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Use of Microbial Biofilms to Monitor the Efficacy of a Stormwater Treatment Train

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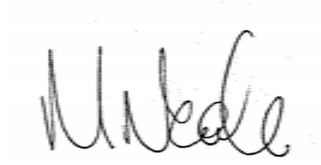

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Use of Microbial Biofilms to Monitor the Efficacy of a Stormwater Treatment Train

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1 Executive Summary

Stormwater is considered to have the single largest impact on the ecological health of urban streams within the Auckland Region. The aim of this study was to test the efficacy of a stormwater 'treatment train' in mitigating the environmental impacts of an open-air car park on the receiving waters of a nearby stream. To achieve this, the structure of bacterial biofilm communities both upstream and downstream of the site of stormwater discharge into the receiving stream were documented and used as a novel indicator of freshwater ecological health. In addition, bacterial communities were sampled within the stormwater pipes (where traditional biological indicators [i.e., fish and macroinvertebrates] are not present) to assess the potential ecological impacts of stormwater at different stages of the treatment train, and to monitor the quality of stormwater throughout the site.

The site of the Albany Busway Park and Ride was used as a case study for the appropriate treatment of urban stormwater. A variety of stormwater treatment strategies have been incorporated throughout the site to provide an integrated treatment train. These include the installation of grassy swales, raingardens, catchpit filters, a large StormFilter treatment device and also a treatment wetland. Stormwater is directed through this treatment train via a network of underground pipes before discharge into Lucas Creek. Much of this soft-bottomed stream consists of high-value, low disturbance sites, and the stream receives a high level of community interest. Lucas Creek contains abundant koura (*Paranephrops planifrons*) and populations of banded kokopu (*Galaxias fasciatus*), long-finned eel (*Anguilla dieffenbachii*) and red-finned bully (*Gobiomorphus huttoni*). However, the surrounding catchment is undergoing rapid development (creating new residential and commercial zones) in the nearby areas of Albany Heights, Fairview Heights and Albany Centre. Effective management strategies are therefore required to minimise the impacts of increasing urbanisation on the ecological health of Lucas Creek.

We monitored changes in bacterial community structure (a sensitive biological indicator of ecosystem health) at 10 different locations within Lucas Creek, both upstream and downstream of the Park and Ride stormwater outlet, and at 9 sites within the stormwater treatment train. Bacterial community profiles were used to provide reliable descriptions of community diversity and composition both within and between all sample sites. A suite a physico-chemical characteristics, including concentrations of biofilm- and-sediment associated metals (e.g., Cu, Zn, Pb) were also recorded for each sampling location.

Concentrations of biofilm-associated Pb, Cu and Zn declined throughout the treatment train. However, concentrations of biofilm-associated Zn remained high (declining to a minimum of 1.3 g kg^{-1} biofilm dry wt. at the end of the treatment train, compared to a maximum of 4.8 g kg^{-1} biofilm dry wt. detected at one sampling location). Unexpectedly, concentrations of arsenic, cadmium, chromium and nickel increased in the later stages of the treatment train (comparing values obtained at the inlet of the stormfilter, and the outlet from the wetland). Indeed, concentrations of biofilm-associated nickel reached a maximum of 190 mg kg^{-1} dry wt. directly downstream of

the StormFilter device. The cause of increased concentrations of these metals closer to the discharge outlet into Lucas Creek remains unclear.

Significant differences in bacterial community structure were detected between sections of Lucas Creek located either upstream, or downstream of the stormwater outlet. However, whilst significant, the extent of these differences was minor. The bacterial community structure within the upstream sections of Lucas Creek was very similar to that within the channel of the stormwater outlet. In addition, bacterial communities within the latter stages of the treatment train were most similar to those within Lucas Creek, suggesting a modification of the stormwater to provide similar environmental conditions to within the stream. Concentrations of the metals (Cu, Pb and Zn) within the sediment of Lucas Creek close to the stormwater outlet remained within ANZECC (2000) guidelines for the protection of freshwater ecological health and did not increase in concentration downstream of the stormwater outlet. Concentrations of Cu, Pb, Zn, As, Cd, Cr and Ni in the stream water of Lucas Creek also remained within the values for the protection of 95% of species in freshwater (ANZECC, 2000). Therefore, both the microbial community, and metal data support that the environmental impacts of the stormwater are reduced throughout the treatment train, ensuring that the recent development of the Albany Busway park and ride car park, and adjoining infrastructure, are causing minimal environmental impact on the receiving waters of Lucas Creek.

2 Introduction

2.1 Background

Stormwater is considered to have the single largest impact on the ecological health of urban streams in the Auckland region (BCG, 2009). Recognising this, the Auckland Regional Council is committed to identifying the environmental effects of stormwater and advocating regional management solutions. Key components of this approach include;

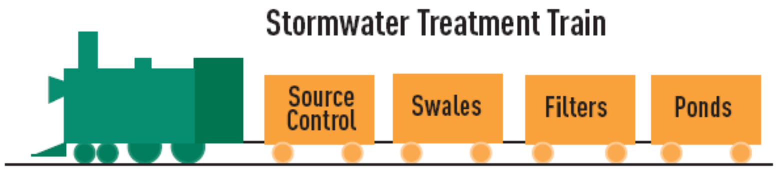
- Improving the cost-effectiveness of existing stormwater treatment practices
- Evaluating new, innovative approaches for removing chemical contaminants from stormwater
- Improving understanding of the cause-effect relationship between stormwater chemical contaminants and effects on life in streams, estuaries and harbours.

In this report, we investigate the efficacy of a treatment train to remove stormwater contaminants originating from the Albany Park and Ride car park located at the northern terminus of Auckland's northern busway. To achieve this, bacterial communities were used as a novel biological indicator of the ecological impact of stormwater at different stages of the treatment train. In addition, bacterial community analysis was used to monitor the effects of the current stormwater discharge on the receiving waters of Lucas Creek.

2.2 Stormwater Sources

Stormwater is a general term applied to water that has accumulated on land as a result of precipitation events and is of concern for two main reasons; (i) flood control and water supply, and (ii) related contaminants carried in the water. The development of land has increased the area of impermeable surfaces (roads, buildings, etc.) that may collect pollutants. These then attenuate until entering rivers and streams following precipitation. The nature of these contaminants is highly variable and site specific. For example, runoff from roofs may contain elevated concentrations of synthetic organic compounds and zinc (from galvanised roofs and gutters) while roads and car parks are major sources of nickel, polycyclic aromatic hydrocarbons (combustions products of gasoline), zinc (from tyres) and copper (from vehicle brake pads). In addition, fertilisers used on lawns are a significant source of nitrates and phosphorus, and herbicides may impact aquatic plant communities in the receiving waters. The complex composition of urban stormwater means that a multifaceted 'treatment-train' approach is frequently seen as a desirable method to manage the cocktail of contaminants present within urban stormwater, a method recently advocated by the Auckland Regional Council (Figure 2.1).

Figure 2.1
Schematic of the stormwater treatment train advocated by the ARC (ARC, 2003).

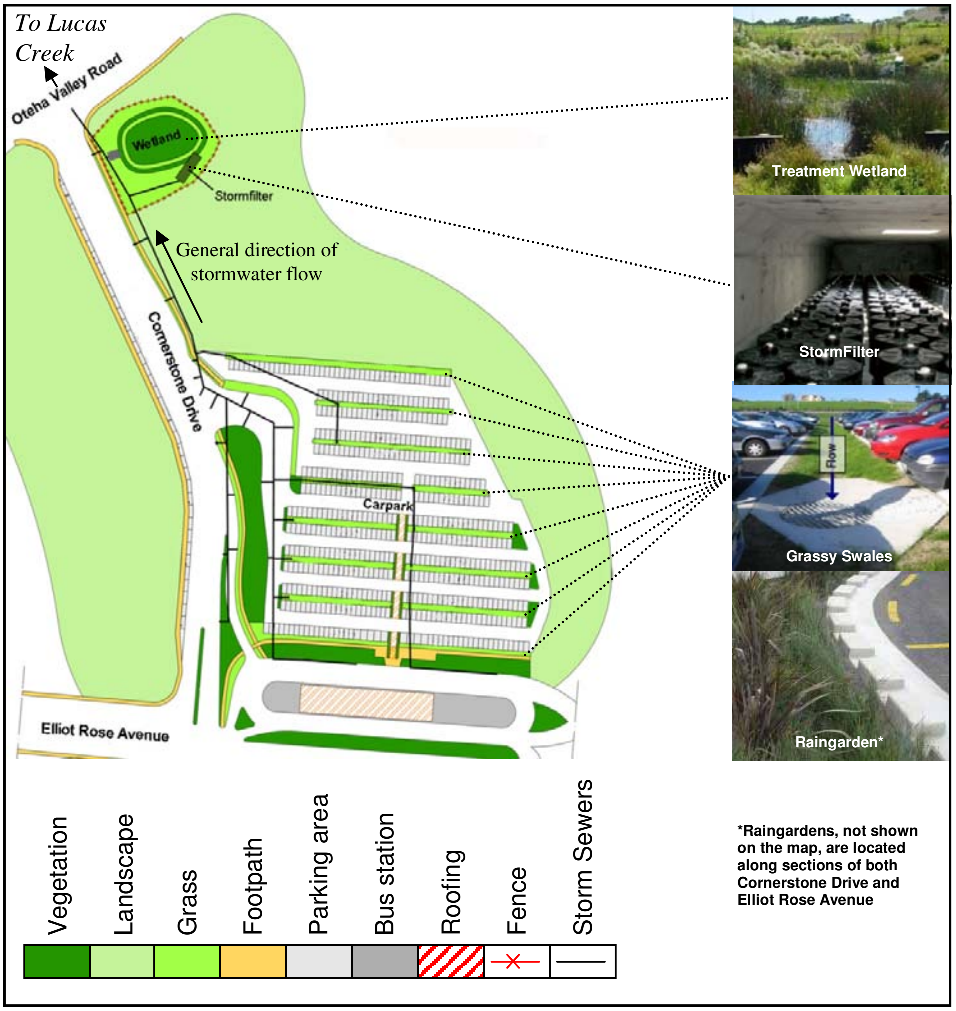


2.3 The Albany Busway: A Case Study in Stormwater Treatment

The objective of this study was to monitor the efficacy of a treatment train to mitigate the biological impacts of stormwater on a freshwater stream. The Albany Park and Ride station (Fig. 2.2) opened in November 2005 as part of the northern busway transport system running alongside State Highway 1 in the North of Auckland. Albany station is an 'offline' station, meaning that it is not connected to the other stations by a physically separated bus route (which currently connects stations from Constellation to Akoranga). It is located within a grassed area in the vicinity of Albany Town Centre, a young and rapidly expanding commercial area. Albany station has dedicated park and ride facilities for ~ 600 cars (located at 36°43'18"S, 174°42'45"E), with another 1000 to be added in later stages to meet future demands.

Different stormwater management strategies are required for the treatment of various stormwater contaminants. For example, biofiltration methods, such as swales and rain gardens are highly effective at removing particulate lead, but have little potential to reduce concentrations of phosphorus and nitrogen within stormwater. For this reason, a stormwater treatment train has been integrated into the Albany Busway site in an attempt to mitigate the effects of the Albany Park and Ride car park on the receiving waters of Lucas Creek. This treatment train, highlighted in Fig. 2.2, includes raingardens, grassy swales and engineered wetlands, as well as more engineered solutions such as Enviropod™ catchpit filters and a 148-cartridge StormFilter (installed by StormWater 360, for more details refer to www.stormwater360.co.nz). Details of each component of the treatment train are provided below.

Figure 2.2:
Map of the Albany Busway site (36°43’18 S, 174°42’45 E) showing stormwater ‘treatment train’. Adapted from a map produced by M. Ort, and re-produced with permission of Auckland Regional Council.



2.3.1 Source Control and use of catchpits

Source control practices are designed to prevent contaminants from entering the stormwater system. In addition to the use of environmentally conscientious practices (proper waste disposal and appropriate treatment of pollutant spills, etc.), 71 Enviropod™ filters have been installed within drains and catchpits throughout the car park, aimed at removing contaminants before they enter the stormwater pipe system. These filter systems consist of a galvanized steel supporting frame housing a removable polyester filterbag, which collect litter, organic debris and pollutants entering the drains, as the stormwater passes through. These filters are an effective, ARC-recognised, pre-treatment device for use in treatment trains, that allow contaminants and debris to be removed from the site for off-site treatment and disposal (for more information, refer to www.enviropod.com)

2.3.2 Raingardens and Swales

Raingardens are located along many of the roads leading into the Albany Busway including Elliot Rose Avenue and the southern end of Cornerstone Drive. These raingardens are designed to drain stormwater from the adjacent roads, into depressions planted with wetland vegetation. Typically, species native to the region are used (mainly grasses, sedges and *Cordyline australis*) as they are more tolerant of the local climatic conditions and are adapted to the prevalent soil and water conditions, negating the need for fertilizer additions. Raingardens reduce the concentrations of contaminants entering the watercourse downstream by enhancing absorption to the soil and encouraging the biological uptake and degradation of contaminants by both plants and associated microbial communities.

Grassy swales provide a similar service as the raingardens and are located throughout the car park, spanning a total length of 600 m. These grassed channels (~1.5 m wide) are also used to separate rows of car parking spaces (without restricting the views throughout the car park), whilst removing contaminants by natural infiltration, absorption and enhanced biological uptake.

2.3.3 Filters

One of New Zealand's largest StormFilters is installed at the Albany Bus Station. The 148-cartridge Stormfilter channels stormwater into an underground chamber and through a series of rechargeable media-filled cartridges (a mixture of zeolite, perlite and granular activated carbon) which trap particulates and adsorbs a wide range of contaminants, including hydrocarbons and heavy metals. These filters are a device which meets ARC TP10 design for the treatment of total suspended solids and contaminants associated with heavy vehicular loads. Whilst StormFilters remain relatively expensive to install, they require infrequent maintenance (every 12-24 months). For more information, refer to www.enviropod.com.

2.3.4 Wetland

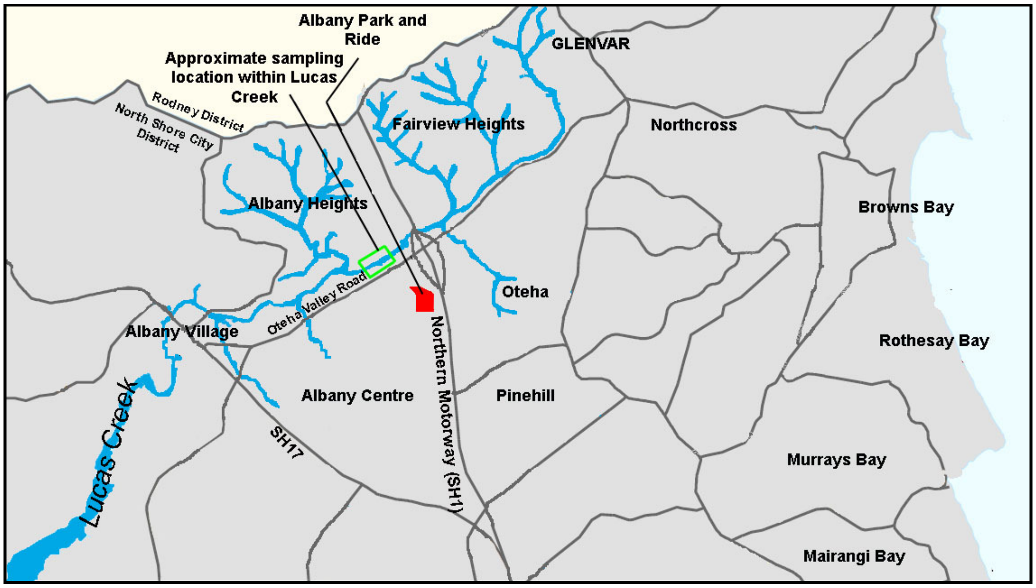
A small polishing wetland is located directly downstream of the StormFilter. The purpose of this wetland is to aid the further removal of stormwater contaminants by enhancing; (i) the retention, settling and adsorption of contaminants within the wetland, (ii) the microbial degradation of pollutants, (iii) plant uptake, and also the degradation of some organic pollutants. Wetlands are of relatively low cost to install and maintain and add both aesthetic and ecological value to the community green space.

2.3.5 Lucas Creek

The major aim of the stormwater treatment train located at the Albany Park and Ride car park is to mitigate the impact of this development on the receiving waters of Lucas Creek (Figure 2.3). Lucas Creek is a soft-bottomed stream draining a catchment of approximately 600 hectares across its 16.3 km length. The large catchment area means that the lower part of the main channel is wider than generally found in North Shore streams (1-5 m).

Lucas Creek contains abundant koura (*Paranephrops planifrons*) and populations of banded kokopu (*Galaxia fasciatus*), long-finned eel (*Anguilla dieffenbachia*) and red-finned bully (*Gobiomorphus huttonii*). However, the catchment of Lucas Creek is undergoing rapid urbanization. Future land use will reduce the area of bush to only 2% of the catchment area, and pasture to less than 1%, compared to a previous cover of 24% in 2005 (NSCC, 2005). Already located within the catchment include Albany Village, North Harbour Stadium, Albany Mega Centre and Northridge Plaza. New residential developments are both planned and currently in progress within the Albany Heights and Fairview Heights areas, and commercial developments continue in the area surrounding the Albany Mega Centre. As a consequence, there are numerous areas of recently exposed earth within the catchment. The catchment is bisected by major roads, including the Northern Motorway (SH1) and Oteha Valley Road, which runs adjacent to the upper reaches of Lucas Creek.

Figure 2.3:
Location of Lucas Creek and its tributaries, shown in blue (widths of the creek and tributaries are not to scale). The location of the Albany Bus Station car park is shown in red and the approximate location of the Lucas Creek sampling site shown in green. Major roads are shown in dark grey.

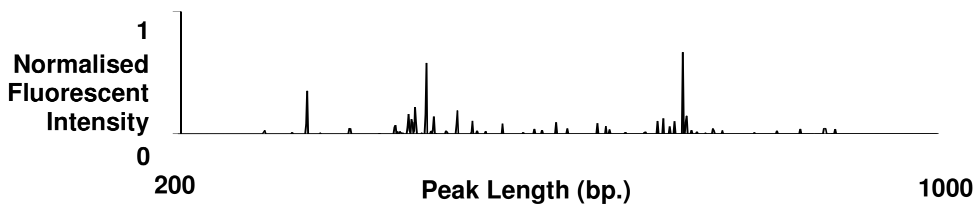


2.3.6 Bacterial Communities as an Indicator of Water Quality

Biological indicators such as communities of fish and macroinvertebrates have been widely used to provide an index of overall ecosystem health (Araujo *et al.*, 2000; Whitfield & Elliott, 2002; Adams *et al.*, 2005; Seilheimer & Chow-Fraser, 2006). However, a number of recent studies have revealed that the analysis of bacterial communities (by Automated Ribosomal Intergenic Spacer Analysis - ARISA) can also provide a sensitive measure of the extent of ecosystem degradation, especially within highly impacted freshwater streams (Lear *et al.*, 2009a; Lear *et al.*, 2009b). This PCR-based method creates a fingerprint of microbial community structure from profiles of the 16S-23S intergenic spacer (IGS) region of the bacterial genome, based upon the length of the amplified nucleotide sequence, which displays significant heterogeneity between species. In the present study, community-specific ARISA profiles are used to provide reliable descriptions of bacterial community diversity and composition (Fig. 2.4) within the enclosed stormwater channels of the Albany Busway treatment train (where traditional biological indicators [i.e., fish and macroinvertebrates] cannot be used) and in the receiving waters of Lucas Creek.

Rather than sampling bacteria within the water column, we assessed communities associated within microbial biofilms. Biofilms are complex assemblages of microorganisms within a protective, adhesive matrix of extracellular polymeric substances, which often account for the large biomass and high diversity of microorganisms that colonise benthic habitats (Romani & Sabater, 2000). The relatively sessile nature of microorganisms within the biofilm increases the likelihood that the abundance of microorganisms within these samples is related to localised influences within the sample site (with a lower representation from transitory bacteria that are continually being washed downstream). The composition of these communities is therefore largely dictated by nutrients, chemical inhibitors, and other growth factors present in the local environment. This analysis of microbial communities introduces a broader temporal aspect than can be achieved with simple chemical and physical monitoring techniques, since the presence of individual organisms are influenced by past, as well as present, conditions.

Figure 2.4:
ARISA profile of a stream biofilm bacterial community within Lucas Creek. Data are peak length of the 16S-23S intergenic regions of bacterial genome (x-axis; in nucleotide base pairs) within the total community, and normalised fluorescent intensity as recorded by a GeneScan automated DNA fragment analyser (y axis; see appendix 9.2). This method creates a 'fingerprint' of the structure of environmental bacterial communities in which each peak may be considered to represent a different bacterial taxon, and peak height represents the relative abundance of each taxon within the total community (note that these assumptions are not strictly true; for a useful review, refer to Bent *et al.* (2007)).



2.3.7 Accumulation of Metals in Microbial Biofilms

Biofilms are known to play a critical role in the transfer of metals and other pollutants into the foodchain (Rhea *et al.*, 2006; Farag *et al.*, 2007). Indeed, concentrations of metals are often greater in stream biofilms than sediments (Schorer & Eisele, 1997; Farag *et al.*, 1998; Holding *et al.*, 2003; Farag *et al.*, 2007) as metals bind strongly to the reactive surfaces on bacterial cell walls or within exuded microbial polysaccharides. In addition, metal containing particles (fine sediments, etc.) are trapped within the microbial biofilm. The strong association between biofilms and metal contaminants mean that they provide a useful, integrative measurement, of the recent exposure of the aquatic community to stormwater pollution events.

2.4 Aims and Objectives

We used the site of the Albany Bus Station car park to test the efficacy of a treatment-train infrastructure in mitigating the environmental impacts of stormwater on the receiving waters of Lucas Creek. Using the ARISA method of bacterial community analysis, we address two major research objectives:

Objective 1: Determination of the efficacy of the stormwater treatment train.

We sample biofilm bacterial communities at different locations within the stormwater pipes of the engineered treatment train. The bacterial community structure within each sample is then characterised to determine where the greatest differences in bacterial community structure occur. The aim of this objective is to determine which structures in the treatment train have the greatest impact in improving the quality of stormwater discharge into Lucas Creek.

Objective 2: To monitor the impact of the Albany Bus Station car park on the receiving waters of Lucas Creek.

We analyse bacterial populations above and below the stormwater discharge outlet from Albany Bus Station into Lucas Creek. Specifically, we compare the similarity between upstream and downstream communities to determine if significantly different bacterial populations reside on either side of the stormwater drain, which would provide evidence of a negative environmental impact caused by the stormwater outlet.

3 Methods

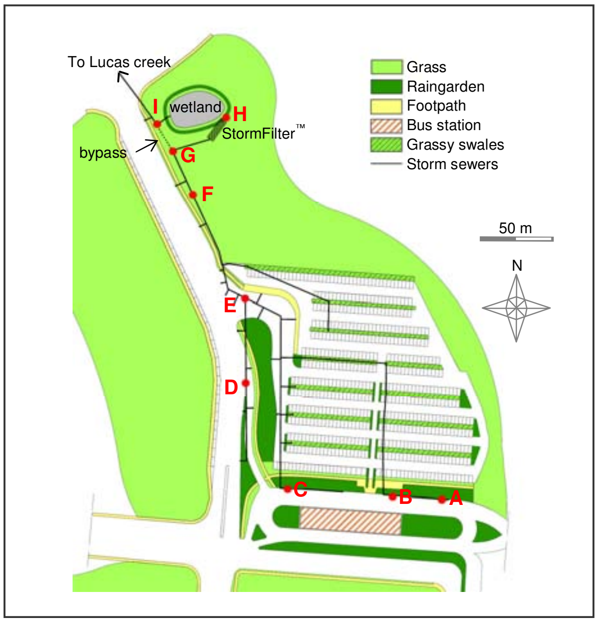
3.1 Site Description

To minimize the impact of the Albany Busway and associated car park on the ecology of Lucas Creek, a stormwater 'treatment' train has been engineered at the site, including raingardens, grassy swales, a retention wetland and the second largest stormfilter currently in operation in New Zealand. Our sampling procedure was designed to monitor changes in biofilm bacterial community structure (a sensitive biological indicator of ecosystem health) and metal content throughout the treatment train and also within the receiving waters of Lucas Creek.

3.1.1 Albany Park and Ride Treatment Train

Manholes provided access to underground stormwater pipes throughout the treatment train (Fig. 3.1). Sites A, B and C drain untreated stormwater from the busway. Site D drains untreated stormwater originating from Cornerstone Drive. Site E drains stormwater from the car park and is downstream of sites A, B, C and D. Sites F and G are located further downstream with additional inputs of untreated stormwater from Cornerstone Drive. Site H is located at the entrance to the wetland, downstream of the StormFilter. Finally, site I is located downstream of the wetland. The biofilm bacterial communities within each sampling site were assessed on 29.01.09 and 26.03.09. The concentration of biofilm-associated metals were analysed for samples taken on 26.03.09.

Figure 3.1:
Map of the Albany Busway site (36°43'18 S, 174°42'45 E) showing sampling locations (where manholes provided access to the stormwater drains). Adapted from a map produced by M. Ort for the Auckland Regional Council.



3.1.2 Lucas Creek

All of the stormwater drained from the Albany Busway is channelled (via the StormFilter and wetland) into Lucas Creek at the location shown (from a stormwater pipe originating downstream (left) of the wetland; Fig 3.2). Untreated stormwater draining Cornerstone Drive and Elliot Rose Road is also channelled into Lucas Creek at the same location (from the stormwater pipe to the left of Cornerstone Drive; Fig 3.2).

The outlet of this stormwater pipe is at site 'S', where the stormwater enters a small open channel (~5 m in length) before entry into Lucas Creek (Fig. 3.3). Within Lucas Creek, five sample sites were located upstream of this stormwater outlet (sites 1 to 5) and five located downstream (sites 6 to 10). Sample sites were located approximately 5 m apart.

Bacterial community structure was obtained for all sample dates and locations within Lucas Creek. Sediment and biofilm associated concentrations of Cu, Zn and Pb were analysed for all sampling locations during 30.01.09. Additional samples were analysed for concentrations of biofilm associated As, Cd, Cr, Cu, Pb, Ni and Zn at sample locations 1, 10 and the stormwater outlet on each sampling date (30.01.2009, 26.02.09 and 26.03.09). Concentrations of metals within the stream water were assessed on only 30.01.09.

Figure 3.2:
Map showing sampling locations (1 to 10) within Lucas Creek (36·43'11 S, 174·42'34 E). Stormwater from the Albany Busway is channelled into Lucas Creek via a drain running under Oteha Valley Road (reaching sampling location 'S').

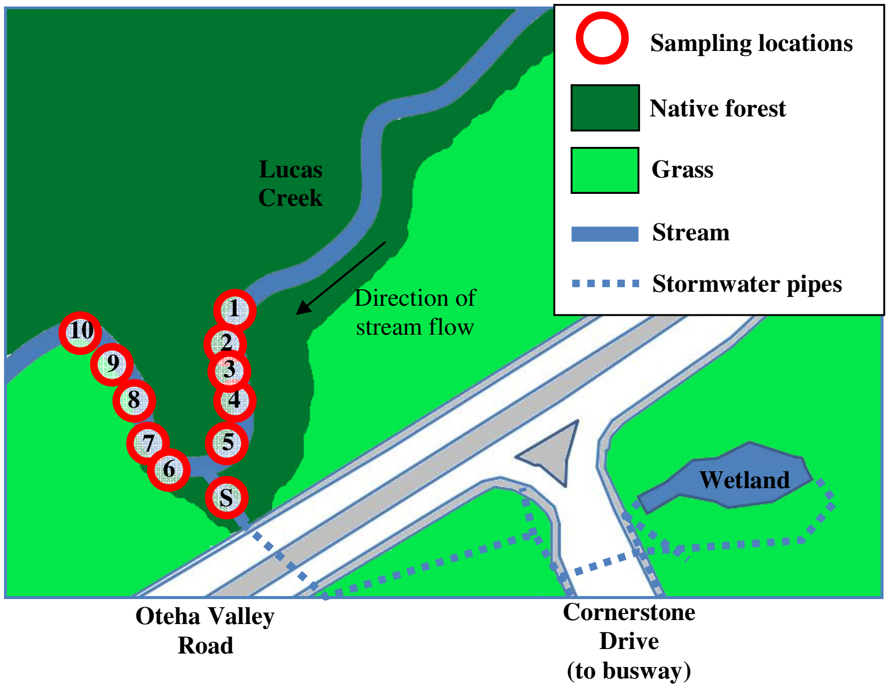


Figure 3.3:
Photograph showing sampling site 'S' within Lucas Creek, the outlet of a drain channelling stormwater from the Albany Busway, into Lucas Creek. Sediment immediately surrounding the outlet is 'red' in colour, presumably from deposited metal oxides.

3.2 Sampling Procedure

Samples were removed from Lucas Creek on three occasions (30.01.09, 26.02.09 and 26.03.09), while samples were removed from the stormwater drain infrastructure of the Albany Busway on two sampling occasions (29.01.09 and 26.03.09) during this period. Within Lucas Creek, biofilm biomass was removed from the surface of six rocks at each sampling site (three for microbiological analysis, and three for the analysis of biofilm associated metals) using the approach outlined in section 3.2.2. In addition, one water sample and one sediment sample was also obtained for each sample site (both *c*. 50 ml). Within the treatment train, six biofilm samples were removed from within the stormwater pipes at each sampling site (three for microbiological analysis and three for the analysis of biofilm associated metals).

3.2.1 Collection of Physico-Chemical Stream Data

Within Lucas Creek, stream physical parameters were recorded during each sampling occasion to measure spatial and temporal differences in a range of environmentally relevant parameters. The flow rate of water was measured 2.5 cm above each rock sampled using a FP101 Flow Probe (Global Water, CA., U.S.A.). Incident light was measured underwater at the surface of each rock sampled using a photometer (Li-Cor LI-185B; Design Electronics, Palmerston North, New Zealand). Water temperature, pH and dissolved oxygen were recorded using a Multi 350i measuring instrument (Wissenschaftlich-Technische Werkstätten, Germany). Stream depth was also noted.

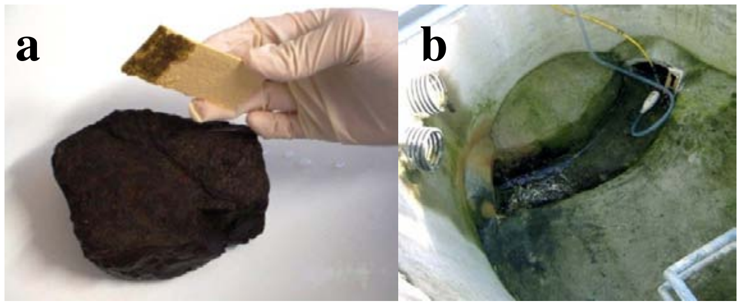
(The analysis of samples for metal data is described in section 3.3)

3.2.2 Collection and Processing of Biofilm Samples

At each sampling location in Lucas Creek, sample rocks were removed from the water and biofilm scraped from the entire surface using a fresh Speci-Sponge™ (VWR International, Arlington Heights, IL, U.S.A.) taking six samples for each sampling location and date (Fig. 3.4). Samples were similarly removed from the base of stormwater drains (swabbing an area of approx. 100 cm^2 for each sample). Following biofilm collection, Speci-sponges™ were placed into individual Whirl-Pak® bags (VWR International, Arlington Heights, IL, U.S.A.) with *c*. 15 ml sterile water to ensure complete immersion, and sealed. Samples bags were transported to the laboratory in darkness, on ice. To separate biofilm biomass from the sponges, samples were macerated using a stomacher (Lab Stomacher 400, Seward, Norfolk, UK) for 90 s at a high speed. Sponges were then squeezed to remove the entire sample material and transferred into centrifuge tubes before the biofilm biomass was pelleted by centrifugation (8000 *g*, 20 min)

Figure 3.4.

A Speci-sponge™ is used to remove biofilm biomass. (a) For stream rocks, the entire surface area of each rock was swabbed using a different sponge. (b) For stormwater pipes, an area at the base of the stormwater pipe (~ 100 cm^2) was swabbed for each sample.



3.3 Processing of Samples for Metal Analysis

To analyse concentrations of metals within biofilm, sediment and water, samples were sent to Hills Laboratories (Hamilton, New Zealand). Stream water samples were tested for concentrations of dissolved trace levels of the heavy metals As, Cd, Cr, Cu, Ni, Pb and Zn, following filtration at 0.45 µm. Samples of biofilm and sediment were dried (at 55 °C) and sieved (mesh size ~250 µM) to remove coarse fractions and to provide a consistent sample fraction which was then sent for analysis. Biofilm and sediment samples were analysed for total recoverable concentrations of As, Cd, Cr, Cu, Ni, Pb and Zn, following nitric/hydrochloric acid digestion using US EPA method 200.2.

3.4 Community Fingerprinting of Bacterial Biomass

DNA was extracted from pelleted biofilm samples within 24 h of collection using a modified method of Miller *et al.* (1999). This method combines a bead-beating methodology with chloroform-isoamyl alcohol extraction, followed by precipitation of the extracted DNA with isopropanol. Further details of this approach are provided in Appendix 9.1. The bacterial diversity of biofilm communities, including the unculturable component, was assessed then using Automated Ribosomal Intergenic Spacer Analysis (ARISA). This PCR-based method creates ‘fingerprints’ of microbial communities from profiles of the 16S-23S intergenic spacer (IGS) region of bacteria, based on the length of the amplified nucleotide sequence. This method enables sensitive descriptions of community diversity and composition to be attained with a high level of taxonomic resolution. This method has recently been used for the evaluation of aquatic bacterial communities (e.g. Jones *et al.,* 2007; Lear *et al.,* 2008; Lear *et al.,* 2009 a, b, c). Further details of this approach are provided in Appendix 9.2.

3.5 Statistical Analysis

GENEMAPPER software (v 3.7) was used to convert fluorescence data (from ARISA) into electropherograms, which enable a comparison of the proportional quantities of different-sized DNA fragments in each sampled community. To visualize multivariate patterns in community structure based on the bacterial ARISA data, multi-dimensional scaling (MDS) was done on the Bray-Curtis matrix. All statistical analyses were done using the PRIMER version 6 computer program (Primer-E Ltd., Plymouth, UK). Further details of the statistical procedures using in this study are detailed in Appendix 9.3.

4 Results

4.1 Stormwater Treatment Train

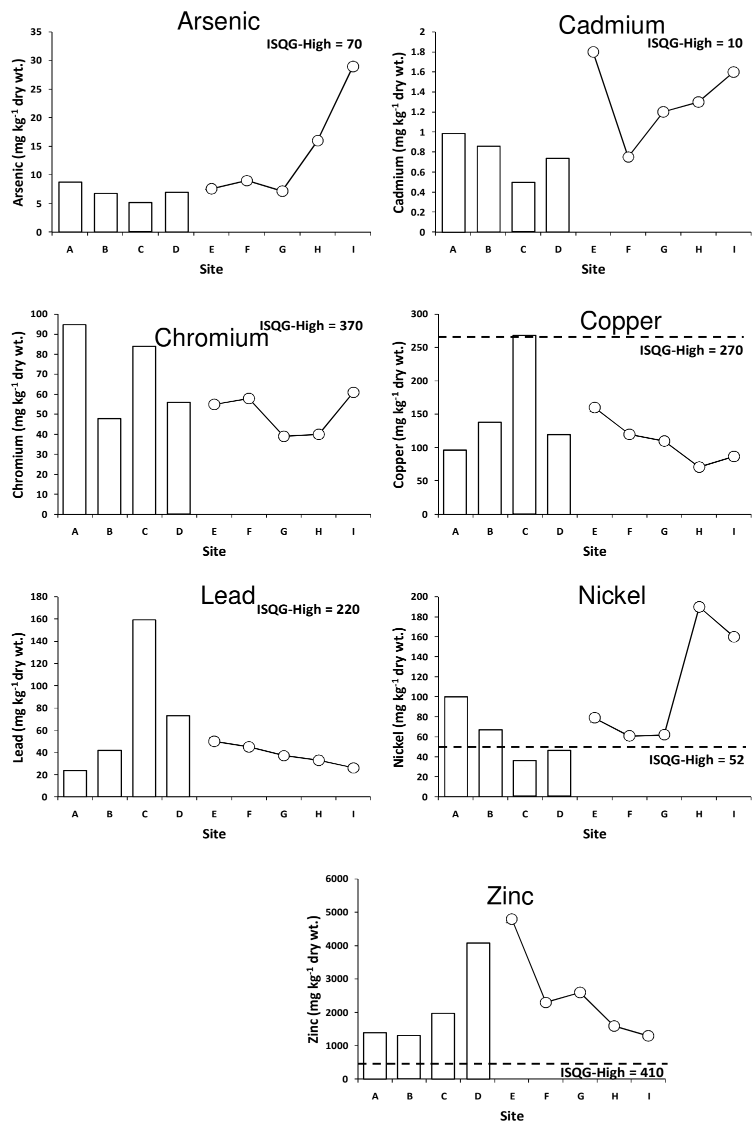
4.1.1 Concentrations of Biofilm Associated Metals

Concentrations of zinc exceeded ANZECC interim sediment quality guideline (ISQG)-High values (ANZECC, 2000) for sediment within every sampling location, reaching a maximum of 4800 mg kg^{-1} dry wt. at site E, directly downstream of the car park (Fig. 4.1). Concentrations of zinc declined further downstream to levels similar to sites located upstream of the car park (1,000-2,000 mg kg^{-1} dry wt. in sites A, B and C). Concentrations of arsenic, cadmium, chromium and lead were below ISQG-high values at all sample locations. However, concentrations of arsenic increased following passage through the StormFilter (site H and I), reaching a maximum of 29 mg kg^{-1} dry wt. (exceeding the ISQG-low trigger value of 20 mg kg^{-1} dry wt.). Similarly, concentrations of nickel increased downstream of the StormFilter (sites H and I), reaching a maximum of 190 mg kg^{-1} dry wt. at site H, nearly four times greater than the ISQH-high value for sediment. Concentrations of copper were generally below the ISQG-high and –low trigger values at every location. Interestingly, there is little evidence for any decrease in the concentration of metals by the StormFilter treatment (i.e. the difference between sampling sites G and H) except for zinc, and possibly copper. Conversely, concentrations of arsenic, cadmium and nickel increased in the biofilm directly downstream of the StormFilter.

ISQG-values for sediment are provided throughout this study as there are currently no recommended trigger values for biofilm associated metals. Therefore, because a biofilm sample exceeds the guideline concentration for sediment may not mean that the community in the receiving waters are of significant risk of ecological impact. Nevertheless, it is worth noting that concentrations of zinc observed within these biofilm samples reach a maximum concentration more than 10 times higher than the ANZECC ISQG-High trigger value for sediment.

Figure 4.1:

Concentrations of As, Cd, Cr, Cu, Pb, Ni and Zn (mg kg^{-1} dry wt. of biofilm) at different sampling locations within the Albany Busway Park and Ride treatment train. Data were collected from pooled samples, combining the biofilm biomass of three sponge samples, and not replicated. The dashed lines show the high interim sediment quality guideline (ISQG-High) values (ANZECC 2000) which indicate possible risk to environmental health. The data for sites E to I are plotted as a line as stormwater passes through each site, sequentially.



4.1.2 Bacterial Community Structure

Multidimensional scaling (MDS) was done to visualise multivariate patterns in bacterial community structure based on the ARISA data generated from each biofilm sample (Fig. 4.2 & 4.3). Similar patterns in bacterial community structure were observed on both sampling occasions where significant differences were detected between sampling sites (PERMANOVA, $P < 0.0001$), but not within sampling sites (PERMANOVA, $P = 0.98$). For both sampling dates, similar bacterial community structures were detected for sites A and B, which were significantly different from sites C and D (PERMANOVA, $P < 0.0001$). The community within site E, located downstream of the car park area was significantly different from that of sites A, B, C and D (PERMANOVA, $P < 0.0001$). No significant differences were detected in bacterial community structure between sites E, F and G (downstream of the car park area), or between sites G and H (which are located at the inlet and outlet of the StormFilter device; PERMANOVA, $P < 0.0001$).

Figure 4.2:

Differences in bacterial community ARISA profiles from different sections of the Albany Busway treatment train sampled in January 2009. Plots are non-metric multidimensional scaling of bacterial ARISA data, derived from a Bray-Curtis matrix of samples. Letters refer to sampling sites detailed in Fig. 3.1. 2D stress = 0.16.

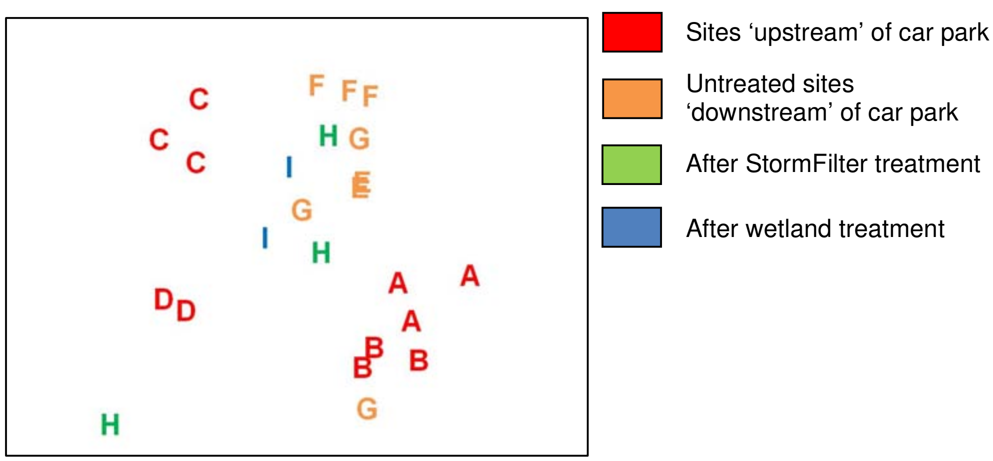
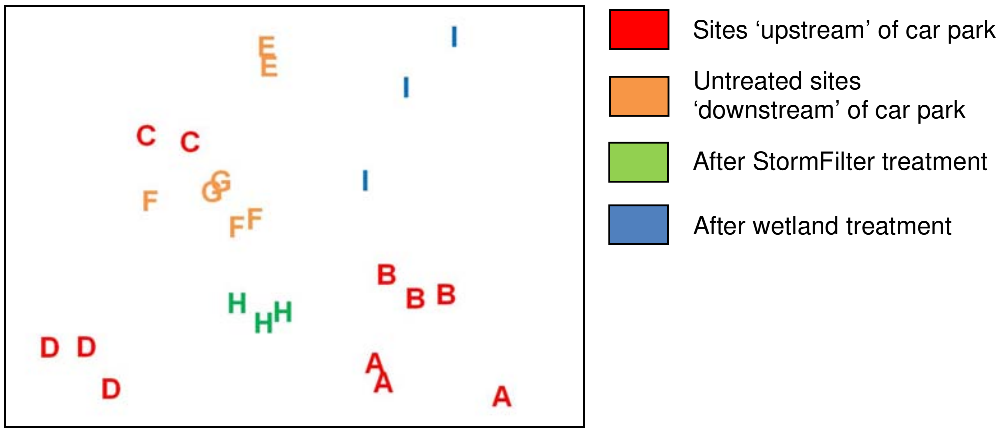


Figure 4.3:

Differences in bacterial community ARISA profiles from different sections of the Albany Busway treatment train sampled in March 2009. Plots are non-metric multidimensional scaling of bacterial ARISA data, derived from a Bray-Curtis matrix of samples. Letters refer to sampling sites detailed in Fig. 3.1. 2D stress = 0.20.



4.2 Lucas Creek

4.2.1 Physico-Chemical Stream Data

Concentrations of biofilm-associated metals were greater than concentrations of sediment-associated metals in every sample obtained (paired t-test, $P \leq 0.05$ for Cu Pb and Zn). Concentrations of Cu, Zn and Pb within the sediment of Lucas Creek were below ISQG-high and –low trigger values (ANZECC, 2000) at every sampling location (Fig. 4.4) Concentrations of metals in either biofilm or sediment did not differ significantly between sampling sites located upstream or downstream of the stormwater outlet (student t-test, $P > 0.05$), except for concentrations of biofilm associated Pb, which were greater in upstream sections (average = 17.8 ± 0.97 mg kg^{-1} dry wt., compared to 13.0 ± 0.58 mg kg^{-1} dry wt. downstream; t-test, $p = 0.008$). In addition, concentrations of all metals were generally similar for the stormwater outlet as for the surrounding sampling sites within Lucas Creek.

Trends in the concentrations of As, Cd, Cr, Cu, Pb, Ni and Zn within Lucas Creek biofilm samples were broadly similar between sampling dates (Fig. 4.5). Concentrations of Cd were below detection, whilst concentrations of Ni and Zn were greater than 50 mg kg^{-1} (dry wt. biofilm) at all sample locations and exceeded ANZECC (2000) ISQG-High guideline values. Concentrations of Zn were at least twice as high within the stormwater outlet than in the sampling locations upstream during January and March. However, there appeared to be little difference in the concentration of any biofilm-associated metals between sampling sites located upstream or downstream of the stormwater outlet (noting that samples were not replicated for any sampling date).

Figure 4.4.

Concentrations of Cu, Pb and Zn (mg kg-1 dry wt.) in sediment (left) and biofilm (right) within: (☐) Lucas Creek sites located upstream of the stormwater outlet; (■) Lucas Creek sites located downstream of the stormwater outlet; (■) the stormwater outlet. Data for each site were collected from pooled samples, combining either the biofilm biomass of three rocks or three 50 mL sediment samples and not replicated. Labels on the x-axis refer to sampling location within Lucas Creek, SWO is stormwater outlet. The dashed lines show the high interim sediment quality guideline (ISQG-High) values (ANZECC, 2000) which indicate possible risk to environmental health.

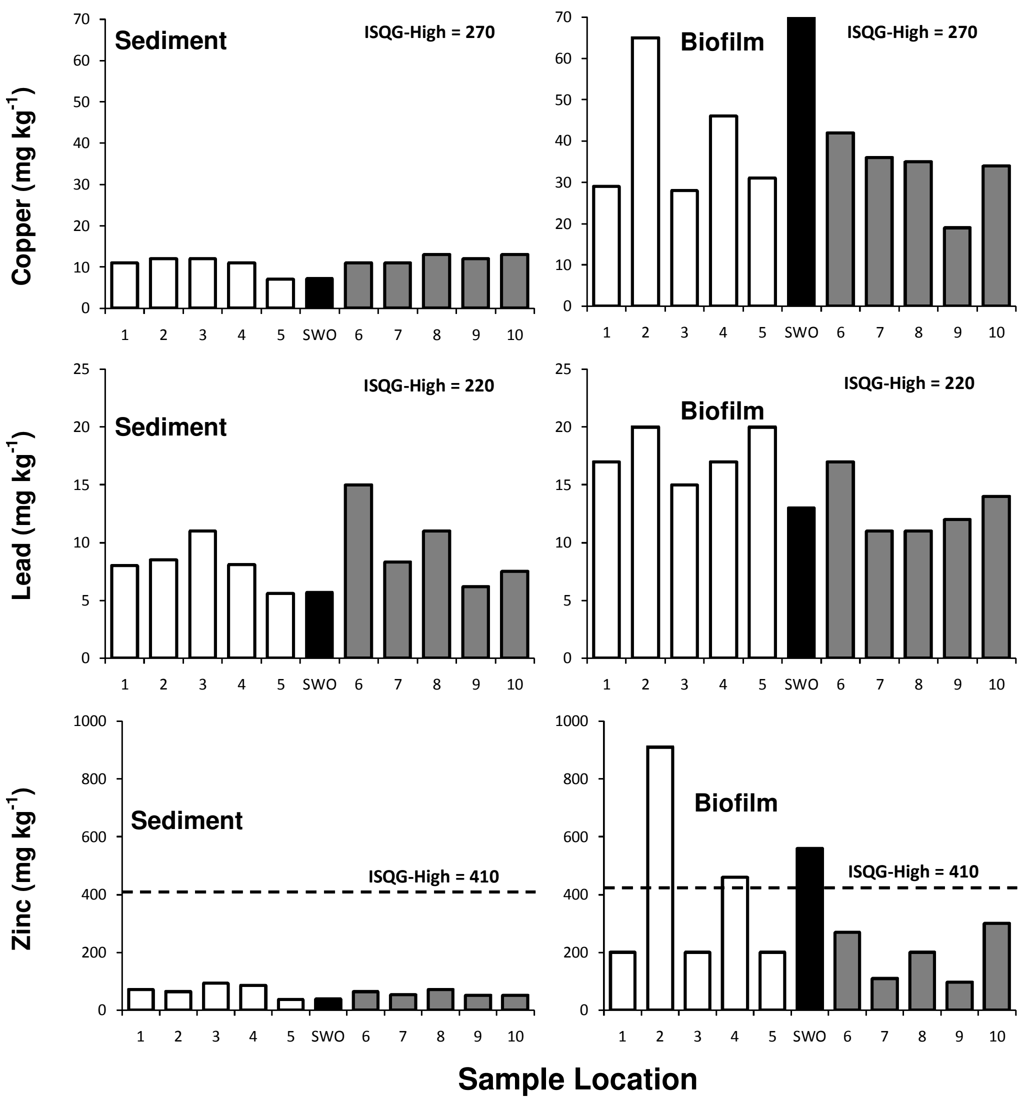
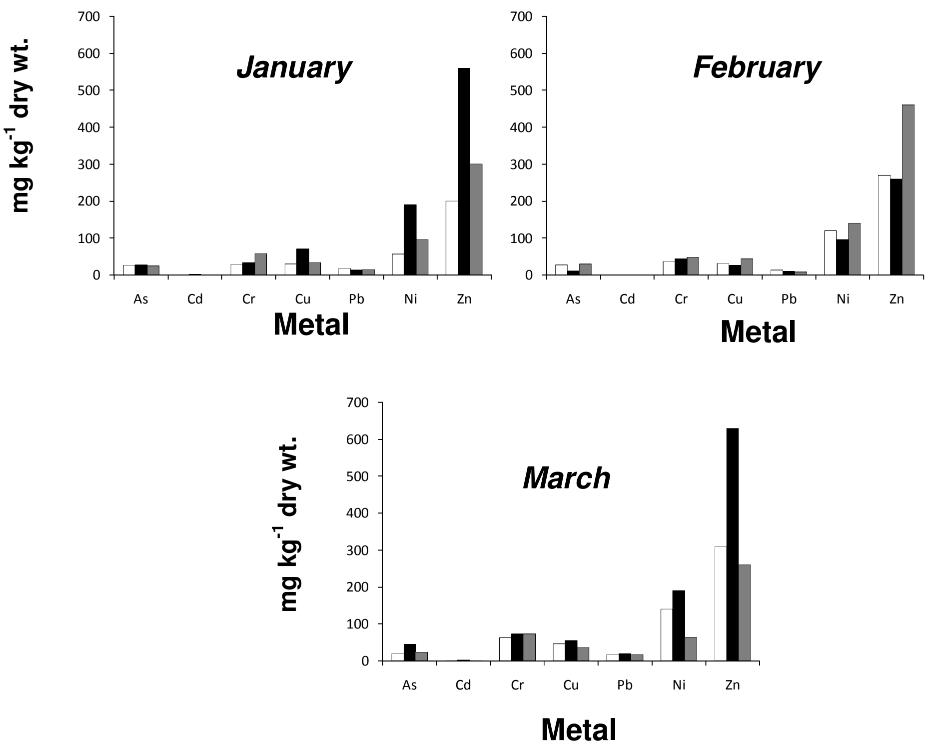


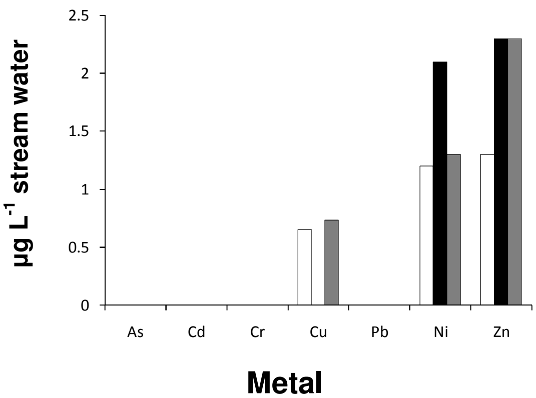
Figure 4.5.

Concentrations of As, Cd, Cr, Cu, Pb, Ni and Zn (mg kg^{-1} dry wt. of biofilm) at different sampling dates within: (☐) Lucas Creek sites located upstream of the stormwater outlet; (■) Lucas Creek sites located downstream of the stormwater outlet; (■) the stormwater outlet. Data for each site were collected from pooled samples, combining the biofilm biomass of three rocks and not replicated. ANZECC (2000) high interim sediment quality guidelines are 70 mg kg^{-1} (dry wt.) arsenic; 10 mg kg^{-1} (dry wt.) cadmium; 370 mg kg^{-1} (dry wt.) chromium; 270 mg kg^{-1} (dry wt.) copper; 220 mg kg^{-1} (dry wt.) lead; 52 mg kg^{-1} (dry wt.) nickel; 410 mg kg^{-1} (dry wt.) zinc.



Concentrations of As, Cd, Cr and Pb were all below detection (<0.001 ppm, < 0.00005 ppm, < 0.0005 ppm and < 0.0001 ppm, respectively) in the streamwater and stormwater outlet (Fig. 4.6). Cu was detected within Lucas Creek, but not within the stormwater, indicating the Cu is derived from a source further upstream. Concentrations of both Ni and Zn exceeded 2 mg L^{-1} water, however none of the metals exceeded trigger values for the protection of 95% of species in freshwater (1.4, 11 and 8.0 μg L^{-1} for copper, nickel and zinc, respectively; ANZECC (2000)).

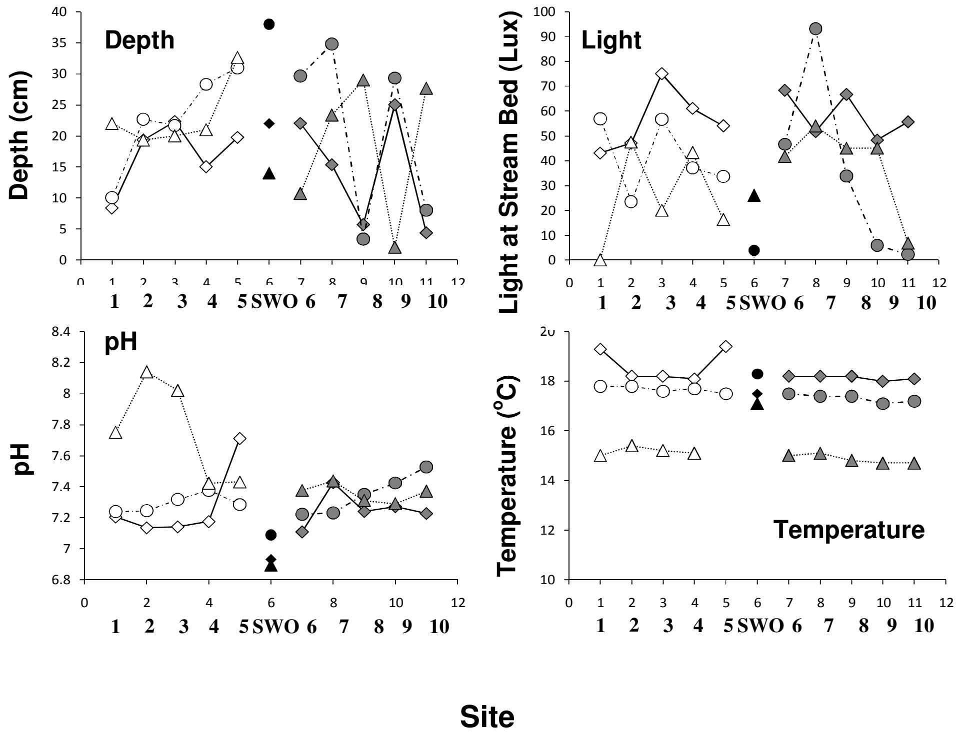
Figure 4.6.
Concentrations of As, Cd, Cr, Cu, Pb, Ni and Zn (µg L- stream water) (☐) within Lucas Creek, upstream of the stormwater outlet; (■) within Lucas Creek, downstream of the stormwater outlet; (■) within the stormwater outlet. Data for each site are not replicated.



The average depth of the stream was 20.0 cm and the average pH of the stream water was pH 7.34 (Figure 4.7; all sampling dates and locations combined). No significant differences in stream depth, pH or light at the stream bed were detected (student t-test, P > 0.05) between sampling dates or sections (pooling data obtained from upstream and downstream sampling locations; sites 1 to 5, and 6 to 10). Significant differences (student t-test, P < 0.05) in the temperature of the stream water were detected between sampling dates, being coolest in March (an average of 15.2 °C, all sampling locations combined), and 0.5 °C cooler within the downstream sampling locations (6 to 10), all sampling dates combined.

Figure 4.7.

Measurements of physical stream characteristics during (◇) January; (○) February; (△) March for sample sections located (white) within Lucas Creek, upstream of the stormwater outlet; (grey) within Lucas Creek, downstream of the stormwater outlet; (black) within the stormwater outlet.



4.2.2 Bacterial Community Structure

Significant differences in bacterial community structure were detected between sample sites located upstream, or downstream of the stormwater outlet (PERMANOVA, $P \leq 0.001$; Fig. 4.8). The bacterial community within the stormwater outlet was marginally more similar to the bacterial communities recorded downstream of the stormwater outlet, than communities upstream (similarities 43 and 41, respectively, where PRIMER similarity values ranged from 0 to 100 [perfect similarity]).

Significant differences in bacterial community structure were detected between stream samples located either upstream, or downstream of the stormwater outlet for each sampling date (PERMANOVA, $P < 0.05$), forming distinct groups on MDS plots (Fig. 4.9). In February and March, there was no difference in the average richness of bacterial taxa within samples between sampling locations upstream or downstream of the stormwater outlet (student t-test, $P = 0.63$ and 0.53, respectively). However, in January, bacterial taxon richness was significantly higher downstream of the stormwater outlet (t-test, $P = 0.02$, for more details of average bacterial taxon richness between sample sites, refer to appendix 9.6, 9.7 and 9.8)

Figure 4.8:
Differences in bacterial community ARISA profiles from sections of Lucas Creek, combining data gathered on each sampling occasion (January, February and March, 2009). Plot is a non-metric multidimensional scaling of bacterial ARISA data derived from a Bray-Curtis matrix of samples. Data points refer to samples from (△) upstream of the stormwater outlet; (▲) downstream of the stormwater outlet; (+) the stormwater outlet. 2D stress = 0.21.

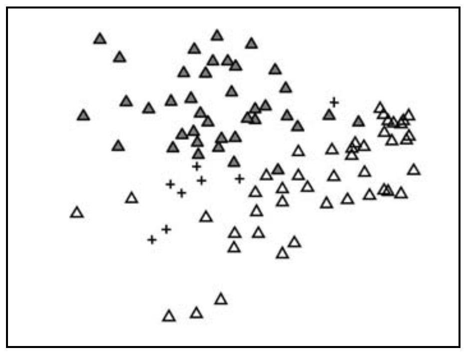
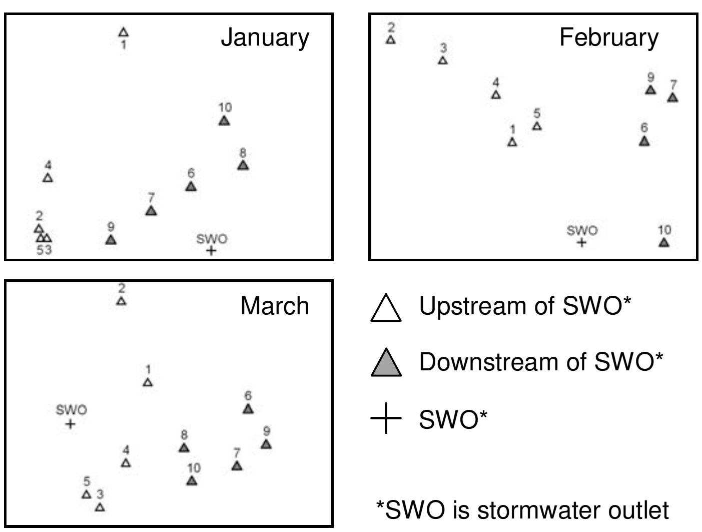


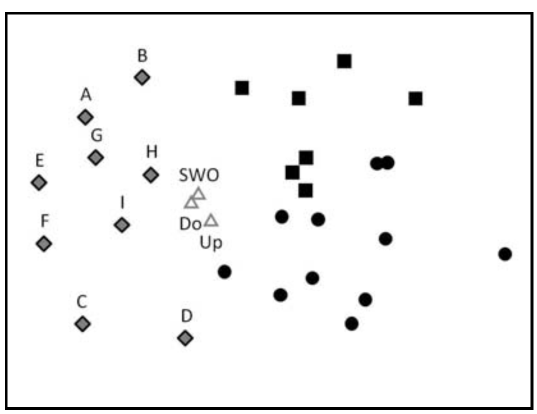
Figure 4.9:
Differences in bacterial community data from different sections of Lucas Creek, sampled in January, February and March, 2009. Plots are non-metric multidimensional scaling of bacterial ARISA data derived from a Bray-Curtis matrix of samples. Data points are averages (an average of three rocks per sampling site). Numbers on plot refer to sampling location within the stream. 2D stress = 0.06, 0.07 and 0.11 for January, February and March respectively.



In Fig. 4.10 bacterial community data collected in this study is compared to data collected from 18 different Auckland streams, located in both rural and urban catchments (data from Lear *et al.* (2009)). Analysis of the ARISA data using multivariate dispersion values (PRIMER MVDISP) revealed similar variability (dispersion value = 1.020) in bacterial community structure among samples obtained from within the stormwater treatment trains (in which the greatest physical distance between sample sites was only 0.3 km), compared to between streams in which the greatest physical distance was ~100 km between sample sites (Ngakoroa Stream (nr. Pukekohe) and the Matakana River (nr. Matakana)) (dispersion value = 0.994).

Samples abstracted from sections of Lucas Creek used in this study varied little in comparison (dispersion value = 0.055) revealing that the stormwater outlet had relatively little effect on the bacterial biofilm community within Lucas Creek. Interestingly, samples from lower sections of the stormwater treatment train (e.g., H and I) were most similar to the communities within Lucas Creek (including sections located upstream of the stormwater outlet).

Figure 4.10:
Differences in bacterial community ARISA profiles from a range of Auckland streams located within: (■) predominantly rural catchments; (●) predominantly urban catchments; (△) sections of Lucas Creek analysed in this study; (◆) sections of the Albany park and Ride treatment train; (Do), sampling sections in Lucas Creek located downstream of the stormwater outlet; (Up), sampling sections in Lucas Creek located upstream of the stormwater outlet; (SWO), stormwater outlet from Albany Busway treatment train into Lucas Creek. Letters A to I refer to sampling locations within stormwater pipes of the Albany Park and Ride treatment train (as detailed in Fig. 3.1). Plot is derived using a Bray Curtis matrix. 2D stress = 0.19.



5 Discussion

5.1 Lucas Creek

This study used the analysis of bacterial biofilm communities to provide a sensitive measure of the ecological impact of stormwater on a freshwater stream. Our study revealed statistically significant differences in bacterial community structure between stream sections located upstream or downstream of a large outlet channelling stormwater from the site of the Albany Park and Ride car park. However, the observed differences in bacterial community structure were relatively small, and no differences in stormwater-associated metals were detected between upstream and downstream sampling locations. This suggests that the stormwater outlet is currently causing minimal disturbance to the ecological health of the receiving waters of Lucas Creek.

5.1.1 Accumulation of Metals in the Sediment and Biofilm

ANZECC (2000) threshold values for the protection of aquatic life (in both sediment and water) were not exceeded for the concentrations of any of the metals monitored in this study. Although concentrations of biofilm-associated zinc and copper were elevated within the stormwater outlet, compared to the stream water, the concentrations of these metals did not increase significantly in the downstream sections of Lucas Creek, presumably due to dilution by the flow of water from upstream. Conversely, concentrations of biofilm-associated lead were lower in the stormwater than in upstream sections of the stream, and were reduced downstream of the stormwater outlet. This suggests dilution of lead in the receiving waters of Lucas Creek by the stormwater from the Albany Busway site. Concentrations of biofilm-associated arsenic, cadmium and chromium were also relatively low within the stormwater outlet and did not increase downstream of the outlet (compared to sections monitored upstream).

High concentrations of trace elements have previously been observed to accumulate within natural biofilm communities exposed to pollutants (Ivorra *et al.*, 1999; Morin *et al.*, 2008). In the present study, concentrations of biofilm-associated metals were consistently higher than concentrations of sediment–associated metals. This may have important implications for aquatic systems, since biofilms are the basis of most aquatic food webs. Therefore, macroinvertebrate communities, particularly those with scraping feeding strategies ingest and accumulate biofilm-associated metals (Farag *et al.*, 1998; Courtney & Clements, 2002), and may provide a concentrated source of metals that can be toxic to predatory fish and other organisms (Kiffney & Clements, 1993). Since aquatic organisms at higher trophic levels are directly affected by intimate contact with microbial biofilm, concentrations of biofilm associated contaminants could provide a more sensitive measure of the effects of human activity freshwater ecosystem health. This warrants further study, since at present, there are no recommended guidelines for acceptable levels of pollutants within microbial biofilms. If, as we expect, the contaminants associated with biofilm communities are capable of providing a more sensitive indicator of the impact of stormwater on freshwater ecological communities, this would provide a significant advance towards the Auckland Regional Councils' goal of 'improving understanding of the cause-effect links between stormwater chemical contaminants and effects on life in streams, estuaries and harbours' (ARC, 2009).

5.1.2 Biofilm Bacterial Community Structure

Only minor differences in bacterial community structure were detected between sampling sites located upstream or downstream of the stormwater outlet. Since no significant differences were noted in concentrations of stormwater-associated metals, these differences may have been caused by variation in physical factors including the reduction in water temperature observed downstream of the outlet, and factors not monitored as part of this study such as differences in substrate composition and habitat heterogeneity. The observed differences in bacterial community structure could also have been caused by certain chemical characteristics of the stormwater, not recorded in this study (such as concentrations of nitrate, phosphorus, or polycyclic aromatic hydrocarbons). Finally, the differences in bacterial community structure between samples sites located either upstream or downstream of the stormwater outlet could be due to the addition of bacteria in to the stream from the stormwater. Different communities of bacteria typically inhabit terrestrial and aquatic environments and during storm events, 'terrestrial' communities of bacteria, originating primarily from sediments surrounding the Albany Park and Ride car park will be washed into the receiving waters of Lucas Creek. These 'immigrant' bacteria will alter the community composition within the stream. Indeed, in January bacterial taxon richness was significantly higher in the downstream sample sections, presumably due to the addition of new populations of the bacteria from the stormwater.

Whatever the cause (urban streams are typically effected by multiple, interacting stressors (Allan, 2004)), the relatively small differences in biofilm bacterial community structure and metal content between the upstream and downstream sections of Lucas Creek provide a strong indication that the stormwater outlet is having little ecological impact on the receiving waters of Lucas Creek. This suggests that either (i) the stormwater contains few ecotoxic contaminants, and/or (ii) the ecology of Lucas Creek is already significantly degraded, such that we observed little effect from the stormwater outlet.

Our study suggests that the former explanation is most likely since we found no evidence of harmful concentrations of stormwater-associated metals within the sediment or water of Lucas Creek. In support of this, Lucas Creek has previously been identified as having good habitat quality, riparian cover and instream habitat, supporting a diverse macroinvertebrate community (ARC 2004).

5.1.3 ARISA Methodology

The findings of this study highlight the potential of ARISA to detect differences in bacterial community structure between complex and varied environmental samples.

The differences in bacterial community structure within sections of Lucas Creek located upstream or downstream of the stormwater outlet differed very little when compared to the differences in bacterial community structure between different Auckland streams. Nevertheless, our ARISA-based technique revealed a remarkable ability to differentiate between the bacterial communities located either upstream or downstream of the outlet, revealing them to be significantly different on all sampling occasions. This supports the use of ARISA as a sensitive and reproducible indicator of the bacterial community structure within freshwater streams. The sensitivity of this approach to detect changes in community structure is likely to be improved by the large number of individual bacterial (many million) analysed within each biofilm sample. The use of 'whole-community' bacterial indicators of stream health offers numerous benefits compared to traditional assessments of macroinvertebrate and fish communities, since:

(i) the small sample size required for analysis means that many replicate samples can be taken from a small sample area (typically the quantity of biofilm obtained from only 10 cm^2 of the stream bed is required for analysis);
(ii) samples are removed from the site with minimal sampling effort;
(iii) samples can be removed with minimal site disturbance, which allows repeated sampling at the same site, with minimal periods of time required for site recovery;
(iv) samples can be removed from sites in which alternative indicators of stream health (fish and macroinvertebrates) are not present (such as in the stormwater pipes examined in this study);
(v) using bacterial ARISA we are able to analyse many hundreds of samples within a few days, a rate which compares very favourably to high-throughput macroinvertebrate methods, and
(vi) the approach is cost effective. The costs of sample analysis are $150/sample (based on the analysis of 16 samples), but are significantly reduced for the analysis of larger sample numbers ($45/sample, based on the analysis of 96 samples).

Despite the many advantages offered by the analysis of bacterial communities, the assessment of macroinvertebrate communities remain the favoured indicators of freshwater ecological health. A key advantage of their use is the ability to use detailed taxonomic information to provide further estimates of water quality. Indeed, for bacterial communities, even where detailed taxonomic information has been gathered, the different functional roles of bacteria within complex environmental communities remain poorly understood, as does the relative sensitivity of different bacterial taxa to various anthropogenic disturbances. Additional research is therefore required to increase the sensitivity of bacterial indicators to gain the maximum potential from the high-throughput analysis of freshwater bacterial communities. However, as shown in this study, the analysis of bacterial communities is particularly suited to the assessment of highly impact environments, in which traditional indictors of stream health (e.g., fish and invertebrates) are largely absent.

5.2 Stormwater Treatment Train

Although the stormwater outlet did not cause significant differences in the concentrations of sediment and biofilm-associated metals in Lucas Creek, elevated concentrations of nickel, and especially zinc were found throughout the stormwater treatment train, reaching a maximum of 4,800 mg kg^{-1} of biofilm (dry wt.). Since concentrations of some metals (most notably of copper, zinc and lead) decreased in the downstream sampling sections of the treatment train, this would seem to highlight the effectiveness of the treatment system in reducing the load of these contaminants entering Lucas Creek.

In support of this, relatively large differences in bacterial community structure were observed throughout the stormwater treatment train. The bacterial communities within sample sites A and B were very different from those detected in C and D, which may reflect differences in contaminant sources between these sites. Sample sites A and B drain stormwater from the bus shelter and may also drain water originating from SH1, channelled down the relatively steep decline which leads directly from the highway to the bus shelter. Sites C and D are located closer to the intersection of Cornerstone Drive and Elliot Rose Avenue, and contained high concentrations of both lead and zinc. Interestingly, concentrations of copper, lead and zinc decreased mostly between sites C to F. Since no 'in-line' treatment devices are present between these sampling locations, reductions in trace metal concentrations are likely to be due to dilution effects as the piped stormwater is supplemented by additional sources of water collected from outside of the bus shelter and car park areas. In addition, much of the stormwater collected at sites downstream of E has passed through some of the 600 m swale system, before entering the stormwater drain.

In the present study, it is not possible to differentiate the relative effects of contaminant reduction by different stormwater treatment structures from those obtained by the dilution (suggested approaches to address this are outlined in section 6.1). Despite the elevated concentrations of especially zinc within the microbial biofilms of the stormwater treatment train, concentrations of copper, zinc and lead within the sediment of Lucas Creek were well within interim sediment quality guidelines (ANZECC, 2000) and concentrations were not significantly different downstream of the stormwater outlet. This provides evidence that the stormwater generated from the Albany Park and Ride car park, and surrounding infrastructure, is not contributing harmful levels of these contaminants to the receiving waters of Lucas Creek.

The Auckland Regional Council seeks to 'evaluate innovative processes for removing chemical contaminants from stormwater' (ARC, 2009). Interestingly, the StormFilter treatment device had little effect in reducing concentrations of biofilm-associated metals throughout the treatment train. Conversely, concentrations of arsenic, nickel and possibly cadmium increased directly downstream of the StormFilter (between sample sites G and H), despite there being no additional source of stormwater between these sampling locations. This would suggest either that: (i) Metals are leaching out of the StormFilter device (originating from perhaps the filter media, or the concrete structure of the underground device); (ii) Processes occurring within the

StormFilter are changing the mobility/reactivity of these metals, such that they are more likely to associate with components of the biofilm further downstream. Indeed, Fassman *et al*. (2009) recently reported that concentrations of dissolved solids increased downstream of the StormFilter. This increase in dissolved solids is of interest since the behaviour of contaminants in aquatic systems is highly dependent on the relative distribution of dissolved and particulate forms; the former exhibiting greater toxic potential as a consequence of enhanced bioavailability. (iii) Additional sources of these contaminants are entering the wetland. Elevated concentrations of certain contaminants could be entering the StormFilter via the backward flow of water from the wetland, which has been reported to occur at this site (Fassman *et al*., 2009). Treated wood has been used to construct barriers within the wetland (see appendix 9.10). It is possible that this wood contains chromate copper arsenate [CCA] preservatives, which could increase concentrations of chromium and arsenic within the wetland (we note however that concentrations of copper remained low throughout the wetland system). The origin of the increased concentrations of biofilm-associated nickel throughout the StormFilter is important to determine since concentrations of nickel were elevated within the stormwater outlet into Lucas Creek (reaching concentrations of 190 mg kg^{-1} biofilm dry wt., four times greater than ANZECC (2000) ISQG-High values). At present, a large mound of disturbed earth, covering some 2250 m^2 is located only 30 m south-east of the wetland (see appendix 9.9). During significant precipitation events, runoff from this mound is likely to enter the wetland. However, since the concentrations of contaminants within this soil have not been monitored, the impact of this mound on the wetland remains unclear.

5.3 General Conclusions

In conclusion, we found little evidence to suggest that stormwater generated from the Albany Park and Ride car park is having a negative impact on the ecology of Lucas Creek since: (i) we observed little difference in biofilm bacterial community structure (used as a biological indicator of stream health) downstream of the major stormwater outlet, and (ii) we did not observe significant increases in the concentrations stormwater-associated contaminants downstream of the stormwater outlet. Concentrations of copper, lead and zinc decreased throughout the stormwater treatment train. However, no one specific device within the treatment train was determined to be responsible for this. In addition, the effects of contaminant dilution via the addition of 'cleaner' stormwater at different sections of the treatment train cannot be quantified.

6 Conclusions and Recommendations

6.1 Implications and Recommendation for Current Management

The concentrations of metals (in both the sediment and water) in the section of Lucas Creek studied are being maintained within the limits prescribed for healthy freshwater systems, and did not increase downstream of the outlet. In addition, bacterial communities, used a biological indicator of stream health, differed little between samples sites located either upstream or downstream of the stormwater outlet into Lucas Creek. These findings suggest that current management strategies to minimize the impact of stormwater originating from the Albany Park and Ride car park, and surrounding infrastructure on the ecology of Lucas Creek are working well.

However, despite finding little evidence of any negative impact from the car park site, the ecology of Lucas Creek remains degraded, with reduced fish and invertebrate fauna in comparison to less impacted reference streams (Parkyn *et al.*, 2005). To improve the ecological health of Lucas Creek, we recommend additional investigations along the length of the stream to see if any specific causes of environmental degradation can be identified, or if the stream ecosystem is instead responding to the wide variety of diffuse stressors that are common within most urban streams (including diverse factors such as stormwater contaminants, altered stream hydrology and connectivity, and losses of riparian vegetation).

To provide a more informative test of the efficacy of treatment trains in mitigating the impacts of stormwater on the receiving waters, we recommend that more studies should be undertaken, across a range of study sites, and including locations with minimal provisions for the treatment of stormwater. These sites will provide a useful control to determine the relative extent to which the impacts of stormwater are reduced by engineered structures in the treatment train, as compared to the other processes (such as additions of less polluted stormwater, which will act to dilute the relative concentrations of contaminants further downstream in the pipe system). This would be improved by the appropriate installation of water contaminant and flow monitoring apparatus, which will help to provide mass balances of contaminant introduction and loss throughout the stormwater treatment train.

6.2 Recommendations for Future Research

Our study revealed that current management strategies are sufficient to minimize the impact of stormwater originating from the Albany Park and Ride car park, and surrounding infrastructure on the ecology of Lucas Creek. However, major developments are planned for the area directly south and west of the car park site, which has been zoned for office/residential/retail and entertainment purposes. Continual monitoring of the site is therefore required to ensure that the current treatment system is capable of mitigating the harmful impacts of stormwater originating from these new developments.

No significant differences in bacterial community structure were detected between sites either side of the StormFilter. In addition, concentrations of biofilm associated metals decreased little across this treatment device, and the concentrations some heavy metals actually increased. It would therefore be prudent to further consider the cost-effectiveness of the installation of these highly engineered structures for the removal of stormwater pollutants. It would also be desirable to elucidate the unexpected source of arsenic, chromium, cadmium and nickel in the latter stages of the stormwater treatment train. Potential contaminant sources that warrant further investigation include the timber barriers located within the treatment wetland, and the mound of disturbed soil located to the south east of the wetland. Determination of the exact contaminant source will aid the better design and maintenance of future stormwater treatment systems.

Many pollution sensitive macroinvertebrate taxa (e.g. caddisflies, mayflies, stoneflies) preferentially inhabit (and graze upon) epilithic stream biofilms (Maxted *et al.*, 2003), which we observed contain far higher concentrations of stormwater-associated metals than the stream sediment. The concentrations of metals, and other contaminants, within stream biofilms may therefore provide a better indicator of their effects of stream ecosystems due to the strong food web links between microbial biofilms and macroinvertebrate taxa with shredding and scraping feeding strategies. Further studies are required to determine the reliability of biofilm-associated contaminants as useful indicator of stream ecological health.

Overall, this study highlights the potential of bacterial ARISA as a rapid and cost-effective tool to monitor the impact of urban stormwater on aquatic ecosystems. In support of other recent studies (Lear *et al.,* 2009a, b, c), this study reveals that bacterial community analysis is a sensitive indicator of ecological health within highly modified environments in which most traditional biological indicators of water quality (i.e., fish and macro-organisms) are absent. We therefore recommend the monitoring of bacterial communities as a part of future studies, which incorporate varied measures of stream hydraulic, biogeochemical and biotic function to provide an integrative measure of stream ecosystem health.

7 Acknowledgements

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9 Appendix

9.1 Extraction of DNA from Biofilm Biomass

DNA was extracted from biofilm samples using a modified method of Miller *et al.* (1999). Up to 0.25 g of each pelleted biofilm sample were individually resuspended in 270 µl phosphate buffer (100 mM [pH 8.0]), 300 µl SDS lysis buffer (100 mM NaCl; 500 mM Tris [pH 8.0]; 10% sodium dodecyl sulphate) and 300 µl chloroform:isoamyl alcohol (24:1) within a polypropylene beadbeater vial (containing 0.5 g each of 0.1 mm and 3.0 mm silica-zirconium beads). Vials were agitated (4 ms^{-1}, 40 s) in a FastPrep machine (Bio 101, Q-BioGene, Australia), allowed to cool for 1 min and then shaken once more. Samples were centrifuged (20,000 *g*, 5 min) and the supernatant (~ 650 µl) combined with 7 M NH_4OAc (360 µl) before being mixed by hand and centrifuged (20,000 *g*, 5 min). The supernatant (~ 580 µl) was combined with 0.54 volumes of isopropanol, mixed, incubated at room temperature for 15 min, and then centrifuged (20,000 g, 5 min). The DNA pellet was then washed twice with 70% ethanol and air-dried. The extracted nucleic acids were resuspended in sterile, nuclease-free water and stored at -80 °C, until analysis.

9.2 Automated ribosomal intergenic spacer analysis of biofilm DNA

The biodiversity of bacterial communities, including unculturable components, was assessed using automated ribosomal intergenic spacer analysis (ARISA). PCR was undertaken on extracted DNA using Promaga GoTaq® Green DNA polymerase master mix (Invitro Technologies Ltd., Auckland, New Zealand) and the universal bacterial primers SDbact (5’-TGC GGC TGG ATC CCC TCC TT-3’) and LD Bact (5’-CCG GGT TTC CCC ATT CGG-3’) (Ranjard *et al.* 2001), with the following amplification conditions: (i) 95 °C for 5 min; (ii) 30 cycles of 95 °C for 30 s, 61.5 °C for 30 s, 72 °C for 90 s and then (iii) 72 °C for 10 min. To enable analysis by ARISA (Ranjard *et al.* 2001) the primer SDBact was labeled at the 5’end with HEX (6-carboxyhexafluorescein) fluorochrome (Invitrogen Molecular Probes, Auckland, New Zealand). PCR products were purified (Zymo DNA Clean and Concentrator Kit, Ngaio Diagnostics Ltd., Nelson, New Zealand) and diluted in sterile water to a concentration of 40 ng μl^{-1} (using a Nanodrop-8000 spectrophotometer; BioLab Ltd., Auckland, New Zealand). An aliquot of this solution was combined with 10 µl Hi Di formamide and an internal LIZ1200 size standard (Applied Biosystems Ltd., Melbourne, Australia), before being heat treated (95 °C, 5 min) and then cooled on ice. To generate ARISA profiles of bacterial community structure, the samples were then run on a 3130XL Capillary Genetic Analyser (Applied Biosystems Ltd.) using a 50 cm capillary and standard genemapper protocol [but with an increased run time (15 kV, 65 000 s)] to record the fluorescent intensity of different sized PCR products (approximating to the abundance of each bacterial ‘taxon’) within each sample.

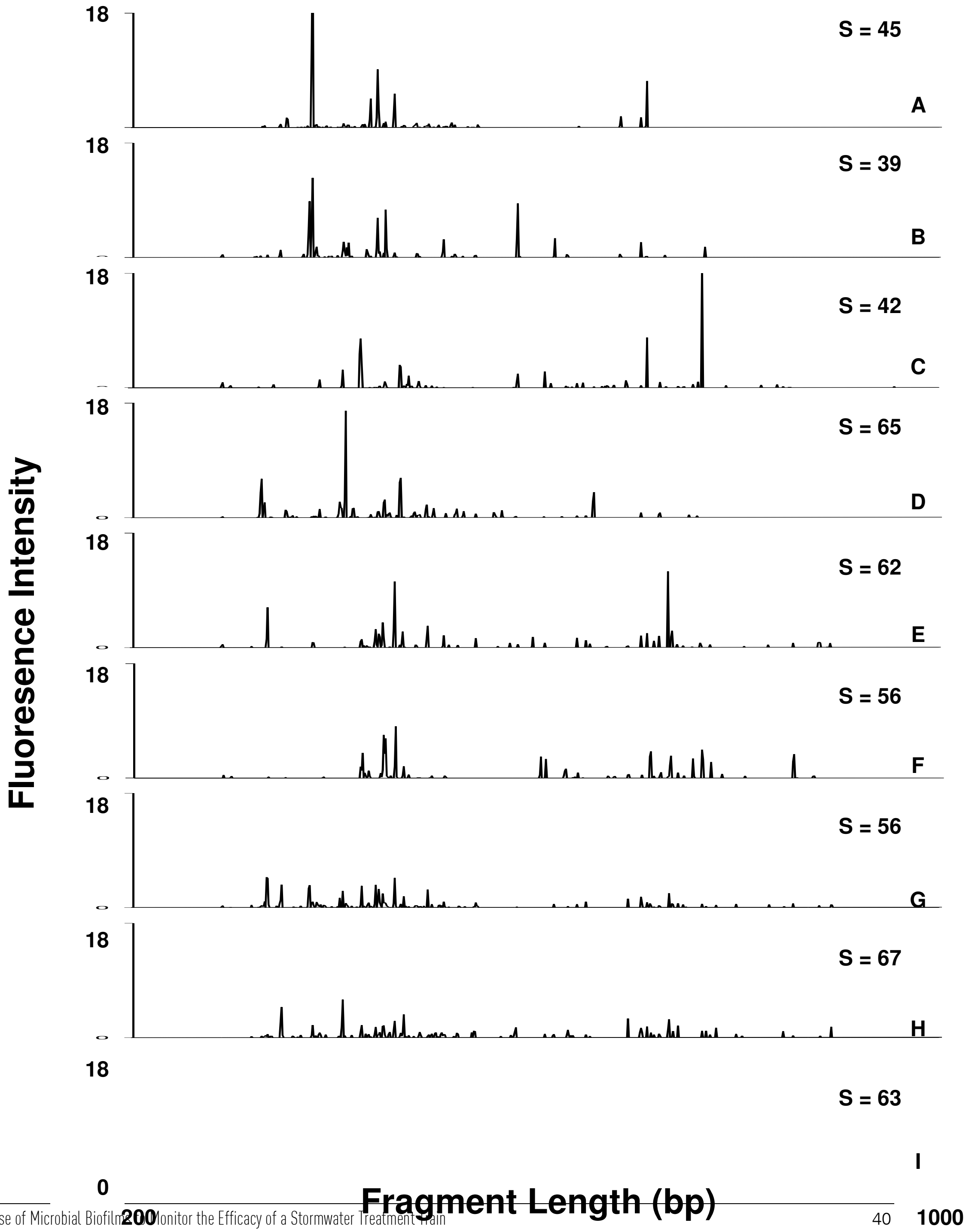
9.3 Quantitative Methods

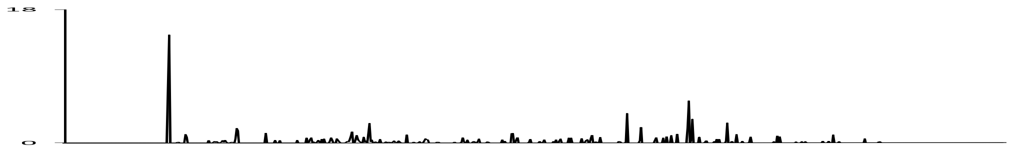
GENEMAPPER software (v. 3.7) was used to convert fluorescence data (from ARISA) into electropherograms, which enable a comparison of the proportional quantities of different-sized DNA fragments in each sampled community. This software was also used to assign a fragment length (in nucleotide base pairs) to peaks, via comparison with the standard ladder (LIZ1200; Applied Biosystems Ltd., Melbourne, Australia). To include the maximum number of peaks while excluding background fluorescence, only peaks with a fluorescence value of 50 U or greater were subsequently analysed. As the 16S-23S region is thought to range between *c.* 140 and 1530 bp (Fisher & Triplett 1999), fragments < 150 bp were excluded from analysis. No samples contained fragments >1000 bp. The total area under the curve was normalized (to 1.0) to remove differences in profiles caused by different DNA template quantities, and peak size rounded to the nearest whole number. Each sample therefore consisted of 850 variables that represent the length (in bp) of the intergenic spacer region of constituent bacteria, thereby creating a profile of the bacterial community structure within each sample.

To visualize multivariate patterns in biofilm community structure based on the ARISA data, multidimensional scaling (MDS) was performed on the Bray-Curtis measure. MDS is a non-metric procedure that is robust to outliers and preserves the rank orders of the relative distances among points in the higher dimensional data cloud as well as possible on a smaller number of dimensions. As well as plotting the relationship between datasets using MDS, the statistical significant of differences between ARISA datasets were analysed using permutational multivariate analysis of variance (PERMANOVA; McArdle *et al.*(2001)). Statistical analyses were completed using the Primer 6 (v. 6.1.11) computer program (PRIMER-E Ltd., Plymouth, UK) with the PERMANOVA+ add-on package (Anderson *et al.* 2008).

9.4 Bacterial ARISA Profiles – Jan 2009 – Treatment Train Samples

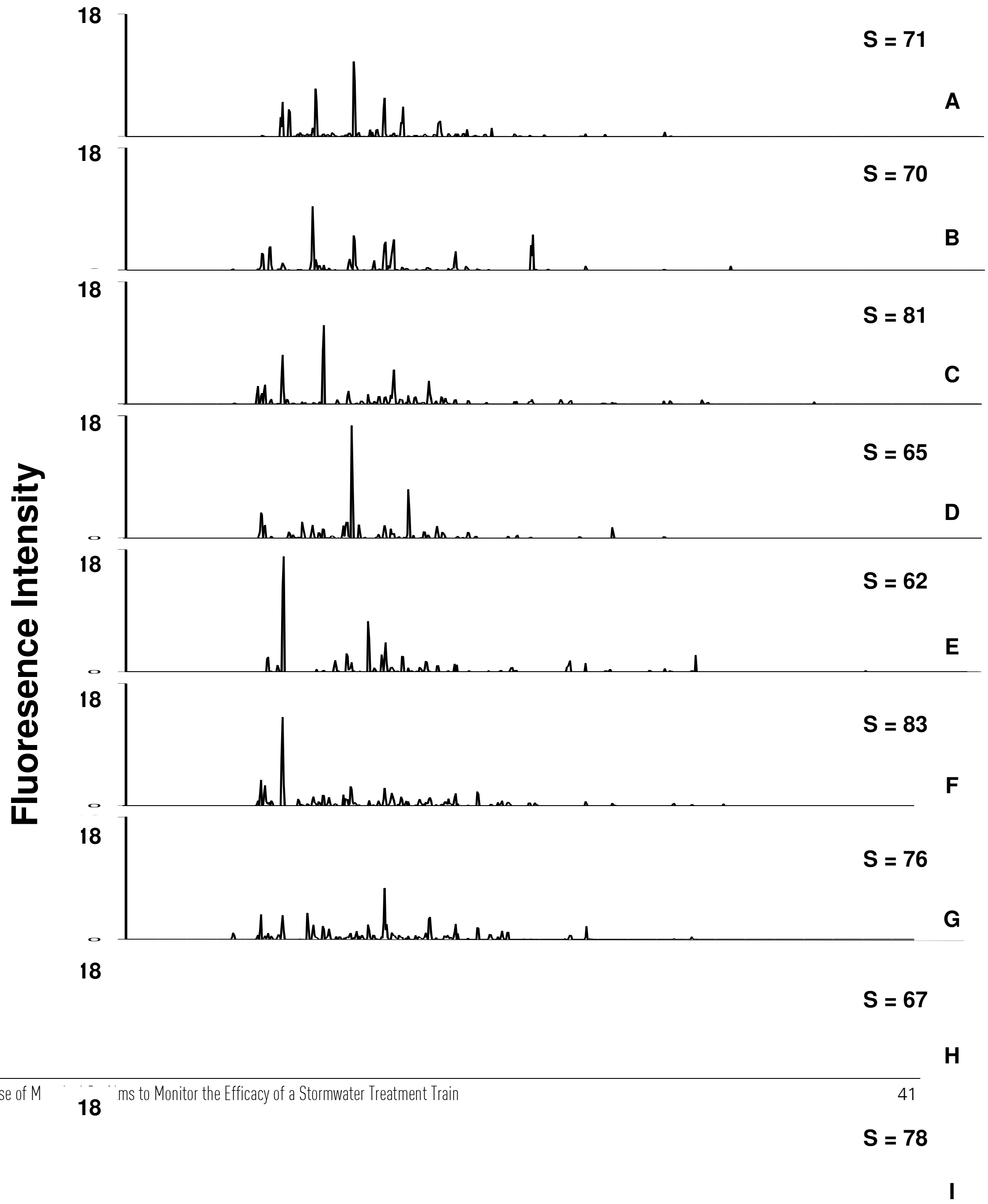
Comparison of ARISA traces obtained from different sections of the Albany Treatment Train (A to I). Data are peak height (fluorescence; y-axes) and fragment length (nucleotide base pairs; x-axes). Data are averaged for replicate samples. S refers to the number of peaks identified (analogous to taxa species richness).

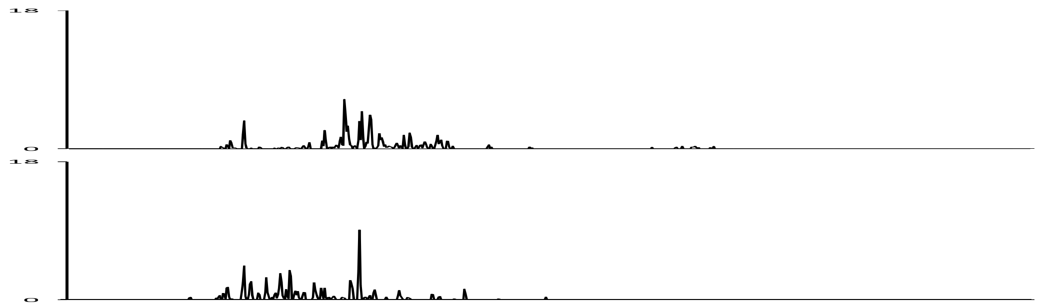




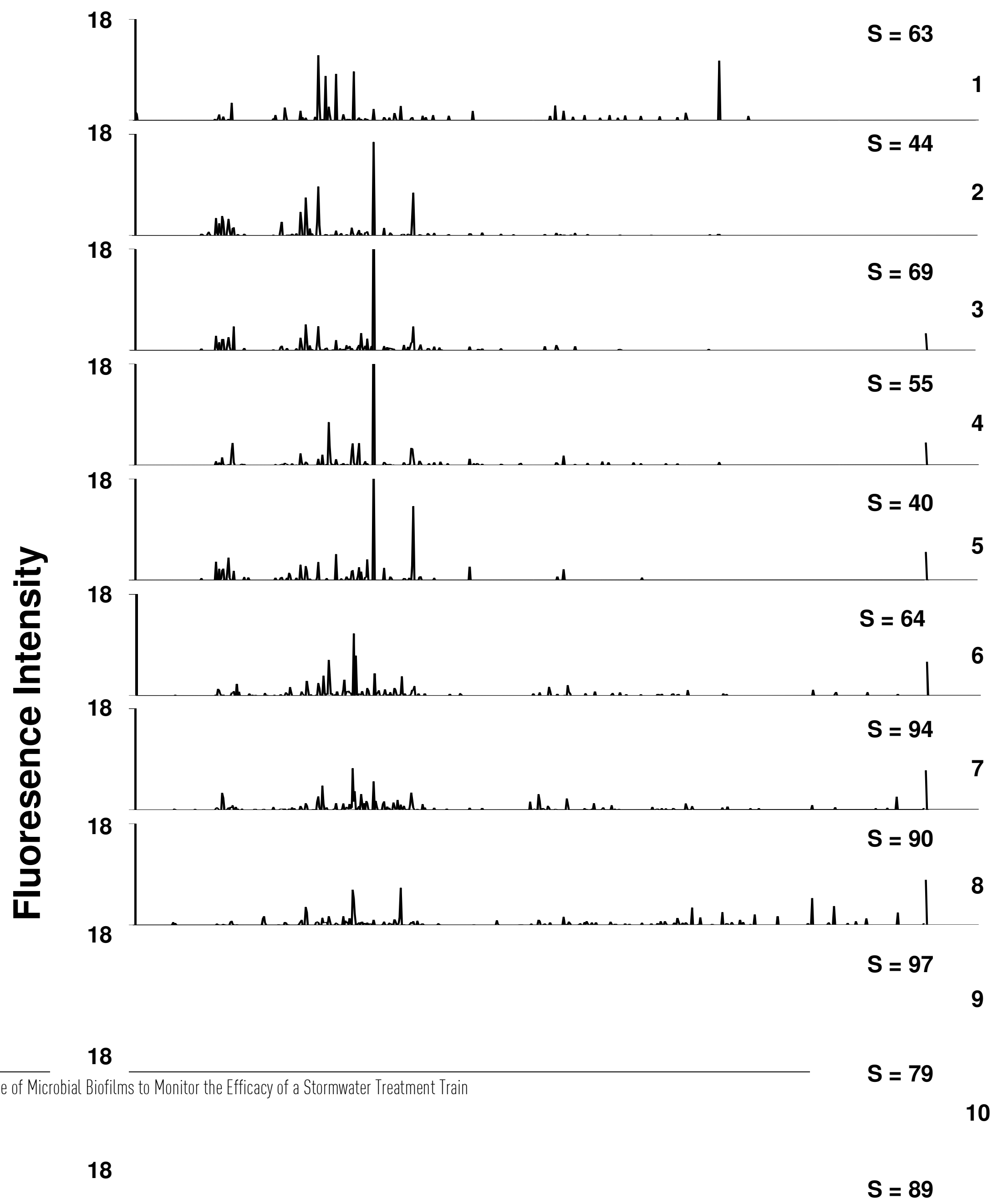
9.5 Bacterial ARISA Profiles – March 2009 – Treatment Train Samples

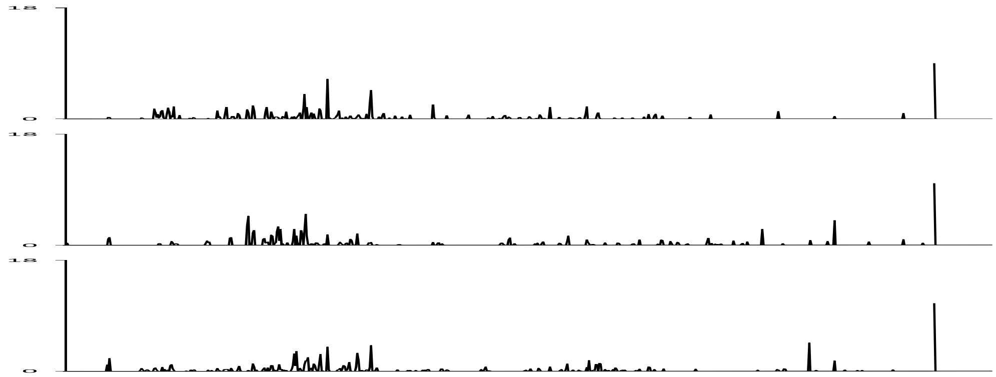
Comparison of ARISA traces obtained from different sections of the Albany Treatment Train (A to I). Data are peak height (fluorescence; y-axes) and fragment length (nucleotide base pairs; x-axes). Data are averaged for replicate samples. S refers to the number of peaks identified (analogous to taxa species richness).



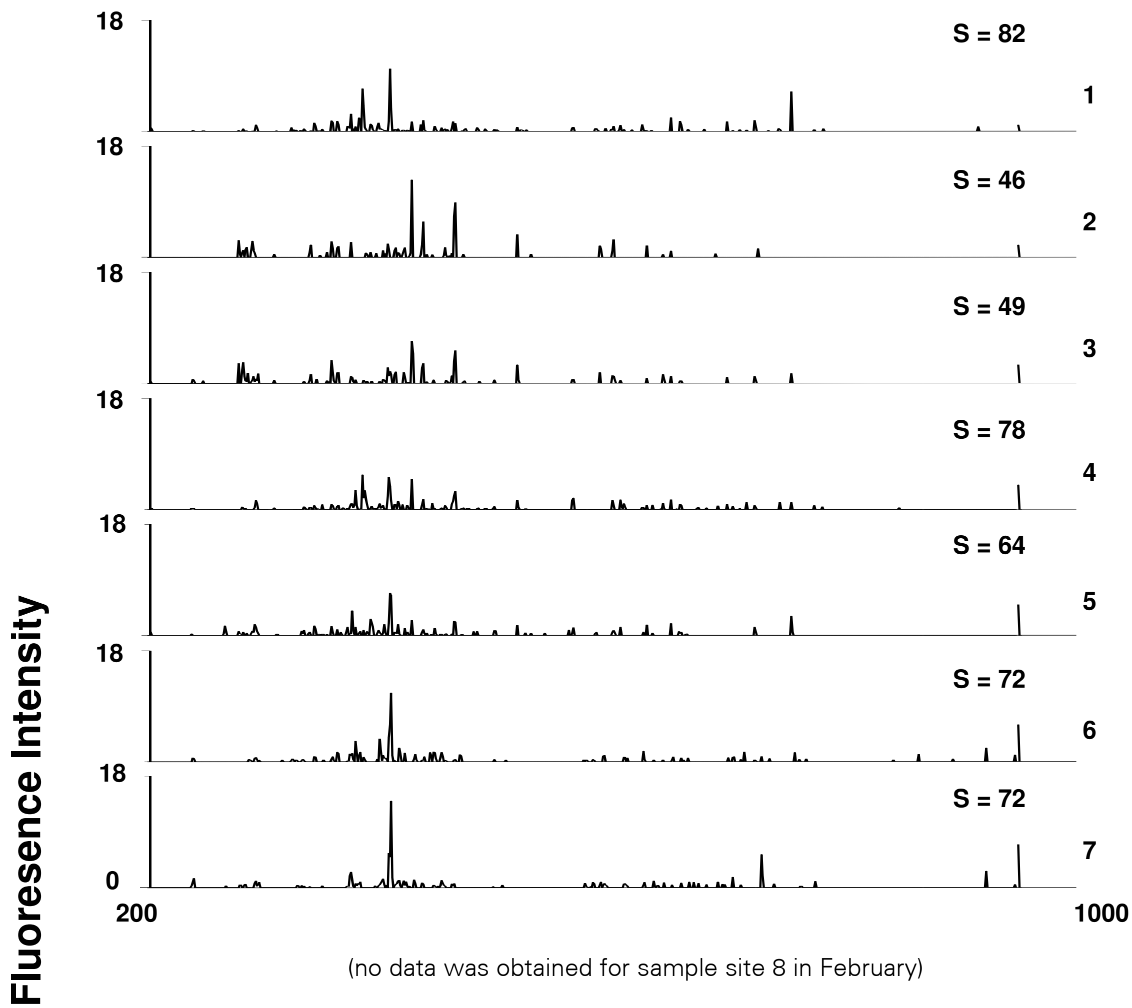


9.6 Bacterial ARISA Profiles – Jan 2009 – Lucas Creek Samples

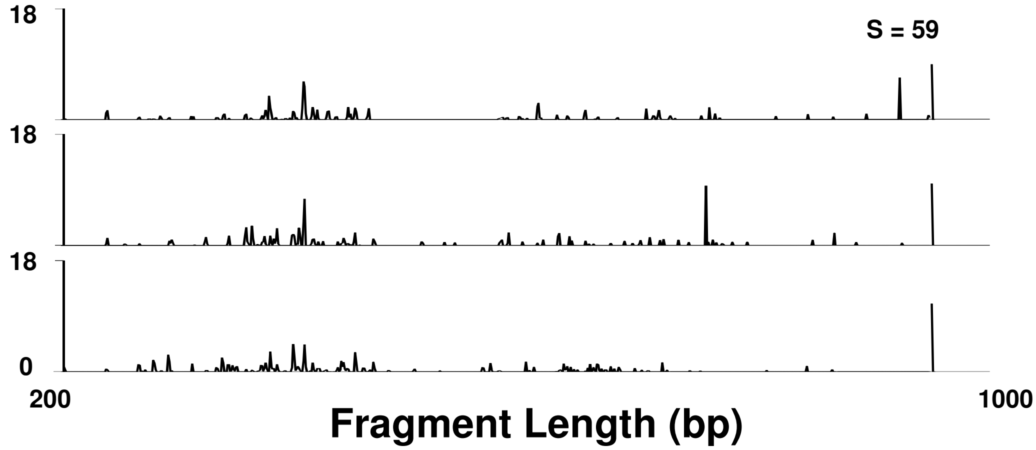




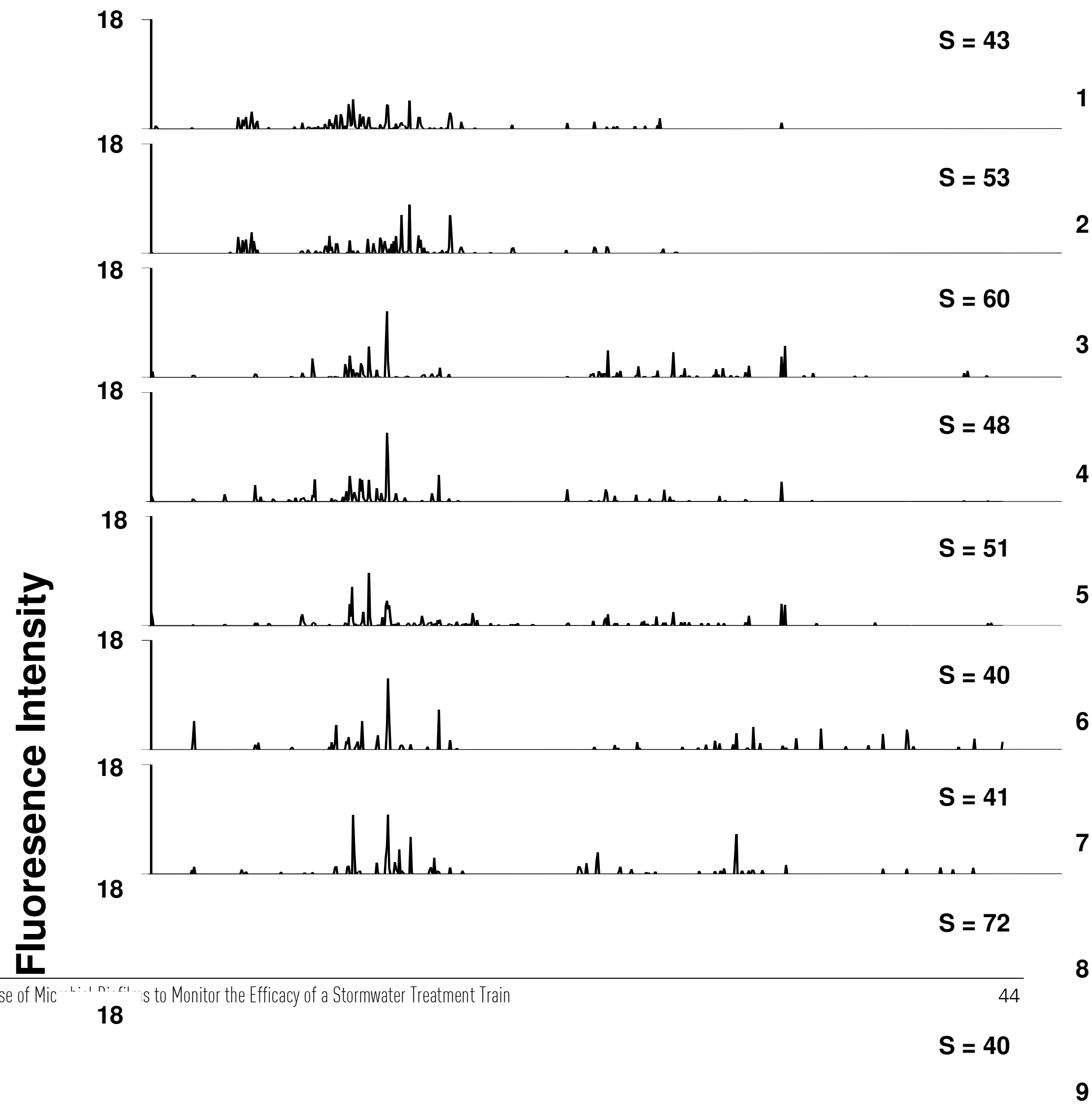
9.7 Bacterial ARISA Profiles – February 2009 – Lucas Creek Samples

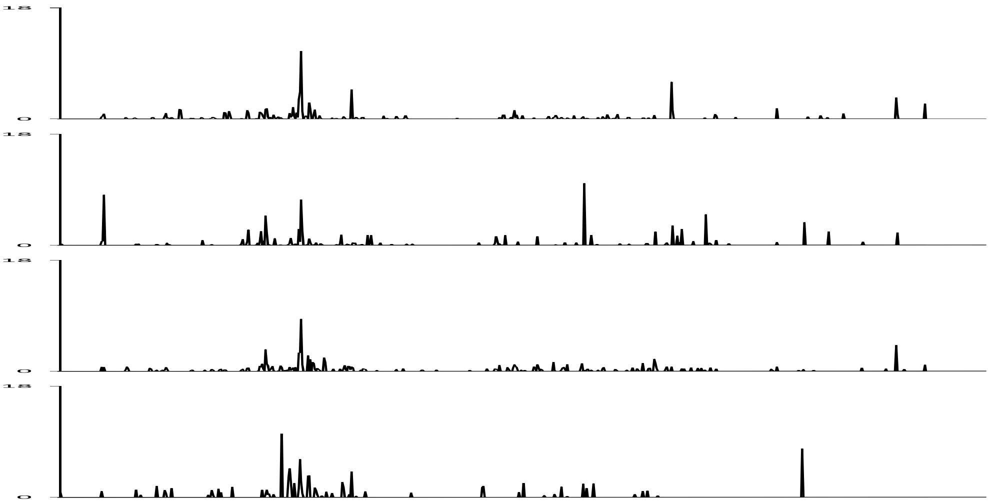


(no data was obtained for sample site 8 in February)



9.8 Bacterial ARISA Profiles – March 2009 – Lucas Creek Samples





9.9 Photograph of treated wood barrier at the western, downstream end of the treatment wetland

9.10 Photograph showing the large mound of disturbed earth south-west of the treatment wetland