highest proportion of carbon available during this phase, is most likely in the form of labile C, or suspended organic matter. During this early period, biofilm biomass was relatively low (Fig 7.3) and yet the high TOC/DOC release (Fig 7.17), suggested that the majority of carbon available, during this stage, is mobile and suspended within the water column, available for filtering species. The Simulid fast reproductive rate combined with generalist feeding behaviours facilitates fast population increases associated with the favourable conditions (Chung Kim and Merritt, 1989).

The dominance of chironomids within the shaded site during the July sampling trip could be a key indicator in the shift of carbon sources and availability. Chironomids are fast colonizers found in high densities in disturbed habitats. These attributes allow them to be among the few taxa able to exploit patchy, ephemeral food resources (Palmer et al., 2000). Biomass of the biofilm is at a maximum during July (Fig 7.3). Additionally, TOC runoff downstream dropped (Fig 7.17). Thus, I hypothesise that the quantity of carbon available as suspended particles is reduced and that the majority of organic food material is located in the form of a thick, allochthonous dominated biofilm (Fig 7.16). As the majority of chironomid species fall within the 'Collector-Gatherer' functional feeding group (Merritt and Cummins, 1996) and feed by collecting and consuming particulate organic matter from the benthos, this shift in biofilm characteristic favours the chironomid feeding morphology/functional feeding group (FFG). This group depends on allochthonous derived detritus in the form of coarse (CPOM) and fine particulate organic matter (FPOM) for both habitat and food (Richardson, 1991; Grubbs and Cummins, 1994), and thus the impact of felling is unsurprising in causing the community shift to one of chironomid dominance.

Interestingly, two of the stream sites were bordered by buffer strips of a greater width than the minimum requirements of the Forestry Commission's Forest and Water guidelines, yet the presence of buffer strips seemed insufficient to eliminate impacts of logging activities on macro-invertebrate communities. This provokes obvious questions concerning the appropriateness of the design, width and nature of

the riparian buffer zones currently being produced within the constraints of these most recent guidelines.

7.5.3 Allochthonous Vegetation

Although the vegetation types present in the survey suggest high soil moisture content, and as a consequence, the anaerobic conditions required for denitrification (e.g. Weller et al., 1994), the soil organic matter content and distribution in relation to ground water flows is not known. It is hypothesised therefore, that reduction in the buffering capacity of the riparian zone was as a consequence of increased redox potential at the depth of groundwater flows. Optimising conditions for nitrogen removal may therefore be a balance of maintaining high soil water content and providing the appropriate content and distribution of organic matter. It is suggested that this could be achieved through tree planting (as trees have deeper root systems, more likely to intercept groundwater flow paths). However as this may then pose a risk to soil moisture conditions due to increased evapo-transpiration from the riparian zone.

Further research into vegetation types and optimising soil water and nutrient levels has the potential to aid in the design of buffer strips within riparian zones. It is suggested that soil moisture conditions should be considered when designing and managing riparian buffer strips. Further corridor design should consider more closely that the specific biological demand for nitrate matches the photosynthetic rate of the vegetation, and thus, allowing for adequate removal of excess nutrients from the soils. Further, it is it appears apparent that current Forest and Water guidelines do not adequately match the hydrological conditions of the ground flow at sites with the type of vegetation present, in order to ensure interaction between vegetation and soil-water is maximized. Therefore, despite the riparian zone design falling within the current Forest and Water guidelines, the ecological response following the event suggested significant alterations to the chemical and biological conditions present both at the felling site and downstream. Therefore, this chapter provides evidence through biological monitoring that increase in corridor width alone, does not appear to contribute sufficiently to amelioration of the water body adjacent to the felling activity.

8 Conclusions

In this study, conifer forestry stream corridor design was considered in relation to both in-stream and riparian zone biodiversity. Additionally, the contribution, availability and source of basal resources in forest stream systems have provided the primary focus of this project. Further, a substantial area of investigation concentrated specifically on, how these basal resources vary with variable site conditions, temporal scale and under the scenario of felling disturbance. These approaches have been combined to provide a data set which examines community diversity at key trophic scales in order to determine how the corridor characteristics and their associated resource availability, affects community structure and system functioning within the confines of current forestry management guidelines. Here I summarise the key findings of each chapter and comment on their relevance to stream corridor design.

Chapter two considered riparian vegetation biomass and diversity. Riparian vegetation biomass, particularly proximate bank-side biomass, was assumed to be directly related to the delivery and quantity of allochthonous material available within the in-stream habitat. The correlation found between overhanging vegetation percent estimates and bank-side vegetation biomass supported the view that there was direct delivery of allochthonous material to the stream from bank-side vegetation sources. However, an attempt to quantify the volume and type of allochthonous material available within the water column (through detritus trapping: see appendix) failed due to the destruction of traps during spate events.

However, previous studies have shown that overhanging material provides a direct source of allochthonous carbon as well as increasing the delivery of drift invertebrates, which can be essential components of consumer diets. Stream consumers, such as fish, depend on terrestrial invertebrates that fall into streams for up to half of their diet (Elliott 1967, 1973; Cloe and Garman, 1996*;* Johansen et al., 2000; Kawaguchi and Nakano, 2001). In addition, overhanging areas of riparian vegetation can also act as refuges for invertebrates and fish (Eklöv and Greenberg 1998), and are often utilised in the emergence of the adult forms of many aquatic invertebrates (see Sabo and Power 2002; Kato et al., 2003*)*. The significant correlation observed between PAR intensity and riparian ground vegetation biomass,

although a foreseeable relationship, nevertheless reinforces the need to maximise light intensity within riparian zones to promote ground-flora and associated overhanging vegetation biomass. However, both the correlation of vegetation biomass with light and % overhang had large variances and low associated r^2 values. As such, the power of these relationships was poor and illustrated the importance of possible confounding variables. The primary variable which appeared to influence these relationships was thought to be seasonality. The increase of biomass production at all sites (irrespective of corridor light regime) within summer sampling points, increased the variance of the relationship. It was also likely that variation in the light requirements of specific vegetation types reduced confidence in the correlations found. However by using direct comparisons between the broad site-type categories (i.e. 'broadleaf' 'open' and 'corridor' etc), found that the pooled data from those sites defined as 'open' had the greatest overall riparian vegetation ground flora biomass.

Successful implementation of the detrital trapping experiments would have provided useful information on quantity and quality of allochthonous material available within the water column and to stream biota, within differing habitat types. In respect to the investigative route taken throughout chapters 3 and 4, improvements to project design would have benefited from prioritising the redesign of detrital trapping experimental equipment, so as to gain more information on resource availability and source. Information on this aspect may well have significantly added to, and complimented information gained on benthic biofilm carbon source and availability. However, consideration of secondary consumers (i.e. benthic macro-invertebrates) was used instead as a proxy for relative food type and availability, through analysis of functional feeding group abundances. The dominance of detrital feeding guilds confirmed the importance of allochthonous resources rather than autotrophic in-stream production. Further, a significant direct linear relationship between riparian biomass and in-stream benthic macro-invertebrate diversity was observed. This result provided further evidence to suggest that allochthonous organic material was the most important resource promoting a diverse invertebrate community assemblage structure. However, the importance of over-storey tree diversity to benthic macro-invertebrates was more questionable as no direct relationship between over-storey tree diversity and invertebrate diversity was found.

A number of past studies have noted reduced diversity of ground-flora species within upland, open, moorland habitats, and specifically the domination of grass species such as *Deschampsia flexuosa* (e.g. Bokdam and Gleichman, 2000). However, the data presented in Chapter 2 suggested that although broadleaf sites overall yielded the highest riparian ground flora vegetation diversity scores (Fig 2.11), vegetation diversity was only significantly reduced at the conifer shaded sites, with all other site types yielding statistically similar diversity scores. Thus, the potential trade-off in vegetation diversity which one might expect to exist with the adoption of wide, light intensive corridors is in fact, minimal here. As such, corridor design aimed at optimising the promotion of both biomass and diversity of riparian vegetation, and through this, macro-invertebrate diversity, should consider orientating management towards more open, light intensive corridors. Vegetation biomass standing crop was greatest at higher altitude locations, within areas of greatest light availability. The Forest and Water Guidelines suggest that 50% of the riparian zone should be kept open. Yet, to promote greater ground vegetation biomass, important to benthic invertebrate populations, I suggest that this area could be increased, especially at the bank edge, where vegetation biomass is directly related to overhanging material.

The analysis of in-stream basal resources was the focus of Chapters 3 and 4. Several different techniques were employed to determine the composition and characteristics of in-stream benthic biofilm growths. This focus was applied in order to determine the source, quantity and quality of in-stream resources, and to gain insight into the functioning of biofilms under varying corridor characteristics. With reference to studies in the literature, relating biofilm composition to functioning (chapters 3 and 4), I considered the functioning of the biofilm in terms of the potential for nutrient drawdown, retention and processing of organic and inorganic pollutants. These chapters form a pilot data set exploring the idea that biofilm characteristics could be optimised to promote increased in-stream buffering and processing of excess nutrients. Although further development of this approach is clearly required, this is an area of stream functioning research which has not previously, to my knowledge, been considered in respect to forestry activities and the diffuse pollution which they may cause (Allan, 1997; Clenaghan et al., 1998, Maitland et al., 19990 and Ormerod et al., 1986). A large number of studies have explored the potential of riparian buffer-zones for reducing in-stream pollution impacts (e.g. Pinay et al., 1990; Naiman and Decamps, 1997; Giller and Malmqvist, 1998; Gordon et al., 1992).

However I have found none exploring the potential of optimising biofilm characteristics as an alternative or complementary approach to buffering downstream impacts of forestry activity. I feel that the research presented here forms the basis for further studies utilising in-stream primary resources as a secondary line of defence against excessive terrestrial nutrient run-off.

The biofilm study of chapters 3 and 4 considered approaches to defining algal contribution to benthic biofilms and the source of carbon available to secondary consumers. Further, through delineation of basal resources, and determination of the variability of biofilm mass, the degree of variation in the proportion of allochthonous and autochthonous resources under a variety of corridor conditions could be identified. In addition, autotrophic contribution was considered in respect to past studies which have noted optimal processing and retention of pollutants within biofilms with high autotrophic proportion. Here, corridor characteristics, and specifically increased areas of light availability, were explored in relation to autotrophic proportion optimisation, and thus, biofilm functioning capacity.

The development of a molar C:N two-source mixing model within Chapter 4 indicated that under normal, non-disturbed conditions, biofilm biomass is made up of ~ 70 – 80% autochthonous material (Fig 4.24 to Fig 4.27). The confidence of this result was high as the result was consistent between both the streams studied (BB and T33). This result provides scope for the wider applicability of this approach in defining the source of carbon available within stream benthic habitats. Whilst this approach was unable to delineate the autotrophic to heterotrophic ratio, it contradicts a large number of findings which suggest that upland afforested streams have allochthonous based resource dominance (e.g. Vannote et al., 1980). The results gained in this study suggest that these low order systems, within afforested catchments are either based on autotrophic production, or extensive internal recycling of resources, but not directly on allochthonous material. As such, this questions the importance or even the relative availability (Dobson and Cariss, 1999) of allochthonous material within such systems.

Relating biofilm characteristics to light (PAR) intensity had mixed results. There were few intra-site specific differences in chlorophyll production between the majority of the undisturbed sites (BB 2004 sites or T33, 2005), despite significant variation in

corridor conditions and light availability. However, T33 biofilms were generally more chlorophyll enriched than BB 2004. This result suggested that either chlorophyll production was not light limited by the specific light intensity provided by each site condition or that alternative factors were influencing algal productivity. Similar studies within the literature have found comparable patterns, noting that algal biomass in streams is as much a function of flow regime (Rounick and Gregory, 1981; Tett et al., 1978) and invertebrate grazing (e.g. Steinman, 1996) as it is of growth rate. Additionally, the level of light required by algae is not only controlled by riparian shading, but also as a function of the turbidity and coloration of the water itself. The methodology used in this study would be improved by paying more attention to the light attenuation within stream waters. This was not considered here as the mean depth of the streams was often less then 30cm and thus it was assumed that Zeu (photic zone cut-off depth) would not be reached within the shallow streams concerned.

However, this raises the issue that corridor design and management must take into account not only the optimum light requirements of the in-stream biota but also the water quality of the specific water-course and specifically the light attenuation within the water column, and may require accounting for the greater light requirements of streams with naturally darker waters. Further, as studies predict a global increase in DOC concentrations in riverine run-off (Freeman et al., 2001; Worrall and Burt, 2004; Worrell and Burt, 2005b), corridor design which does not account for the variability in water colouration, turbidity or DOC concentrations, may need updating.

Changes in the proportional contribution of carbon to chlorophyll *a* (C:Chl) has the potential to cause significant shifts in the biofilm functioning, providing information on the relative contribution of autotrophic carbon. The high C:Chl found at BB 2005 suggested that there was significant input from C-rich allochthonous material. Conversion of chlorophyll *a* to algal cellular carbon (using the middle conversion factor of 60) was useful in determining biomass of algal material within the biofilms at each site. However, data presented here indicated that only a very small proportion of biofilm material is derived from algal cellular origin: approximately 2.5% for T33, compared to 5.6 % for BB 2004 and 0.6 % for BB 2005. Although the conversion factor (CF) has been found to be variable under a number of common environmental variables (e.g. light, temperature, algal species and pH), exploration

of the potential variation in CF did not cause significant variation to relative algal concentrations compared to the remaining mass of organic matter. The low autotrophic proportion of biofilm found at these sites is similar to the findings of a number of previous studies (e.g. Frost and Elser, 2002; Bowman et al., 2005) where algal cells were a minor component of 'periphyton'. However, there appears to be much variation in the literature, as equally Frost et al. (2005) and Hamilton et al. (2001), described a high algal component to periphyton. This inconsistency between studies describing the proportion of autotrophic material within biofilms, indicates that it is not possible to make assumptions about biofilm content, specifically that periphyton is primarily autotrophic. The relative proportion of the autotrophic biofilm component significantly influences functioning of the biofilm and processing of potential pollutants (Sabater and Romani, 2000). Consequently, temporal and spatial variation in biofilm content should be considered as important an aspect of stream ecology to survey as other approaches to quantifying stream health, basal resource availability and potential vulnerability to habitat modification. However, the low autotrophic component to the stream biofilms indicated that biofilm functioning was only minimally reliant on algal production.

As the autochthonous proportion was derived though a molar C:N mixing model, and the autotrophic component of that autochthonous material derived through chlorophyll conversion, this allowed for the remaining proportion to be defined as heterotrophic. With this information, it was possible to suggest that approximately 25% of biofilms under non-felled conditions were allochthonous, ~5% was autotrophic and ~70 % of material was heterotrophic in character. This high heterotrophic proportion is surprising and suggests significant levels of internal processing from bacteria, fungi and protozoa. Further, this result also suggested that the biofilm at the sites studied was largely self-sufficient as there was little 'raw material' in the biofilm matrix in comparison to the heterotrophic component. However, in comparison to past studies, biofilms here had significantly lower autotrophic to heterotrophic component than the 3:1 described as optimal for functioning by Sabater and Romani (2000). Following the felling event at BB, in 2005, the allochthonous component often reached closer to ~50 - 60% of the biofilm biomass. Further, the autotrophic component dropped to around 1% of the biofilm material.

The high proportion of heterotrophic material is largely derived due to the high autochthonous estimations (especially in undisturbed sites). Confidence in this result is high due to the significant difference in molar C:N between terrestrial and aquatic material (and the consistency of these signatures across both spatial and temporal scales: Redfield, 1934). However, it was not clear how much the molar C:N of allochthonous material is altered when processed within the stream system. It is possible that with extensive transformation and processing of allochthonous material by aquatic organisms, the protein content of the material increases, and in doing so, the molar C:N decreases. As such, the material (originally allochthonous) resembles an autochthonous signature. As this transformed material cannot be defined as autotrophic, it was defined as heterotrophic, when it may, in fact, be highly processed material of allochthonous origin. However, although this was a possible scenario, it also demonstrates the significant internal processing and recycling of material and the reduced requirement and utilisation of 'raw' allochthonous material. This further demonstrated the need to maximise retention of material within forest streams as unprocessed allochthonous material is a small component of biofilm biomass.

Yet caution should be given towards the alternative approaches employed for surveys of biofilm characteristics. Although microscopic analysis was useful in this research in providing information on community composition, the technique has limitations in determining proportional contribution from algal cells. My comparison of techniques illustrated that quantities of autotrophic material are often greatly overestimated due to cells being relatively more conspicuous than homogeneous detrital material. Additionally, there is no viable way of determining the origin of unidentifiable detrital material.

Felling at the uppermost Black Burn site (BBCF) within the winter of 2004/2005 had significant impacts on biofilm composition (and thus, potentially on functioning). This event was considered in greater detail in Chapter 7. Unfortunately, the unexpected nature of the event meant that data was limited in areas of water chemistry, and much of the work can consequently only be viewed as pilot data.

The abundance of *Sphaerotilus natans* (a colonial bacterium, often referred to as 'sewage fungi') immediately following felling proved a useful indicator of chemical

and physical modification of stream conditions post-felling, and specifically, significant nutrient enrichment. Detrimental effects of the sewage fungi bloom were observed on invertebrate individuals caught as part of the invertebrate sampling programme, which were encased in filamentous growths. Further, evidence from data collected independently by the Scottish Environmental Protection Agency (SEPA) indicated that total organic carbon (TOC) and dissolved organic carbon (DOC) showed significant increases within the main Minnoch tributary at a site approximately 7km downstream of Black Burn. Although other factors may have contributed to this peak (e.g. seasonal DOC flux), the relative consistency of DOC and TOC at all other times of the year, and the rapid increase coinciding with the felling event, is certainly suggestive of a correlation. This event illustrates the potential for carbon (and potentially other nutrients) to increase in downstream locations following felling.

Despite a significant allochthonous contribution to biofilms post-felling, the very high biofilm biomass found at BB during July 2005 reflects high levels of in-stream retention of externally derived material. Consequently, even biofilms dominated by allochthonous material were found to be important in retaining much of the excess organic material released into the stream system. Retention of organic material within the biofilm removes material from the water column and may reduce impacts downstream. Further, retention of material within the benthos increases the opportunity of on-site processing and may make resources available within systems where inputs of coniferous needles mean that nutrient availability may be low (Dobson and Cariss, 1999).

During felling, it appears that canopy cover and riparian characteristics become increasingly important in controlling biofilm character and thus autotrophic contribution and production. For example, using Chl *a* as a proxy, autotrophic production was higher at the clear-felled and open sites. The functionality of the biofilm, in terms of nutrient retention, was considered most efficient at BBCF due to: 1) the greatest mean biomass (indicating organic matter retention in the benthos), 2) high chlorophyll production (indicating a autotrophic proportion to aid in biofilm function), from low overall autotrophic biomass (suggesting that the algal growth was not significantly contributing to this increase in biofilm dry-weight), 3) the first of the sites to have a significant algal population recovery (Chlorophyeae), and 4) the

site showing the fastest significant reduction in sewage fungus production (indicating relative nutrient depletion). Thus, it is suggested that a greater light availability in-stream at the clear-felled and open sites facilitated the recovery of the autotrophic component of the stream biofilm, which in turn, may have increased the functionality of the biofilm. From this, one might infer that increased light intensity may promote more effective autotrophic functioning of the biofilm and a faster period of recovery post-felling.

However, although 'opening' of the canopy may promote biofilm functioning during all felling events; such benefits cannot be assumed to continue downstream if corridor design differs. BBSH, the lowermost site and the only site at BB, that did not fall within the minimum conditions described within the Forest and Water Guidelines (Forestry Commission, 2003), had limited autotrophic production. Further, despite being positioned at the greatest distance from the disturbance event, the shaded site demonstrated the slowest biotic recovery (as evidenced by chlorophyll recovery rates and the dominance of a nutrient enrichment, anoxic environment-adapted invertebrate species - chironomid bloom). This spatial variation in biofilm functioning reinforces the need to maintain corridors at a width to allow for autotrophic production in-stream and increase the buffering potential of biofilms, thus protecting water quality against pollution and disturbance events upstream as well as on-site.

Chapter 5 considered the ability of benthic macro-invertebrates to indicate in-stream conditions, basal resource dominance, availability and utilisation. Further, measures of diversity within a variety of corridor conditions indicated relative optimal conditions. Specific site types (i.e. corridor, open, clear-felled and broadleaf) proved insignificant in predicting changes in diversity. Multivariate analysis also indicated that catchment, season and specific streams separated groups of assemblage type, but not by site types within any one stream system. Variables related to corridor design (i.e. corridor width, tree height, tree diversity and % light) all had relatively shorter arrows, in the CCA ordination. However, secondary factors which are often dependent on corridor design were found to be of greater influence (e.g. % sewage, % algae, % overhanging vegetation and temperature). This decoupling of the physical variables (light and corridor variables) with allochthonous and autochthonous production may have been due to confounding variables such as altitude, pH and season. Therefore, it is difficult to ascertain how very specific

alterations in corridor design will influence in-stream and riparian diversity without experimental manipulation of controlled sites, and reduction of confounding variables. Rather, it seems more appropriate, from the results presented here, that with the manipulation of general variable trends, it may be possible to improve in-stream and riparian ecology while still being aware of the variance associated with multiple variables and an ecological approach.

Invertebrate diversity was not found to be positively correlated with algal diversity or cover. Instead, the majority of invertebrate taxa were positively associated with riparian tree species diversity and overhanging vegetation cover. Thus, it is concluded that community composition as a result of afforestation (either on-site or upstream), has resulted in a relatively low dependence on autochthonous primary production. Significantly, despite wide-ranging availability of autotrophic production, the majority of invertebrate communities appear to rely on allochthonous resources. However, without more focused research on diet analysis (e.g. isotope or gut contents analysis), the nature of the consumer diet cannot be guaranteed and may be subject to variation of local autotrophic productivity. Further, the contribution of algal material to benthic periphytic material has already (chapters 3 and 4) been shown as variable. Despite the often high degree green colouration of the benthos (used as rapid estimates of algal cover at sites), the actual contribution (and therefore, overall availability of algae) cannot be assumed to be high. A more focussed future approach should consider both the actual content of algae within periphyton at all sites, and also take into account possible opportunistic variation in invertebrate diets and not just FFGs.

Much of my research to characterise autochthonous resources indicated that the availability of autochthonous material is not always significantly influenced by the immediate corridor characterises of the site. There was evidence to suggest some spatial variation of chlorophyll production, with greater production at the two CF sites of T33, and the CF BB site of 2005. However, when data from all biofilms were pooled, the proportional contribution of the autotrophic component remained predominantly low at all sites of the entire sampling period. Only occasional samples from BBOP/CO, 2004 showed any significant autotrophic contributions, comparable with biofilm studies in the literature (e.g. Romani and Sabater, 2000). The reduced site-specific variation in autotrophic biomass (even with variation apparent in site

design) casts doubt on the direct coupling between light availability and algal biomass. Light does not alone influence algal productivity, and variables such as invertebrate grazing, flow and pH may also have influenced the standing crop of autotrophic material. Further, any direct relationship between corridor design and the in-stream algal biomass may have been masked by the influence of turbidity, and in-stream nutrient availability. Therefore, one can conclude that even with modification to corridor design, the impact on autotrophic resources is not predictable and not necessarily reflected in community structure. Thus, to promote biodiversity of invertebrates and the abundance of native salmonids, a more predictable approach may be the maximisation of allochthonous energy inputs and quality. This could be achieved either through the increase of riparian over-story tree diversity, or through corridor widening and the promotion of riparian ground vegetation biomass.

Chapter 6 considered salmonid population variation. Salmonids represented the most economically important taxa considered within this project. Both Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) are considered to be of such high conservation value as to fall within the protection of several different conservation schemes, including Local Biodiversity Action Plans (LBAP), and the Bladnoch Special Area of Conservation (SAC). Yet despite this current focus of protection, these species populations are still vulnerable and show evidence of local population decline (SNH, 2007). Within the study, Atlantic salmon fry were stocked into a number of streams within the Cree (2004) and Bladnoch (2005).

The recovery success, and associated survival rates were low for the salmon stocked. Due to this low recovery, meaningful conclusions about the effects of corridor characteristics and design on salmon fry survival was difficult to draw. In addition, the unexpected presence of brown trout within several sites (which had been chosen due to their apparent isolation from natural populations) confounded results. The differences in habitat preferences and tolerances to abiotic variation meant that it was not possible to use trout as a proxy for salmon survival. However, the presence of trout in a number of sites did not appear to cause additional reduction in salmon survival success (owing to the fact that the most successful of sites also included healthy trout populations).

Intra-stream variation in salmon growth and survival showed some relationship with in-stream macro-invertebrate distribution and relative population abundance. However, ordination analysis of habitat variables indicated that light availability was the strongest parameter influencing salmon size categories, with the largest salmon fry at the most light-intensive sites. Yet, although multivariate analysis indicated weak associations between salmon fry and corridor characteristics, using direct correlation analysis, it was not possible to relate salmon abundance directly to any of the corridor design parameters.

The trout populations appeared to be positively influenced by higher pH, conductivity, alkalinity, benthic invertebrate diversity and abundance. Yet, direct linear regression analysis suggested that only light availability could be used to directly predict trout abundance (negative linear relationship). It was not possible to relate trout abundance directly to any other corridor design parameter. However, even using light (the strongest correlation variable), provided only very limited predictive powers as there was a high level of variance associated with the relationship.

Salmon growth was greater within light intensive sites, whereas trout abundance was correlated with shadier areas. Abundance of salmon was very variable and could not be directly related to light regime, but rather stocking. Consequently, as the overall survival likelihood is greater for larger fish (e.g. Parker, 1971; Juanes, 1994; Elliott, 1989*a*, 1989*b;* Thorpe, 1977, 1989; Wright et al., 1990), the limited results here can be used to tentatively suggest that through promoting open canopies, a mean increase in the relative size of Atlantic salmon, and an increase in population survival, should occur. However this may reduce populations of trout. Whether this is an acceptable trade-off may be geographically subjective (area-specific), and thus local policies and species-specific priorities may also be important in corridor design.

Other implications for management

The primary objective of this project was to explore the aspects of riparian characteristics which would promote the greatest diversity and system functioning in forest streams in Scotland. Apparent in this consideration of different trophic levels, and of allochthonous and autochtonous production, is that maximising the benefits

to one trophic group may be to the detriment of others. Light regime was often fundamental in shaping production and community structure within these ecosystems. However, using light to directly predict the standing crop and characteristic of autochthonous material proved problematic. It is likely that the confounding effects of water colouration, shading by overhanging vegetation, invertebrate grazing, nutrient availability and allochthonous inputs, reduced the direct relationship between light and either autochthonous of autotrophic production. Autotrophic material appeared of little importance to the invertebrate community studied. Thus, it may not be considered a priority in respect to this trophic group. However, increased production of autotrophic material within benthic biofilms has in previous studies, been found to positively influence the functionality of biofilms. Autotrophic biomass has been found to aid retention of organic material, and hence the subsequent removal of that material from the water column. This activity may be an important pollution control mechanism and important in positively influencing the rate of recovery of sites post felling. However, here, estimated functioning of the biofilm material in relation with autotrophic content was variable. The considerable retention of allochthonous material post felling at BB sites, by chlorophyll depleted biofilms, indicated successful drawdown of C-rich material by biofilms, even with low algal contribution. However recovery of the stream as evidenced through biotic indicators provided evidence that sites with open canopies, and greater autotrophic mass, recovered at a greater speed than the downstream, shaded sites. Thus, the spatially specific increase in autotrophic content did appear to positively influence the stream ecological status. Therefore, I would suggest that maximisation of autotrophic material should be considered in corridor design as a route to contributing to pollution control and buffering.

The production of riparian ground vegetation biomass was positively influenced by increased light availability. In addition, vegetation diversity was not detrimentally influenced by high light levels. Allochthonous material appeared to be the primary resource utilised by the majority of benthic invertebrate taxa. Therefore, I conclude that an increase in priority should be given to optimising conditions to riparian ground flora through canopy removal. It is likely that delivery of this resource to the stream, and the relative influence of riparian ground cover were positively related to direct bank-side biomass and specifically, the level of vegetation overhang. The high number of detritus associated macro-invertebrate species suggested that the

assemblages found were reliant on allochthonous material. However, the strong associations with overhanging vegetation and often corridor width, suggests that the preferential allochthonous resource, is derived from ground vegetation rather than riparian trees. I suggest that this preference was responsible for detectable light-associated community compositions, and that riparian biomass, rather than autotrophic production was responsible for much of the positive relationship which occurred between light and both invertebrate and salmon populations and growth. However, there remained a high level of variance in these relationships, making firm conclusions and predictions difficult. Much of the variance may have been due to a variable response and association between algal cover and light. Further, assigning the primary baseline resource to food chains of specific communities and trophic groups may have been complicated by the co-relatedness of autotrophic in-stream production, and riparian production. Additionally, seasonal variation was likely to confound any results from light intensities, water temperatures and autotrophic biomass estimates, independently of site type design.

Although two out of the three sites at Black Burn were within the minimum requirements of the Forest and Water Guidelines (Forestry Commission, 2003), the lack of trees with deep rooting systems (e.g. broadleaf species, such as willows) may have contributed to the significant release of organic material post-felling. Stabilisation of the bank may have reduced in-stream delivery of POM, and other nutrients (as evidenced by the high allochthonous content of the biofilm and the ecological response from sewage fungi and dominant invertebrate taxa). Additionally, the highest vegetation diversity was found within broadleaf sites. As such, I feel that optimal corridor design should integrate broadleaf areas, in order to utilise these benefits. However, the often positive associations of invertebrate diversity, riparian biomass, salmon size, autotrophic in-stream cover estimates, with light (PAR) intensity, emphasises the need to also prioritise open, light intensive corridor designs. Therefore, I would recommend a combined approach to management and design of conifer corridors, which integrates both broadleaf and open unplanted areas. Specifically, the importance of riparian ground-flora which appeared within many of the analyses means that for the majority of sites, I would recommend planting broadleaf trees adjacent to coniferous plantations, in order to aid in bank stabilisation and ground water interception, post felling. Further, to maximise light availability, immediately adjacent to the stream channel and increase biomass of

riparian vegetation and aid in maximising autotrophic biofilm proportion (and thus in-stream buffering by biofilms), I suggest the limitation of all tree growth near the stream edge and removal of coniferous species. This approach should result in high biomass production of riparian ground flora, within the aquatic – terrestrial transition zone, and in-stream autotrophic production within biofilms, two aspects that appear, from my research, fundamental to ecosystem functioning in a system where timber harvest occurs periodically.

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10 Appendices

05/05/2005	Total dry-weight	% Deciduous	% coniferous	% peat
AIRIES	305.25	46.09	53.91	0.00
BBB	10.19	98.68	1.32	0.00
BBCF	208.66	13.01	69.06	17.93
BBOP	191.19	5.15	42.40	52.45
BBSH	2.33	94.15	5.85	0.00
FILI	505.20	26.00	74.00	0.00
GT1COR	140.15	71.54	28.46	0.00
GT1SH	358.25	57.86	42.14	0.00
GT3	153.70	91.92	8.08	0.00
PCF1	251.91	77.81	22.19	0.00
PCF2	262.41	81.39	18.61	0.00
PCOR	7.25	72.06	27.94	0.00
RBR	47.32	32.06	67.94	0.00
ROP	19.22	19.42	80.58	0.00
RSH	644.11	11.21	88.79	0.00
SPPBR	76.61	98.48	1.52	0.00
SPPSH	56.24	80.21	19.79	0.00
T33CF1	47.16	98.26	1.74	0.00
T33CF2	5.01	100.00	0.00	0.00
T33SH	50.68	82.21	17.79	0.00
WOC	41.86	97.72	2.28	0.00
01/06/2005				
AIRIES	600.00	85.99	14.01	0.00
BBB	25.23	32.37	67.63	0.00
BBCF	175.87	12.03	69.49	18.49
BBOP	44.65	0.00	0.00	100.00
FILI	66.96	64.32	35.68	0.00
FILI	488.20	90.47	9.53	0.00
GT1 COR	350.00	91.61	8.39	0.00
GT1COR	130.10	96.48	3.52	0.00
GT1SH	460.00	95.33	4.67	0.00
GT3	23.39	100.00	0.00	0.00
PCF2	156.27	99.12	0.88	0.00
ROP	9.15	77.86	22.14	0.00
RSH	125.34	15.78	84.22	0.00
RSH	260.00	92.48	7.52	0.00
SPPBR	16.72	100.00	0.00	0.00
SPPSH	20.28	77.59	22.41	0.00
T33CF1	11.35	91.15	8.85	0.00
T33CF2	116.94	85.60	14.40	0.00
T33SH	48.26	65.62	34.38	0.00
WOC	1037.00	96.00	4.00	0.00

Fig 10.1. Detrital trap results. Traps were lost, damaged and/or altered by flow, resulting in the dry-weight organic matter results being only a guide to the possible range in allochthonous material available within the catchment sites

Table 10-1 algal taxa classifications, identification codes and TWINSPAN group classifications. Where Chlorophyceae (f) = filamentous taxa.

Twinspan Group	Taxa	Code	Classification
1	Chlorella	CHL	Chlorophyceae
1	cocconeis	COC	Diatom
1	Sphaerotilus	SPH	sewage fungus
2	Navicula	NAN	Diatom
2	Stigeoclonium	STIG	Chlorophyceae (f)
2	Synedra	SYN	Diatom
3	Cladophora	CLAD	Chlorophyceae (f)
4	Amphipleura	AMP	Diatom
4	Ankistrodesmus	ANK	Chlorophyceae
4	Batrachospermum	BAT	Rhodophyceae
4	Coelastrum	COE	Chlorophyceae
4	Cymbella	CYM	Diatom
4	Diatoma	DIA	Diatom
4	Euglena	EUG	Euglenophyta
4	Gonyostomum	GONY	Raphidophyceae
4	Microspora	MICR	Chlorophyceae (f)
4	Microthamnion	MITH	Chlorophyceae
4	Nitzschia	NIT	Diatom
4	Peridinium	PER	Dinophyceae
4	Pinnularia	PIN	Diatom
4	Stephanodiscus	STE	Diatom
4	Tabellaria	TAB	Diatom
4	Trebonema	TREB	Xanthophyceae
4	Ulthrix	ULTH	Chlorophyceae (f)
4	Uroglena	URO	Chrysophyceae
5	Anebaena	ANE	Cyanobacteria
5	Aphanochaete	APH	Chlorophyceae
5	ceratium	CER	Dinophyceae
5	Characium	CHA	Chlorophyceae
5	Chlamydomonas	CHLA	Chlorophyceae
5	Closterium	CLO	Desmidacea
5	Cosmarium	COS	Desmidacea
5	Cyclotella	CYC	Diatom
5	Draparnaldia	DRA	Chlorophyceae (f)
5	Fragularia	FRA	Diatom
5	Frustullia	FRU	Diatom
5	Gloeocystis	GLO	Chlorophyceae
5	Gomphonema	GOM	Diatom
5	Gyrosigma	GYR	Diatom
5	Melosira	MEL	Diatom
5	Meridion	MER	Diatom
5	Mesotaenium	MES	Desmidacea
5	Mougeotia	MOU	Chlorophyceae (f)
5	Oedogonium	OED	Chlorophyceae
5	oocystis	OOC	Chlorophyceae
5	Palmodictyon	PAL	Chlorophyceae
5	Pediastrum	PED	Chlorophyceae
5	Staurastrum	STAU	Desmidacea
5	Stichococcus	STIC	Chlorophyceae
5	Surirella	SUR	Diatom
5	Trentepohlia	TRE	Chlorophyceae (f)

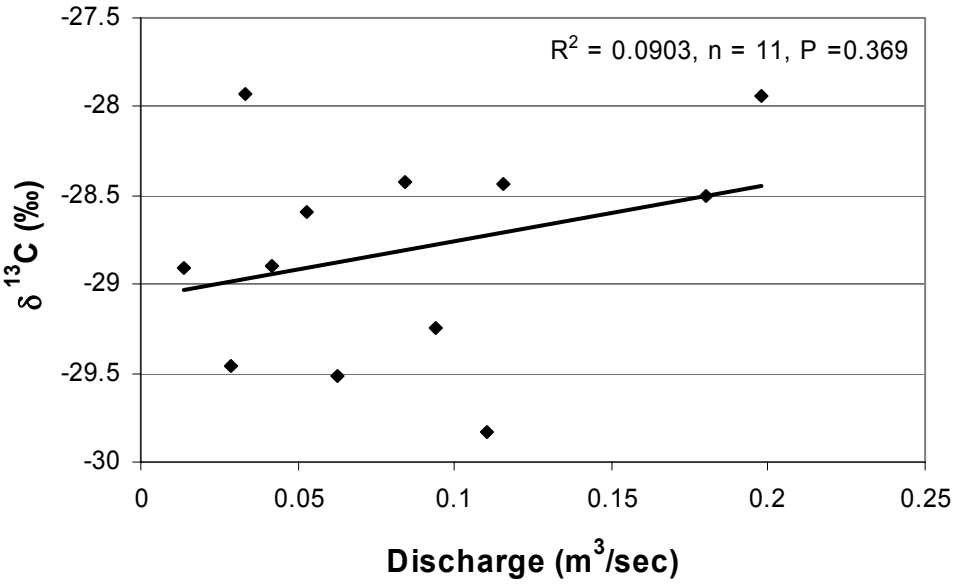

Fig 10.2. Discharge measurements as back calculated from SEPA gauge on Minnoch with $\delta^{13}C$ of BB 2004/2005 data, indicating a positive linear trend, yet r^2 indicates no significance to the relationship

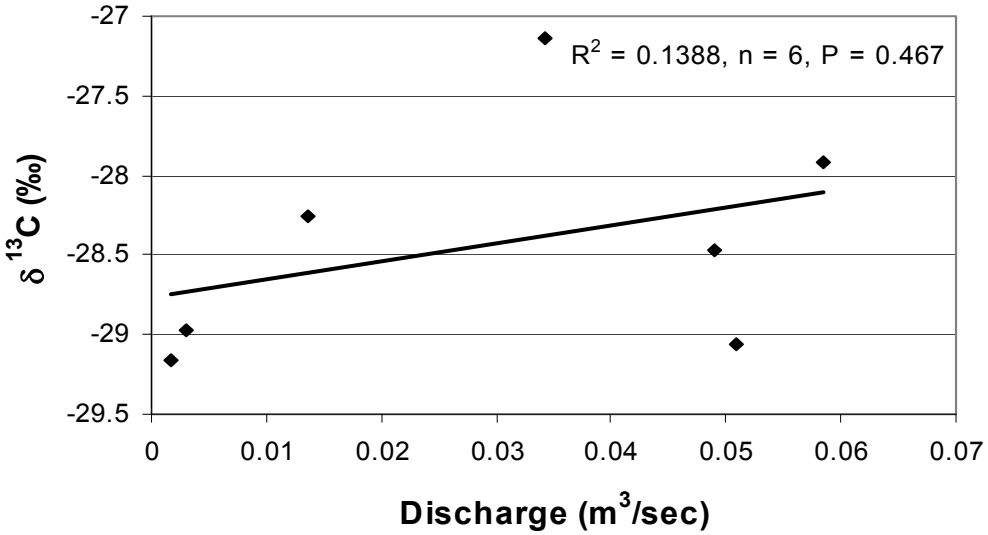

Fig 10.3. Discharge measurements as back calculated from SEPA gauge on Bladnoch with $\delta^{13}C$ of T33 2005 data, indicating a positive linear trend, yet r^2 indicates no significance to the relationship